Response of Three Hymenopteran Parasitoids Introduced for Fruit Fly Control to a Gall-Forming Tephritid, Procecidochares alani (Diptera: Tephritidae)

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The response of three larval–pupal parasitoids, Diachasmimorpha longicaudata (Ashmead), Diachasmimorpha tryoni (Cameron), and Tetrastichus giffardianus Silvestri, to the Hamakua pamakani gall fly, Procecidochares alani L., was determined in the laboratory. We also observed responses of these parasitoids to their normal rearing hosts, Bactrocera dorsalis (Hendel) and Ceratitis capitata (Weidemann). D. tryoni landed on pamakani galls or on dishes containing P. alani larvae as frequently as on dishes containing C. capitata. In contrast, D. longicaudata only rarely landed on the galls. D. tryoni and D. longicaudata oviposited in galls in fewer than 1% of our observations. D. longicaudata probed P. alani larvae as frequently as B. dorsalis larvae, but no parasitoid offspring were observed. D. tryoni oviposited more frequently in C. capitata than P. alani. No D. tryoni developed in P. alani larvae. T. giffardianus landed on pamakani galls and P. alani larvae more frequently than any other host substrate. In contrast, T. giffardianus entered galls with artificially opened windows one time in 10 observations. We observed 12% parasitism of P. alani larvae dissected from the galls and 20% of parasitism P. alani in the windowed galls. We discuss the implications of our results for future augmentative or classical biological control studies.

_key words:_ biological control; tephritidae; parasitoids; Procecidochares alani; Diachasmimorpha longicaudata; Diachasmimorpha tryoni; Tetrastichus giffardianus; risk assessment; nontarget effects.

**INTRODUCTION**

One of the landmark cases of successful biological control introductions includes several hymenopteran parasitoids of the oriental fruit fly Bactrocera dorsalis (Hendel) and the Mediterranean fruit fly Ceratitis capitata (Weidemann) in Hawaii (DeBach and Rosen, 1991). Significant reductions of fruit fly populations were reported within 1 year after the release of these parasitoids (Newell and Haramoto, 1968). About six parasitoid species are responsible for significant parasitism of these fruit flies. One egg–pupal parasitoid, Biosteres arisanus (Sonan), and five larval–pupal species attack B. dorsalis and C. capitata (Wong et al., 1984). The larval species include the opine braconids Diachasmimorpha longicaudata (Ashmead), Diachasmimorpha tryoni (Cameron), Psyytalia incisi Silvestri, Biosteres vandenboschi (Fullaway), and the gregarious eulophid Tetrastichus giffardianus (Silvestri). Some species are very common in ripe and rotting fruits that have fallen from trees (Purcell et al., 1994a). Efficient mass-rearing techniques have been developed for these species (Greany et al., 1976; Wong and Ramadan, 1992; Purcell et al., 1996). The potential effectiveness of augmentative releases of fruit fly parasitoids is of great theoretical and practical interest (Knipping, 1995; Sivinski, 1996). Augmentative release of D. tryoni successfully reduced C. capitata populations in peaches and loquats (Wong et al., 1991; Wong et al., 1992). Augmentative release of D. longicaudata in Florida reduced populations of Anastrepha suspensa (Loew) in suburban dooryard fruits (Sivinski et al., 1996).

Despite numerous advantages of biological control, concerns have been expressed about potentially adverse effects on populations of native insects (Howarth, 1983; Howarth, 1985; Howarth, 1991). The parasitoids of tephritid fruit flies are not host specific. These parasitoids were originally collected from several tephritid species, although most were from the genera Bactrocera and Ceratitis (Clausen et al., 1965). One potentially adverse effect might be on native tephritid gall formers, Phaeogramma spp., and the flower head feeders, Trupanea spp. (Loope and Medeiros, 1992; Asquith, 1995).

Concerns also exist over possible effects on tephritids
that were introduced to control weeds in Hawaii. For example, parasitism of the lantana gall fly Eutreta xanthochaeta Aldrich by D. tryoni was observed shortly after the gall fly was introduced to Hawaii for control of the invasive lantana Lantana camara L. (Bess and Haramoto, 1972). Recent surveys in certain areas of Kauai indicate that D. tryoni is the dominant parasitoid of the lantana gall fly (Duan et al., in press).

Other tephritids used for biological control of weeds in Hawaii include Procecidochares utilis (Stone) and Procecidochares alani Steyskal, which were introduced from Mexico (Hapai, 1977, Davis et al., 1992). P. utilis controls Ageratina adenophora (Spreng.) R. King and H. Robinson, the Maui pamakani weed (Bess and Haramoto, 1958). On Hawaii island, P. alani is responsible for the control of Hamakua pamakani Ageratina riparia (Regel) R. King and H. Robinson (Hapai and Chang, 1986). On Maui, Bess and Haramoto (1959) observed incidental parasitism of P. utilis by D. tryoni and D. longicaudata when pamakani occurred near host fruits of C. capitata. Similarly, on Hawaii island, P. alani was recorded as a host of D. tryoni (Funasaki et al., 1988). Both lantana and pamakani occur in Australia, which is where D. tryoni originated. We do not know whether D. tryoni had already coevolved with these gall flies in their native home before they were introduced.

Procecidochares spp. lay eggs between leaves at the tips of growing shoots (Bess and Haramoto, 1959; Hapai, 1977). Hatching larvae crawl down the leaves and penetrate the tender meristematic tissues. The size of a gall depends on the number of larvae inhabiting the gall. We observed ranges of 8–17 mm in length and 4–7 mm in diameter for P. alani within Hamakua pamakani galls (M. F. Purcell, unpublished data). Larval and pupal development occurs within the gall. Before pupation, the larva cuts a channel, leaving a thin membranous layer, called the “emergence window.” The adult fly breaks the window and emerges out of the gall. Galls are typically inhabited by an average of three larvae of various ages, and feeding chambers often coalesce (M. F. Purcell, unpublished data).

To address concerns about impacts of the parasitoids introduced for control of pest tephritids in Hawaii, protocols were developed to evaluate the ovipositional response of several parasitoids to nontarget species in Kauai (Duan and Messing, 1996). These methods are currently being considered for evaluation of host specificity of new parasitoids being introduced for classical biological control of fruit flies (R. H. Messing, unpublished data).

The objective of the present study was to evaluate the potential impact of three parasitoid species on P. alani. In the laboratory, we exposed late instar larvae of P. alani in their natural substrate (intact galls), in shaved galls, or in artificial diet to three species of parasitoids, D. longicaudata, D. tryoni, and T. giffardianus. We compared ovipositional probing behavior and landing rates in these substrates with behaviors exhibited to their normal rearing hosts in artificial diet. After exposure to parasitoids, we determined the survival and development of fly and parasitoid progeny in each substrate and host.

MATERIALS AND METHODS

Exposure tests with D. longicaudata and D. tryoni. The opine parasitoids D. longicaudata and D. tryoni were obtained from the insectary of the USDA-ARS Tropical Fruit and Vegetable Research Laboratory in Honolulu. These parasitoids have been reared in the laboratory for several hundred generations (Wong and Ramadan, 1992). Parasitized puparia were flown to Hilo on a weekly basis using standardized packing and shipping methods (Purcell et al., 1994b). Parasitized puparia were transferred to wooden cages (25 × 25 × 25 cm) and held at 22 ± 2°C, 60 ± 2% RH, with a 400-lx fluorescent cool light during photophase and ambient photoperiods. The day length during these tests ranged between 11 and 13 (11–13 L: 13–11 D). Natural light was also provided during photophase through two 1 × 2 m glass windows with venetian blinds partially open to create a diffused light source. Emerged adult parasitoids were given water and honey ad libitum. Tests with D. longicaudata were done between 16 May and 19 July 1995 and those with D. tryoni between 21 September and 22 November 1995. All wasps used in these tests were sexually mature (5–8 days old) and deprived of any hosts before testing.

Stems containing mature galls of P. alani (at least 6 mm in length, no emergence windows) were randomly collected from plants growing in the Hamakua district on Hawaii island. Infested pamakani stems were cut in the field about 6 cm below the gall and immediately placed in plastic buckets filled with water. The galls were used for testing within 24 h after field collection. We used methods described in Duan and Messing (1996) to expose fly larvae in three different host substrates and conduct behavioral observations. Twenty parasitoids were selected at random and introduced into each of three Plexiglas cages (30 cm³). After 30 min, each cage was provisioned with a different host substrate: (1) intact galls, using 10 freshly collected galls, each with a stem about 6 cm long below and 1 cm long above the gall; (2) shaved galls with tissue on one side of each gall reduced to only a thin layer of plant tissue to simulate an emergence window; (3) artificial Tanaka diet (Tanaka et al., 1969), consisting of 10 late second to third instar larvae of P. alani, dissected from pamakani galls and placed inside a screen-covered petri dish (9.5 cm in diameter) containing 1 g each of moistened fresh diet and fermented diet (allowed to ferment for at least 4 days at 22°C); and (4) third instar...
lalvae of normal rearing larval species in Tanaka diet (same proportions of fresh and fermented diet were added, as described in No. 3). In the case of D. longicaudata, the normal rearing host was B. dorsalis, and with D. tryoni, the host was C. capitata. To evaluate pupation and eclosion rates of P. alani in the absence of parasitoids, two control treatments were added; the first was for P. alani in “intact” galls and a second for B. dorsalis or C. capitata larvae in diet. All treatments were replicated 10 times.

During a 3-h observation period, we recorded the frequency of substrate visits and ovipositor-probing behaviors of female parasitoids on each substrate (Duan and Messing, 1996). Hosts were exposed to parasitoids for 24 h and then removed from the cages. Larvae were examined for the presence of oviposition scars to determine the proportion of larvae with scars. One week later, the puparia were removed from either diet or the galls and transferred into a plastic cup partially filled with sand to allow for development and emergence of insects. Puparia were held for 4 weeks, and all progeny eclosing from puparia were identified. Any other parasitoid species found to have attacked P. alani before field collection of the pamakani stems were identified using published keys (Yoshimoto, 1965). Uncommon species were identified by John Beardsley, of the University of Hawaii, Department of Entomology, and Michael Schauf at the Systematic Entomology Laboratory, USDA, Washington, DC. Unemerged puparia were dissected and contents examined for undeveloped flies or parasitoids.

Exposure of hosts to Tetrastichus. Tests with T. giffardianus were performed between 22 November 1995 and 30 January 1996. The ambient photoperiod was 11:13 (L:D). Parasitoids were obtained from our rearing colony initiated in the Hilo laboratory in 1994, using methods described by Purcell et al. (1996). We used smaller Plexiglas cages (10 cm²) for exposure tests, because T. giffardianus wasps are minute (<2 mm) and we wished to maximize the chances of parasitoids locating the substrate. There were five treatments consisting of different hosts or substrates. The first treatment contained five pamakani stems with windowed galls, each placed in a 2-ml vial filled with water. Each gall window was artificially opened using a scalpel to allow T. giffardianus access to larvae. Forty sexually mature female T. giffardianus, 1-3 days old, previously deprived of hosts, were randomly selected from the colony and introduced into each cage. The second treatment consisted of ten 6-day-old B. dorsalis larvae, which were placed inside a clear plastic cup and positioned at the bottom of a Plexiglas cage. The cup was covered with screen to prevent escape of larvae, but allow entry of T. giffardianus. The third treatment consisted of 10 third instar P. alani larvae, which were dissected out of galls and placed into a cup with screened top. No artificial diet was provided to larvae in the last two treatments. Two controls were included for each substrate treatment to evaluate pupation and eclosion of P. alani and B. dorsalis in the absence of parasitoids. After 24 h, most of the larvae had pupated and were then transferred into cups with sand. Each host was examined for presence of oviposition scars, and number of scars per host was counted. We also recorded the proportion of hosts that contained one or more oviposition scars.

Only the frequency of T. giffardianus visits inside the opened gall window was recorded, because it was impossible to view females after they entered a windowed gall. In treatments containing larva or pupa, we recorded the frequency of landings on larvae rather than ovipositions, because we could not see them insert their ovipositors without magnification (we reasoned that illumination while viewing with a microscope would significantly alter probing behaviors). Four days later, galls were dissected, and hosts were placed into cups filled with sand and held for eclosion of insects. Larvae or puparia occurring in only the windowed chambers were saved. The others were discarded, because larvae were completely enclosed by plant tissue and would not be attacked by T. giffardianus.

Analysis. A small number of replicates was discarded in certain treatments if pupation rates of larvae were below 40%. Thus, to allow for comparisons of treatments with different sample sizes, we used the Kruskal–Wallis test to compare substrate visits and ovipositor probes among the four treatments. A χ² approximation was used to determine significance levels (SAS, 1994). In D. longicaudata tests, we omitted the third treatment (P. alani in Tanaka diet) because of high larval mortality rates. Tukey–Kramer’s pairwise comparison test was used to separate means of all treatments (SAS, 1994). The proportions of larvae that died, pupated, or yielded flies and parasitoids were calculated. These variables were also analyzed using Kruskal–Wallis and Tukey–Kramer pairwise comparisons. Additionally, proportions of parasitoid-exposed larvae or pupae with oviposition scars were similarly analyzed.

RESULTS AND DISCUSSION

Behavioral observations of three parasitoids. D. longicaudata made significantly more substrate visits and ovipositor probes on dishes containing artificial diet with either B. dorsalis or P. alani larvae than in larvae within intact or shaved galls (Figs. 1A and 1B; χ² = 13.48; df = 3; P = 0.004 for substrate visits; χ² = 20.5; df = 3, P = 0.0001 for ovipositor probes). For D. tryoni, the frequency of visits did not differ between hosts and substrates (Fig. 2A). However, females probed more into C. capitata larvae in diet than P. alani in diet.
or P. alani within shaved or intact galls ($\chi^2 = 20.8, df = 3, P < 0.0001$; Fig. 2B). In contrast, T. giffardianus females landed more frequently on galls than either B. dorsalis larvae or P. alani larvae ($\chi^2 = 12.0, df = 2, P = 0.002$; Fig. 3). Entry of a T. giffardianus female into the gall window occurred only once in all 10 observation periods.

Survival of fly and parasitoid progeny. In tests with the two opine parasitoids, pupation rates of P. alani were generally lower than pupation of B. dorsalis larvae but still exceeded 50% (Figs. 4A and 5A). This reduction was probably because some larvae within the galls were first to second instar larvae. Since immature larvae are not susceptible to attack by these opine parasitoids, the mortality of these larvae was considered inconsequential to the results of these tests. Although mature galls were collected, as many as five larvae may inhabit the same gall, and not all are the same age (Hapai and Chang, 1986). In contrast, pupation rates of P. alani from windowed galls exposed to T. giffardianus were 80% or higher (Fig. 6A). This was most likely because larvae were more advanced in age, since galls in these tests were already windowed. Also, larvae occurring in other chambers within the gall were discarded, because the chambers prevented T. giffardianus from gaining access to these larvae.

Progeny of D. longicaudata and D. tryoni were never observed from P. alani larvae in intact galls, shaved galls, or artificial diet (Figs. 4B and 5B). Less than 5% of P. alani larvae exposed to D. longicaudata contained oviposition scars (Fig. 4C). Parasitism of B. dorsalis larvae by D. longicaudata was 22% and parasitism of C.
capitata larvae by D. tryoni was 36%, which showed that these parasitoids produced viable progeny. These rates approximated parasitism rates occurring in quality control tests from the insectary reared parasitoids (M. F. Purcell, unpublished data). Also, the proportion of larvae with ovipositional scars from intact or shaved galls was not higher than scars found on larvae in control (no parasitoid) treatments (Figs. 4C and 5C). This indicated that the scars on these larvae were from other parasitoids in the field that attacked these larvae before galls were collected. However, 46% of P. alani larvae in dishes had scars (Fig. 5C). This suggested that D. tryoni probed larvae, but did not lay eggs, because eggs or larvae were never found.

T. giffardianus parasitized more B. dorsalis than P. alani larvae either in the windowed galls or in the cups (Fig. 6B). However, 20.4% of P. alani inside galls were parasitized by T. giffardianus. Also, 12% of P. alani larvae in the cups were parasitized by other species (E); means ± SEM.
surprising, given that only one gall entry was observed during the 10 observation tests. However, since parasitoids were confined to cages with the hosts for 24 h, some parasitoids apparently entered the galls and attacked larvae when we weren’t observing them (during the remaining 21 h). These results indicated two things: the gall substrate was acceptable to T. giffardianus for host location and P. alani larvae were physiologically suitable hosts for development of this eulophid parasitoid.

Recovery rates of P. alani flies did not differ between D. longicaudata exposed or control (no parasitoid) treatments (Fig. 4D). In contrast, fewer adult B. dorsalis flies were recovered from larvae exposed to D. longicaudata. This indicated that the parasitoids inflicted significant mortality to their normal rearing hosts, but not to P. alani.

Three species of parasitoids were recovered from P. alani, which attacked larvae before field collection of galls. These parasitoids included the endoparasitoids Eurytoma tephritidis Fullaway (Eurytomidae), Eupelmus cushmani Crawford (Eupelmidae), and the ectoparasitoid Bracon terryi (Braconidae). These parasitoids have been reported on Procecidochares spp. (Bess and Haramoto, 1959; Hapai, 1977). Two of these species, E. tephritidis and B. terryi, were accidentally introduced into Hawaii (Bess and Haramoto, 1959). The third species, E. cushmani, was purposely introduced for biological control of a coleopteran pest, Anthonomus eugenii Cano, but has attacked other immigrant species (Funasaki et al., 1988). Parasitism rates of larvae in the fall (16.7–51.1%, Fig. 5E) and winter (18.8–44.4%, Fig. 6E) months were at least double the rates observed when tests were conducted with D. longicaudata in the spring and summer months (8.4–22.5%, Fig. 4E). Could the greater background parasitism of P. alani have influenced the response of the parasitoids we tested? It is doubtful, because these parasitoids are relatively indiscriminate and will readily attack hosts previously parasitized by other species (Pemberton and Willard, 1918; van den Bosch and Haramoto, 1953).

The limited ovipositional response of the two opine parasitoids to pamakani galls indicate that P. alani is at low risk if augmentative releases of D. longicaudata or D. tryoni are made. Our results differ from laboratory exposure tests done with D. longicaudata on E. xanthochaeta (Duan and Messing, 1996). They observed a weakly positive ovipositional response and obtained 56% parasitism by D. longicaudata. The reasons for the difference in parasitoid response are unclear. Both lantana and pamakani, although predominantly found in pastures, sometimes occur in habitats of fruit trees which harbor tephritid flies. Thus the potential exists for transference of these parasitoids to gall flies. We know that ovipositional behavior of opine parasitoids is strongly affected by physical and chemical cues of the fruit in which the tephritid larvae occur (Messing and Jang, 1992). Thus, it is possible that pamakani galls lack important physical and chemical cues used by these parasitoids in host location. Both galls are similar in size, but their physical characteristics are quite different. Lantana galls are composed of mostly woody tissue, whereas pamakani is soft and succulent. Also, much is to be learned about possible associations of these parasitoids with gall flies in their country of origin, i.e., tropical America.

The positive response of the eulophid parasitoid T. giffardianus to P. alani may indicate that this nontarget fruit fly could be harmed by mass releases of this

![FIG. 6. Survival of P. alani and parasitoid progeny after exposure to T. giffardianus: Percentage pupated (A), parasitized (B), with oviposition scars (C), flies recovered (D), and parasitized by other species (E); means ± SEM.](image_url)
parasitoid. We must reemphasize that confining parasitoids with the galls in small laboratory cages greatly increases the probability of parasitoids locating the host and is a very conservative test. Thus, if a positive ovipositional response is recorded, it does not mean that this parasitoid would respond the same way in nature (see Sands, 1993, and Simmonds, 1994, for further discussion of constraints of host range testing of parasitoids in confined laboratory cages). However, laboratory tests are a logical starting point in host range testing and should be followed by field cage testing if positive responses are observed.

In subsequent field cage studies in which T. giffardianus was presented with Hamakua pamakani galls, less than 1% were parasitized (M. F. Purcell, unpublished data). Similarly, in field cage studies with lantana galls, Duan et al. (1997) showed that D. longicaudata preferred hosts in guava fruits and rarely visited lantana galls. Also, E. xanthochaeta populations were not reduced as a result of mass releases of D. tryoni in Maui (Wong et al., 1991). Rates of parasitism were the same in both control and release areas. The authors suggested that the reason for no effect was due to major differences between the two habitats occupied by non-target and target fruit flies (lantana occurs in pasture lands and fruit flies occur in fruit trees).

Field surveys of the extant parasitoids on Hamakua pamakani indicate that T. giffardianus and other parasitoids introduced for pest tephritids do not attack P. alani (Duan et al., in press). Four pest tephritids are recorded as hosts of T. giffardianus in Hawaii (Pemberton and Willard, 1918; Liquido et al., 1994; Purcell et al., 1996). T. giffardianus may have parasitized other Bactrocera species found in cucurbits in Africa, but records were unclear (Clausen et al., 1965). Further tests in the field are needed to ascertain effects of mass releases of T. giffardianus on non-target tephritids.

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