Response of the Egg-Larval Parasitoid, *Fopius ceratitivorus* Wharton (Hymenoptera: Braconidae) to the Gall-Forming Tephritid Fly, *Eutreta xanthochaeta* (Diptera: Tephritidae)

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Abstract. We investigated the potential impact of the imported biological control agent *Fopius ceratitivorus* Wharton, on the non-target beneficial tephritid, *Eutreta xanthochaeta* on the lantana weed, *Lantana camara*. In a no-choice test, where the wasp was offered nothing but infested lantana weed, and in a choice test, where the wasp was offered both the non-target fly and its normal host, *Ceratitis capitata* (Wiedemann), *F. ceratitivorus* showed no positive response and caused neither parasitism nor mortality to *E. xanthochaeta* eggs or larvae. Infested plants exposed to *F. ceratitivorus* were reared until all flies eclosed, over which time not a single wasp emerged, indicating that *F. ceratitivorus* is unable to recognize the microhabitat of this gall-forming tephritid. These results, in addition to previous work with two other non-target tephritids, suggest minimal risk of environmental impact from this new biological control agent.

The response of *F. ceratitivorus* Wharton to the lantana gall fly, *Eutreta xanthochaeta* (Aldrich), was studied as part of an assessment of potential non-target impact of new parasitoids of tephritid fruit flies in Hawaii.

F. ceratitivorus is an egg-larval parasitoid of tephritid fruit flies from Eastern Africa, only recently discovered and described (Wharton 1999, Wharton et al. 2000). The wasp was introduced to Hawaii to improve biological control of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) for the following reasons: (1) it is co-evolved with its host, *C. capitata* (Wharton 1999, Wharton et al. 2000) and; (2) its behavior of attacking eggs located near the surface of infested fruits and vegetables offers high potential for control (Lopez et al. 2003, Bokonon-Ganta et al. 2005).

F. ceratitivorus was introduced to the Hawaii Department of Agriculture Quarantine Facility in May 2002 for studies on its host range and biology. These studies demonstrated that *F. ceratitivorus* attacks the Mediterranean fruit fly, *C. capitata* but cannot successfully develop in three other pest tephritid species, *Bactrocera cucurbitae* (Coquillett), *B. dorsalis* (Hendel) and *B. latifrons* (Hendel) (Lopez et al. 2003; Bokonon-Ganta et al. 2005). In addition, these studies revealed that *F. ceratitivorus* attacks host eggs, and very rarely first instars of *C. capitata* (Lopez et al. 2003, Bokonon-Ganta et al. 2005).

Although *F. ceratitivorus* is promising as a biocontrol agent of the Mediterranean fruit fly, the need to introduce new biological control agents should take into account the risks of candidates to non-target tephritids and other beneficial species. Previous studies on the response of *F. ceratitivorus* to two non-target tephritid flies: *Trupanea dubautiae* (Bryan), infesting flowerheads of the endemic Asteraceae shrub *Dubautia raillardioides* Hillebrand (Wang et al. 2004) and *Procecidochares alani* Steyskal, infesting the Hamakua pamakani weed *Ageratina riparia* (Regel) (Asteraceae)) demonstrated that this parasitoid appears unlikely to attack these two species (Wang et al. 2004, Bokonon-Ganta et al. 2005).

The lantana gall fly is a host specific gall-forming tephritid introduced to Hawaii in 1902 to control the lantana weed *Lantana camara* L. (Verbenaceae) (Perkins and Swezey 1924,

Funasaki et al. 1988). The fly, together with other biological control agents has achieved partial to substantial control of lantana in Hawaii (Perkins 1966, Funasaki et al. 1988).

Previous studies showed that lantana gall flies were occasionally attacked by three larval fruit fly parasitoids, *Diachasmimorpha tryoni* (Cameron), *D. longicaudata* (Ashmead), and *D. kraussii* (Fullaway) (Hymenoptera: Braconidae) (Clancy et al. 1952; Duan et al. 1996, 1998; Duan and Messing 1996; Duan and Messing 2000). *D. tryoni* was introduced to Hawaii from Australia in 1913 to control *C. capitata* (Silvestri 1914, Clausen 1956, Wong et al. 1991). *D. longicaudata* was introduced into Hawaii from Southeast Asia in 1947 to control the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Clancy et al. 1952). *D. kraussii,* a parasitoid of *Bactrocera tryoni* (Froggatt) and several other endemic Australian tephritids, was first introduced into Hawaii in 1949 for biological control of *B. dorsalis* but failed to become established. It was re-introduced into the Hawaii Department of Agriculture Insect Quarantine Facility in 1998 for further studies, which revealed that *D. kraussii* successfully parasitized *Bactrocera latifrons* (Hendel) and *C. capitata,* but not *B. dorsalis* (Messing and Ramadan 2000).

This paper reports on experiments testing the non-target impact of *F. ceratitivorus* against the beneficial biocontrol agent, *E. xanthochaeta*.

Materials and Methods

The test was carried out in the quarantine facility at the University of Hawaii at Manoa, under a temperature of 28 ± 2°C and 60–80% RH. A laboratory colony of the lantana gall fly (Figure 1A and B) was initiated from galls collected at 300–1000m in Kokee State Park on Kauai in August 2005. Adult flies were maintained in rearing cages (40 x 25 x 25 cm) in the insectary at 28 ± 2°C and 60–80% RH, under a 12:12 hour LD regime. The flies were fed with a diet made of three parts sucrose, one part protein yeast hydrolysate (Enzymatic, United States Biochemical Corporation, Cleveland, OH), and 0.5 part torula yeast (Lake States Division, Rhinelander Paper Co., Rhinelander, WI), and were given water in wet cotton wicks. Honey was also offered as additional food for adults. Subsequent fly populations were reared on potted flowering lantana plant, grown in 14-cm diameter pots and used when 20–30 cm high. Adult flies were introduced to the cages, allowed to oviposit, and then removed from the cage two weeks later. The plants were kept in the cage until emergence of a new generation of adult flies, about 45 days from the time of release of the parent flies.

Tests were conducted from October 2005 to March 2006. Both choice and no-choice tests were conducted. In the choice tests, a papaya fruit unit infested with the Mediterranean fruit fly was introduced in the test cage. The fruit was placed on a vial on a stand at about the same height as the infested lantana plant in the middle of the cage approximately 10 cm apart. In the no-choice tests only the test plant was provided.

Naïve, mature, mated 2-week-old *Eutreta xanthochaeta* (5 pairs/plant) were released in a clear plexiglas cage (40 x 25 x 25 cm) which held a potted lantana plant. Shortly after release of the flies, observations were made on their oviposition behavior including probing and oviposition attempts into the plant stem and terminal bud.

Forty-eight hours following release of the flies, 20 pairs of naïve; mature (7–10 d old) *F. ceratitivorus* were released onto the infested potted lantana plant in the cages described above. Usually female *Eutreta xanthochaeta* mate and deposit eggs 2–4 days following emergence and continue to lay eggs for up to 2 weeks when fed honey and water (Nakao and Hin Au 1974). The egg incubation period of *E. xanthochaeta* is 3–5 days (Nakao and Hin Au 1974, quoting an unpublished report of the Hawaii Department of Agriculture). Thus, parasitoids were released in the cages while *E. xanthochaeta* eggs were 1–2 days old.

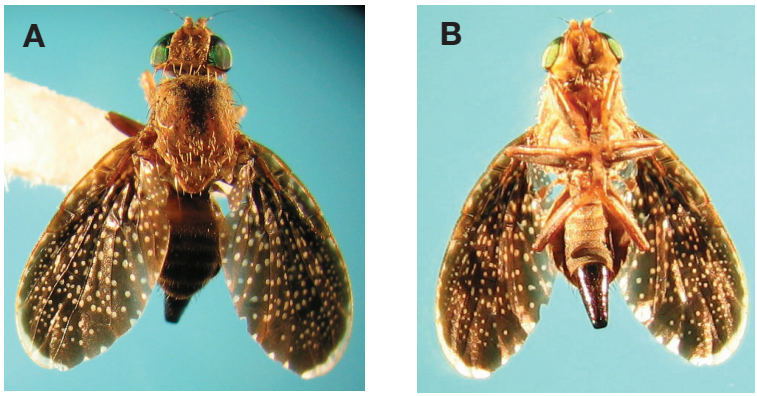

Figure 1. Dorsal (A) and ventral (B) view of female, gall-forming tephritid fly, *Eutreta xanthochaeta* (Diptera: Tephritidae).

Parasitoids were provided with streaks of pure honey (Sioux Honey Ass., Sioux City, IA) on the top of the rearing cages. For detailed parasitoid rearing procedures, see Bokonon-Ganta et al. (2005).

Upon release of parasitoids onto potted plants, responses of the parasitoids to eggs and larvae of *E. xanthochaeta* on the lantana plant and *C. capitata* eggs on the section of infested papaya were observed for 5 min every 2 h three times a day for 3 consecutive days (at 10:00 a.m., 12:00 a.m., and 2:00 p.m.). The number of times parasitoids were observed visiting or probing on terminal shoots or stems searching for *Eutreta xanthochaeta* eggs or larvae inside stem galls was recorded.

In addition to behavioral observations, parasitoids were left continuously in test cages until all of them died. At that time (about three weeks after fly release) swellings (2–3 mm diam.) were seen on terminal shoots. This confirmed infestation of the plants and insured the exposure of the wasps to different fly developmental stages of the non-target fly including eggs, early and mature larval stages. Plants were kept in cages until most flies eclosed, then all galls were dissected to determine the presence of unemerged flies, or parasitoids. All uneclosed puparia within the galls were dissected.

These tests were replicated four times using new plants and parasitoids of different generations. In the choice test, the position of the piece of papaya and lantana plants was rotated after 2 replicates.

Results

In both choice and no-choice tests, *F. ceratitivorus* showed no positive response to *E. xanthochaeta* eggs or larvae on potted lantana plants, and caused neither parasitism nor mortality to the non-frugivorous fly (Tables 1 and 2).

The wasp did not show any ovipositional responses to infested stems or galls. In all infested plants exposed to *F. ceratitivorus* all flies eclosed, and not a single *Fopius ceratitivorus* emerged, indicating that *F. ceratitivorus* is unable to recognize the microhabitat of this gall forming tephritid.

Table 1. Behavioral responses of *Fopius ceratitivorus* to *Lantana camara* plants containing lantana Gall fly, *Eutreta xanthochaeta* eggs and larvae in the absence (no-choice test) or presence (choice test) of papaya fruit infested with *Ceratitis capitata* eggs (normal host of *F. ceratitivorus*).

	Frequency of visits [a] to substrate		Frequency of ovipositor probes [b] in	
Test condition	Lantana	Papaya fruit	Lantana galls	Papaya fruit
No-choice	0	-	0	-
Choice	0	9.75 ± 0.25	0	7.25 ± 0.48

[a] Number of times *F. ceratitivorus* were observed visiting terminal shoots or stems searching for *Eutreta xanthochaeta* eggs or larvae inside stem galls; or medfly eggs inside papaya fruits.
[b] Number of times *F. ceratitivorus* were observed inserting ovipositor into lantana galls searching for *Eutreta xanthochaeta* eggs or larvae; or medfly eggs inside papaya fruits.
Tests were replicated 4 times for both choice and no-choice tests. Values are expressed as mean ± S.E.

Discussion

This study addresses concerns regarding non-target impacts of introduced natural enemies, which have resulted in a tightening of regulations against classical biological control (Messing 1999). In particular, classical biological control in Hawaii has been subject to increasing scrutiny and debate because of the highly sensitive island fauna and flora (Funasaki et al. 1988, Howarth 1991).

Our results show that *Fopius ceratitivorus* lacks normal ovipositional response to the gall forming fly, *E. xanthochaeta*. Previous studies showed that other opiine fruit fly parasitoids including *D. tryoni* and *D. longicaudata* were found to attack *E. xanthochaeta* in the field (Clancy 1950; Duan and Messing 1996, 2000; Duan et al. 1996, 1998) while *D. kraussii* was found to attack *E. xanthochaeta* in laboratory tests (Duan and Messing 2000). These are all larval parasitoids attacking late developmental stages of their respective hosts (Pemberton and Willard 1918; Ramadan et al. 1989, 1994; Rungrojwanich and Walter 2000; Messing and Ramadan 2000).

F. ceratitivorus is an egg-larval parasitoid, attacking eggs and very rarely first instars of *C. capitata* (Lopez et al. 2003, Bokonon-Ganta et al. 2005). To our knowledge, not a single case of an egg-larval parasitoid attacking non-target tephritid flies has been reported to date, despite several intensive field surveys in Hawaii (Duan et al. 1996). Adult gall-forming tephritids lay eggs on the tips of growing shoots of their host plants and hatching larvae bore into stem tissues and eventually induce spheroid galls on the apical region of plant stems. *Fopius ceratitivorus* deposits its eggs inside host eggs inserted in fruit, and does not recognize or attack fly eggs exposed or inserted between folded leaves at the plant tips.

Results from our laboratory experiments demonstrate that *F. ceratitivorus,* exposed to a range of fly stages including eggs, early and late larval stages in infested *L. camara*, completely lacks oviposition responses to the non-target fly, *E. xanthochaeta*.

In addition to the gall fly tested here, laboratory studies with other non-target non-frugivorous tephritid fruit fly species revealed that *F. ceratitivorus* lacks ovipositional response to the non-target native Hawaiian tephritid, *Trupanea dubautiae* (Bryan), infesting flowerheads of the endemic Asteraceae shrub *Dubautia raillardioides* Hillebrand (Wang et al. 2004);

Table 2. Oviposition responses of *Fopius ceratitivorus* to *Lantana camara* plants containing lantana Gall fly, *Eutreta xanthochaeta* eggs and larvae in the absence (no-choice test) or presence (choice test) of papaya fruit infested with *Ceratitis capitata* eggs (normal host of *F. ceratitivorus*).

Test condition	Number of lantana galls	Number of Medfly puparia	Gall flies emerged	Medfly eclosed	Parasitoids emerged
No-choice	3.25 ± 0.25	-	3.25 ± 0.25	-	0.00 ± 0.00
Choice	3.25 ± 0.25	128.75 ± 20.09	3.25 ± 0.25	112.25 ± 17.98	11.5 ± 2.07

Tests were replicated 4 times for both choice and no-choice tests. Values are expressed as mean ± S.E.

and to *Procecidochares alani* Steyskal, infesting the Hamakua pamakani weed *Ageratina riparia* (Regel) (Asteraceae) (Bokonon-Ganta et al. 2005). Based on the current and previous studies we conclude that *F. ceratitivorus* poses minimal risk to non-frugivorous flies in Hawaii. Therefore, utilization of *F. ceratitivorus* in biological control programs targeted against *C. capitata* would likely have no harmful impacts on non-target species.

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Literature Cited

Bokonon-Ganta, A.H. M.M. Ramadan, X.G. Wang, and **R.H. Messing.** 2005. Biological performance and potential of *Fopius ceratitivorus* (Hymenoptera: Braconidae), an egg-larval parasitoid of tephritid fruit flies, newly imported to Hawaii. Biological Control 33:238–247.

Clancy, D.W. 1950. Notes on parasites of tephritid flies. Proc. Hawaiian Entomol. Soc. 14: 25-26.

Clancy, D.W., P.E. Marucci, and **E. Dresner.** 1952. Importation of natural enemies to control the oriental fruit fly in Hawaii. J. Econ. Entomol. 45:85–90.

Clausen, C.P. 1956. Biological Control of Fruit Flies. J. Econ. Entomol. 49:176-178.

Duan, J.J., and **R.H. Messing.** 2000. Host specificity tests of *Dichasmimorpha kraussii* (Hymenoptera: Braconidae), a newly introduced opiine fruit fly parasitoid with four nontarget tephritids in Hawaii. Biological Control 19:28–34.

Duan, J.J., and **R.H. Messing.** 1996. Response of two opiine fruit fly parasitoids (Hymenoptera: Braconidae) to the lantana gall fly (Diptera: Tephritidae). *Environ. Entomol.* 25:1428–1437.

Duan, J.J., R.H. Messing, and **M.F. Purcell.** 1998. Association of the opiine parasitoid *Diachasmimorpha tryoni* (Hymenoptera: Braconidae) with the lantana gall fly (Diptera: Tephritidae) on Kauai. Environ. Entomol. 27:419–426.

Duan, J.J., M.E. Purcell, and **R.H. Messing.** 1996. Parasitoids of non-target tephritid flies in Hawaii: implications for biological control of fruit fly pests. Entomophaga 41:245–256.

Funasaki, G.Y., P.Y Lai, L.M. Nakahara, J.W., Beardsley, and **A.K. Ota**. 1988. A review of biological control introductions in Hawaii: 1890 to 1985. Proc. Hawaiian Entomol. Soc. 28:105–160.

Howarth, R.G. 1991. Environmental impacts of classical biological control. Annu. Rev. Entomol. 36:485–509.

Lopez, M., J. Sivinski, P. Rendon, T. Holler, K. Bloem, R. Copeland, M. Trostle, and **M. Aluja.** 2003. Colonization of *Fopius ceratitivorus*, a newly discovered African egg-pupal parasitoid (Hymenoptera: Braconidae) of *Ceratitis capitata* (Diptera: Tephritidae). Fla. Entomol. 86:53–60.

Messing, R.H. 1999. The impact of nontarget control on the practice of biological control. In: Nontarget Impact of Biological Control. Ed. by Follett, P. A.; Duan, J. J. Norwell, MA; Kluwer Academic Publishers, 45–55.

Messing, R.H., and **M.M. Ramadan.** 2000. Host range and reproductive output of *Diachasmimorpha kraussii* (Hymenoptera: Braconidae), a parasitoid of tephritid fruit flies newly imported to Hawaii. *In*: Area-Wide Control of Fruit Flies and Other Insect Pests, K. H. Tan [ed.], Penerbit Universiti sains Malaysia, Penang, 713–718.

Nakao, H.K., and **Au, S. Hin.** 1974. Oviposition tests with *Procecidochares* n.sp. (Family Tephritidae) a biological control candidate for *Ageratina (Eupatorium) riparia* (Regel) K. and R. Honolulu, USA; Hawaii Department of Agriculture, unpublished report, 2 pp.

Pemberton, C.E., and **H.F. Willard.** 1918. A contribution to the biology of fruit-fly parasites in Hawaii. Journal of Agricultural Research 15:419–467.

Perkins, B.D. 1966. Status and relative importance of insects introduced to combat lantana. Ph.D. dissertation, University of Hawaii, Honolulu.

Perkins , R.C., and **O.H. Swezey.** 1924. The introduction into Hawaii of insects that attack lantana. Bull. Hawaii Sugar Palnters's Assoc. 16:1–83.

Ramadan, M.M., T.T.Y. Wong, and **J.C. Herr.** 1994. Is the oriental fruit fly a natural host for the opiine Parasitoid *Diachasmimorpha tryoni* (Hymenoptera: Braconidae)? Environ. Entomol. 23:761–769.

Ramadan, M.M., T.T.Y. Wong, and **J.W. Beardsley.** 1989. Survivorship, potential, and realized fecundity of *Biosteres tryoni* (Hymenoptera: Braconidae), a larval paraistoid of Ceratitis capitata (Diptera: Tephritidae) Entomophaga 34:291–297.

Rungrojwanich, K., and **G.H. Walter.** 2000. The Australian fruit fly parasitoid *Diachasmimorpha kraussii* (Fullaway): life history, ovipositional patterns, distribution and hosts (Hymenoptera: Braconidae: Opiinae) Pan-Pacific Entomologist 76:1–11.

Silvestri, F. 1914. Report of an expedition to Africa in search of the natural enemies of fruit flies (Trypaneidae) with descriptions, observations and biological notes Territory of Hawaii Board of Agriculture and Forestry, Division of Entomology Bulletin 3:1–146.

Wang, X.G., A.H. Bokonon-Ganta, M.M. Ramadan, and **R.H. Messing.** 2004. Egg–larval opiine parasitoids (Hymenoptera: Braconidae) of tephritid fruit fly pests do not attack the flowerhead-feeder *Trupanea dubautiae* (Diptera: Tephritidae). Journal of Applied Entomology, 128:716–722.

Wharton, R.A. 1999. A review of the old world genus *Fopius*, with description of two new species reared from fruit-infesting Tephritidae. J. Hymen. Res. 8:48–64.

Wharton, R.A., M.K., Trostle, R.H. Messing, R.S. Copeland, S.W. Kimani- Njogu, S. Lux, W.A. Overholt, S. Mohamed, and **J. Sivinski.** 2000. Parasitoids of medfly, *Ceratitis capitata*, and related tephritids in Kenyan coffee: a predominantly koinobiont assemblage. Bull. Entomol. Res. 90:517–526.

Wong, T., M. Ramadan, D. Mcinnis, N. Mochizuli, J. Nishimoto, and **J. Herr.** 1991. Augmentative releases of *Diachasmimorpha tryoni* (Hymenoptera: Braconidae) to suppress a Mediterranean fruit fly (Diptera: Tephritidae) population in Kula, Hawaii. Biol. Cont. 1:2–7.