SCIENTIFIC NOTE

A Survey for Potential Biocontrol Agents of *Bactrocera cucurbitae* (Diptera: Tephritidae) in Thailand

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Abstract. Limited investigations on parasitoids of the melon fly, *Bactrocera cucurbitae* (Coquillett), infesting five species of Cucurbitaceae and seven species of Solanaceae were conducted in Thailand to determine natural occurrence of biological control agents. Fruit samples were collected during January-February 1996, and tephritid puparia were imported into the State of Hawaii Quarantine Facility for fly and parasitoid emergence. *Cucumis sativus* L., *Luffa acutangula* (L.), and *Momordica charantia* L. were commonly infested by *B. cucurbitae. Bactrocera tau* (Walker) was dominant only in *L. acutangula*. Infestation of ripe, cultivated fruit of *M. charantia* was 72.3 *B. cucurbitae*/kg fruit and parasitoids were pre-dominantly *Psyttalia fletcheri* (Silvestri) (Hymenoptera: Braconidae), with parasitization rates up to 12.4%. *Bactrocera cucurbitae* infesting *M. charantia* in northern Thailand yielded an unidentified *Aceratoneuromyia* species, (Hymenoptera: Eulophidae). Unlike other eulophids that attack *B. cucurbitae*, which require the presence of *P. fletcheri* in the same puparium to suppress host immunity, this parasitoid was able to develop alone. Average fecundity was 135 offspring/female and mean parasitoids /host puparium was 21.1. It was also developed in the laboratory on the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), with an average life span of 23 d in both host species. Six species of solanaceous fruit were mainly infested by *Bactrocera latifrons* (Hendel), with infestation rates ranging from 4 to 17 flies/kg fruit. *Psyttalia fletcheri* and unidentified opiine, *Bitomus* species, eclosed from *B. latifrons* infested bird chili, *Capsicum frustescens* L. Only *Lycopersicon esculentum* Mill. produced *B. cucurbitae*, lightly parasitized by *P. fletcheri*.

Key words: *Aceratoneuromyia* sp.; *Bactrocera cucurbitae; B. latifrons; B. tau; Bitomus* sp.; biocontrol; *Cucurbitaceae*; *Psyttalia fletcheri*; *Solanaceae*; Thailand

Introduction

The Indo-Malayan tephritid, melon fly, *Bactrocera cucurbitae* (Coquillett), (Diptera: Tephritidae), has been a major pest of cucurbit and solanaceous fruit in the Hawaiian islands since 1895, and continues to be troublesome for Hawaii's fruit and vegetable growers (Back and Pemberton 1917, Newell et al. 1952, Liquido et al. 1990). All Southwest Pacific nations and Australia are seriously threatened by the recent intrusion of this pest into the region (Waterhouse 1993). The presence of large populations of melon fly in Hawaii increases the risk of accidental introductions into the mainland United States. Annual losses due to crop damage, costs of control measures, and restrictions or loss of export markets are estimated in the millions of dollars (Waterhouse 1993).

In Hawaii, all cultivated species of Cucurbitaceae (e.g. cucumber, watermelon, cantaloupe, pumpkin, bitter melon, zucchini, and oriental squash) are frequently damaged by melon flies, and when fly populations are high they also breed on Solanaceae (tomato, eggplant, and peppers), Fabaceae (string beans), Rutaceae (citrus), Myrtaceae (guava), Rosaceae (loquat, peach), and Passifloraceae (passion fruit) (see ref. in White and Elson-Harris 1992). A variety of other fruit and vegetables are sometimes injured. Feral hosts of melon fly in Hawaii are primarily wild bitter melon *(Momordica charantia* L.) and more recently, melon fly populations have been thriving on the fruit of ivy gourd, *Coccinia grandis* (L.), (Uchida et al. 1990, Ramadan unpub. data).

Early biological control efforts in Hawaii resulted in the introduction and establishment of the opiine larval endo-parasitoid *Psyttalia fletcheri* (Silvestri), (= *Opius fletcheri* Silvestri [Hymenoptera: Braconidae]), from Southern India in 1916 (Fullaway 1920). *Psyttalia fletcheri* provided a fair level of control until 1920, but its effectiveness has since declined (Newell et al. 1952, Nishida 1955, Harris and Lee 1989).

Three other opiine parasitoids were also introduced to aid in biological control of *B. cucurbitae: Diachasmimorpha albobalteata* (Cameron) (=*Biosteres* [or *Opius*] *angaleti* [Fullaway]), from North Borneo in 1951; *Diachasmimorpha dacusii* (Cameron) (=*Biosteres* [or *Opius*] *watersi* [Fullaway]) from North India in 1950; and *Diachasmimorpha hageni* (Fullaway) (=*Biosteres* [or *Opius*] *hageni* [Fullaway]) from Fiji in 1950 (Nishida 1955). The three species were propagated in the laboratory on *B. cucurbitae* and the first two species failed to become established after being released in the field (Clancy 1952). Apparently, *D. hageni* was not released. The larval parasitoid *Aganaspis daci* (Weld) (=*Trybliographa daci* Weld) (Hymenoptera: Eucoilidae) introduced from Queensland, Australia in 1949, has been reported as a primary parasitoid of melon fly (Nishida 1955). The opiine parasitoid *Fopius skinneri* (Fullaway) (=*Biosteres* [or *Opius*] *skinneri* [Fullaway]) was released in 1950 and listed as a melon fly parasitoid because of its tendency to parasitize tephritid larvae in Cucurbitaceae rather than other fruit (Waterhouse, 1993).

Unlike three other tephritid pests in Hawaii, *B. cucurbitae* is extremely resistant to several braconid and chalcid parasitoids (Nishida and Haramoto 1953). In the literature, the opiine parasitoids; *Diachasmimorpha longicaudata* (Ashmead), *Diachasmimorpha kraussii* (Fullaway), *Psyttalia incisi* (Silvestri), and *Fopius arisanus* (Sonan), and three eulophid larval parasitoids; *Tetrastichus giffardianus* Silvestri, *Tetrastichus dacicida* Silvestri, and *Aceratoneuromyia indica* (Silvestri) (= *Syntomosphyrum indicum* Silvestri) are erroneously cited as primary parasitoids of the melon fly. However, host range tests have shown that these parasitoid species may only emerge from *B. cucurbitae* that have been previously parasitized by *P. fletcheri,* which disrupts the hosts' immune system (see ref. in Nishida and Haramoto 1953, Ramadan unpub. data). More recently, *B. cucurbitae* was also found to be immune to the African opiines *Psyttalia humilis* (Silvestri) (*ex*. *C. capitata* infesting coffee), and a *Psyttalia phaeostigma* (Wilkinson) (*ex*. *Dacus ciliatus* [Loew] infesting common zucchini in Kenya, Samira Mohamed, ICIPE, Nairobi, Kenya, pers. comm.).

Among the chalcids attacking melon fly puparia are; *Dirhinus auratus* Ashmead, *D. anthracia* Walker (=*D. giffardii* Silvestri), *D. himalayanus* Westwood (= *D. luzonensis* Rohwer) (Hymenoptera: Chalcididae), *Pachycrepoideus vindemmiae* (Rondani) (= *P. dubius* Ashmead), *Spalangia afra* Silvestri, *S. cameroni* Perkins, *S. endius* Walker (= *S. stomoxysiae* Girault, *S. philippinensis* Fullaway), *S. grotiusi* Girault, and *S. nigra* Latreille (Hymenoptera: Pteromalidae), (Boucek 1963, Narayanan and Chawla 1962, Nishida 1955). These are nonspecific pupal parasitoids, undesirable in classical biocontrol programs because of their wide host range. Some are purposely released in Hawaii for biocontrol of tephritid (*P. vindemmiae* from Philippines in 1914) and muscid flies (*S. cameroni* from California in 1964, *S. endius*, from Philippines in 1914 and California in 1964, and *S. nigra* from California in 1967 [Timberlake 1922, Boucek 1963]). They play a minor role in attacking the melon fly and were frequently reported to also parasitize drosophilid puparia, act as hyperparasitoids of tachinid flies, and attack opiines in parasitized tephritid puparia (Nishida 1955, Ramadan pers. data).

Studies on field parasitism of *B. cucurbitae* in Hawaii have shown that *P. fletcheri* alone is not adequate to suppress larval populations to manageable levels, and growers of cucurbits continue to rely on costly chemicals to control this pest. Consequently, the search for other biocontrol agents was resumed in 1996, but due to limited resources, only preliminary explorations were conducted for a brief period. Here we report on a survey to obtain melon fly parasitoids from Thailand, one of the native countries of melon fly. The search focused on fruit species in the families Cucurbitaceae and Solanaceae, the most commonly injured fruit in Hawaii. Fruit of these families in Thailand are known to harbor *B. cucurbitae* and other tephritid pests including; *Bactrocera tau* (Walker), *Bactrocera latifrons* (Hendel), *Bactrocera zonata* (Saunders), *Bactrocera correcta* (Bezzi), *Bactrocera tuberculata* (Bezzi), and *Bactrocera umbrosa* (Fabricius) (White and Elson-Harris 1992).

Materials and Methods

Areas explored in Thailand, during January-February 1996 (cool-summer season), were the northern region around Chiang Mai Province (three localities near Mae-Jo and Chiang Mai city, elevations 200–1800 m), the central highlands (Bangkok, Nakhon Pathom, and Ratcha Buri, elevation 100–1000 m), and southern lowland region bordering the Malay Peninsula (Rattaphum, Hat Yai, Pattani, Narathiwat, Yaring, Bannang Sata, Yala, and Betong, elevation <100–500 m).

Ripe and green fruit with oviposition scars or signs of larval infestation were collected from roadsides, fields, and local markets. Fruit samples were weighed and placed in wire and nylon mesh cages provided with saw dust for pupation. The puparia were sifted and placed in vials with cloth covers for shipment to Hawaii. A single shipment was hand carried to Hawaii by the second author, and puparia were held in the Honolulu Quarantine Facility, State of Hawaii Department of Agriculture for fly and parasitoid eclosion. Microscopic examination of the puparia revealed that two *B. cucurbitae* individuals contained gregarious endo-parasitoids and these were held separately until parasitoid emergence. As they emerged, adults were fed on streaks of honey, and host specificity tests were conducted.

The gregarious parasitoid has been identified as *Aceratoneuromyia* sp. (Hymenoptera: Eulophidae, John LaSalle, CSIRO, Australia). Voucher specimens of puparia and parasitoids are deposited in insect collections of the State of Hawaii Department of Agriculture. *Aceratoneuromyia* sp. (44 wasps, 86.4% females) were held in one-liter plastic containers with organdy covers and provided with water and honey for ovarian maturation. After 2 days the females were exposed to mature third instar laboratory-reared *B. cucurbitae* for oviposition. Mature fly larvae in their wheat-based artificial diet were sandwiched between tissue papers and placed in the parasitoid container. Female *Aceratoneuromyia* sp. found their way to the hosts and attacked them as they exited the diet. Subsequently, parasitized puparia (i.e. those with oviposition scars and darker than the control puparia) were isolated individually in shell vials with cotton stoppers (1/4 drams, BioQuip) for rearing of F1 progeny. One month after exposure any uneclosed puparia were dissected to determine the rates of immature mortality and composition of parasitoid cadavers. Voucher specimens of the opiine and eulophid parasitoids are placed in parasitoid collections of Robert Wharton, Department of Entomology, Texas A&M University, and John LaSalle, CSIRO Entomology, Canberra, Australia, respectively.

Results and Discussion

In this report we summarize the results obtained from 2,464 puparia collected from five cucurbit and seven solanaceous fruit species (Table 1). Thailand cucurbits were commonly infested by *B. cucurbitae* and *B. tau*. The majority of *B. cucurbitae* eclosed from commercially grown *M. charantia* and produced an average of 72.3 flies / kg of fruit. The second significant host in Thailand was cucumber, *Cucumis sativus* L., harboring 33.2 *B. cucurbitae* / kg of fruit. *Bactrocera tau* varied from 0.6 to 9.0 flies / kg of fruit in the five cucurbit species and was the dominant species only in *Luffa acutangula*. The overall infestation in cucurbit fruit consisted of 94.4% *B. cucurbitae* and 5.6% *B. tau;* no other tephritid species emerged from the cucurbits sampled during January-February 1996 (cool-summer season). Moderate numbers of *P. fletcheri* emerged from *B. cucurbitae* infesting cultivated *M. charantia*, up to 12.4% (Table 1).

Aceratoneuromyia sp. is a new, previously unreported addition to the larval parasitoids known to attack *B. cucurbitae* in Northern Thailand. We also reared it on the Mediterranean fruit fly, *C. capitata*, with an average life span of 23 days on both host species. Unfortunately, F1 parasitoid offspring were exclusively males; most likely parental cohorts were not inseminated. Dissections of uneclosed puparia showed that all pharate adult cadavers were also males. This was our first experience with this Thailand species and we presumed that it would perform as its related species of Tetrastichinae, which mate immediately and mature their ovaries about two days after emergence (personal observation). However, this colony came from a very few cohorts (i.e., only two melon fly puparia) and probably they were not adapted to mate under laboratory conditions. The lack of mating and low offspring sex ratio problems are usually encountered with wild parasitoid cohorts during the initial rearing attempts under laboratory conditions (personal observations). It is also possible that parental male cohorts were adversely affected by the x-ray doses to puparia during the trans-shipment. A mean of 21.1 offspring were able to develop per puparium of *B. cucurbitae* (range 2–50 parasitoids / puparium) and the average fecundity was 135.4 offspring/female (Table 2). A related species in Hawaii, *T. giffardianus*, has an average of 6.4 progeny per puparium of *Bactrocera dorsalis* (Hendel) or *C. capitata* (Ramadan and Wong 1990).

Six species of solanaceous fruit were slightly infested by *B. latifrons* alone, with rates ranging from 4.0 to 17.3 flies / kg fruit. A handful of *B. dorsalis* eclosed from *Solanum erianthum* D. Don. (1.3 flies / kg), and *B. cucurbitae* only emerged from tomatoes, *Lycopersicon esculentum* Mill. (0.4 flies / kg fruit). *Psyttalia fletcheri* and an undescribed *Bitomus* species (Hymenoptera: Braconidae: Opiinae) eclosed from *B. latifrons* infested bird chili, *Capsicum frustescens* L. (5.6% parasitism) and the overall parasitism by *P. fletcheri* from infested solanaceous fruit was 2.2%. Nonetheless, emergence of *P. fletcheri* and *Bitomus* sp. from *B. latifrons* is a new field record from Thailand.

This report indicates that North Thailand is a region with the potential to provide new biocontrol agents for *B. cucurbitae* in Hawaii. Eulophid parasitoids in particular are desirable biocontrol agents because of their entry into the fruit, attacking larvae in a refuge that is usually missed by opiine parasitoids with short ovipositors. They are also known for gregarious development and high female biased offspring (Ramadan and Wong 1990). We recommend that, in addition to this new eulophid species, other natural enemies such as *Fopius skinneri, D. dacusii, D. albobalteata, D. hageni,* and *Aganaspis daci* should be reassessed. The melon fly and its host plants are available in Thailand throughout the year. Nevertheless, exploration was easier during January-February to avoid the monsoon and hot summer seasons. Usually, May to October months displayed heavy rains and high temperature in this country, which makes fieldwork difficult (B. Napompeth, personal comm.). Future expeditions in the Indo-Malayan region for biological control agents should also carefully consider natural occurrence of parasitoids during different seasons of the year. Thailand collaborators at the National Biological Control Research Center and Kasetsart University are on the watch for this and other melonfly parasitoids for shipment to Hawaii.

Presently, the pest status of *B. cucurbitae* in Hawaii is exacerbated by the extensive use of parasitoid-killing insecticides and the widespread invasion of the feral host *C. grandis,* resulting in population outbreaks (Uchida et al. 1990). The current record of infestation on Oahu island is 226 flies / kg *C. grandis* and 8.7% parasitism. Infestation of *M. charantia* appears to be increasing with recent records of 183 flies / kg and only 1.6% parasitism. Only *P. fletcheri* can develop successfully in larvae of *B. cucurbitae* in Hawaii (Ramadan pers. data).

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Table 1. Tephritid fruit flies and parasitoids extracted from cucurbits and solanaceous fruit collected from Thailand during January–February 1996.

Host plant (Common name)	Locality	No. samples[a]	Total weight (kg)	Total fruit fly puparia	Emergence (%)	Fruit fly (numbers)	Parasitoids (numbers, sex ratio)
Family: Cucurbitaceae							
Cucumis sativus L. (Cucumber)	Hatyai, Rattaphum, Betong, Nakhom Pathom	7 - - +	16.7	762	92.5	*B cucurbitae* (554) *B. tau* (150)	0
Luffa acutangula (L.) Roxb. (Angled gourd)	Hatyai, Betong	2 -	-	102	41.2	*B. cucurbitae* (4) *B. tau* (34)	*P. fletcheri* (3, 66.7%__), *Psyttalia* sp. (1_)
Luffa cylindrica (L.) Roem (Disk rag gourd)	Betong, Yala	4 -	-	35	91.4	*B. cucurbitae* (32)	0
Luffa sp.	Narathiwat	1 -	-	4	50.0	*B. cucurbitae* (2)	0
Momordica charantia L. (Wild form bitter melon)	Rataphum, Betong, Narathiwat	3 -	0.75	71	84.5	*B. cucurbitae* (56) *B. tau* (4)	0
Momordica charantia L. (Cultivated form bitter melon)	Malakino, Nakhon Pathom, Ratchaburi, Chiang Mai (nr. Mae jo),	4 + + -	14.5	1392	86.0	*B. cucurbitae* (1013) *B. tau* (9)	*P. fletcheri* (173, 50.9% __), *Aceratoneuromyia* sp. (44 [86.4 %__]: *ex*. 2 *B. cucurbitae* puparia)

Family: Solanaceae							
Solanum aculeatissimum Jacquin (Cockroach berry)	Hatyai, Rattaphum, Betong	5 -	0.5	9	77.8	*B. latifrons* (7)	0
Solanum torvum Swartz. (Terongan)	Hatyai, Rattaphum, Bannang, Yala, Betong	8 + + -	2.5	32	87.5	*B. latifrons* (28)	0
Solanum nigrum L. (Black night shade)	Rattaphum	1 +	70 fruit	10	40.0	*B. latifrons* (4)	0
Solanum erianthum D.Don. (Potatotree)	Ton nga, Yala, Betong	4 - - +	1.6	2	100.0	*B. dorsalis* (2)	0
Solanum incanum L. (Sparrows brinjal)	Yala, Pattani	2 +	2.0	11	72.7	*B. latifrons* (8)	0
Capsicum frustescens L. (Bird chili)	Hatyai, Rattaphum	2 +	0.75	18	83.3	*B. latifrons* (13)	*P. fletcheri* (1_), *Bitomus* sp. (1_)
Lycopersicon esculentum Mill. (Tomato)	Narathiwat, Chiang Mai (nr. Mae jo)	3 -	3.0	16	81.3	*B. cucurbitae* (12)	*P. fletcheri* (1_)

[a]Infested fruit were collected from roadsides, fields, or local markets. Plus (ripe) or minus (green) signs denote degrees of ripeness in fruit samples.

Table 2. Offspring characteristics of *Aceratoneuromyia* sp. (unmated females) reared on third instar *Bactrocera cucurbitae*.

Parameter	No. parasitized puparia examined	Mean ± SEM
Number of progeny per host puparium		
Emerged parasitoids	27	13.9 ± 1.4
Total parasitoids	64	21.1 ± 1.1
Immature parasitoid mortality (%) by stage		
Pharate adults	57	54.5 ± 5.7
Pupa	57	12.2 ± 3.5
Prepupa	57	9.5 ± 3.1
Larva	57	23.7 ± 5.2