Aphidiid Parasitoids Twenty Years Post-Release in the Mariana Islands [1]

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Abstract— Despite the release of approximately 6,500 *Aphidius colemani* Vierick and *Diaeretiella rapae* (McIntosh) parasitoids as biological control agents for a variety of aphids on Guam and Saipan in 1998, no long-term establishment on either island was observed. However, the aphidiid parasitoids *Lipolexis oregmae* Gahan and *Lysiphlebus testaceipes* Cresson were found parasitizing *Aphis gossypii* Glover on a variety of crops in follow-up surveys in the years immediately following the initial aphidiid releases. The presence of *L. oregmae* is apparently the result of a fortuitous introduction, while the presence of *L. testaceipes* likely resulted from a previous introduction as an aphid biological control agent. Aphidiid surveys conducted on Guam and Saipan from 2016 through 2018 again found *L. oregmae* parasitizing *A. gossypii*, *Aphis craccivora* Koch and *Cerataphis* sp, and *L. testaceipes* infesting *A. gossypii*, *Aphis (Toxoptera) citricidus* (Kirkaldy), and *Hysteroneura setariae* (Thomas) on a variety of host plants. No *A. colemani* or *D. rapae* were found in any collections made from any island. The genotype of aphidiid parasitoids has largely been ignored in past biological control studies on aphids and should be considered when matching the natural enemy to the targeted aphid species.

Introduction

All aphids documented in the Mariana Islands of Guam, Saipan, Tinian, and Rota are exotic species that have been introduced inadvertently (Pike *et al*. 2000). Aphids often cause serious damage to crops and ornamental plantings through direct feeding, the vectoring of plant pathogens, and by promoting the growth of mold and other invasive insects, such as ants, which exhibit attendance behavior towards the aphids. Farmers in the Marianas typically rely on repeated applications of chemical insecticides to control these and other insect pests.

Biological control offers an attractive potentially cost-effective alternative to repeated applications of chemical insecticides. Numerous previous studies have shown that biological control of aphids using parasitoids in the family Aphidiidae can mitigate damaging aphid pest populations effectively in island agro-ecosystems (Volkl *et al.* 1990, Starý and Van Harten 1992, Starý *et al.* 1994, Starý *et al.* 1996). The aphidiids *Aphidius colemani* Viereck and *Diaeretiella rapae* were released as biological control agents on Guam in 1998, but aside from a single recovery of *A. colemani* on Saipan a year later, have not been recovered since. However, the aphidiid parasitoids *Lipolexis oregmae* (Gahan), *Lysiphlebis testaceipes* (Cresson) and *Aphidius matricariae* Haliday were collected from Guam, with the former two species found on a variety of aphids on Guam and Saipan, and the latter being represented by a single specimen (Miller *et al.* 2002). The purpose of the present study was to examine the current state of aphidiid populations on Guam and Saipan nearly twenty years after the first releases of *A. colemani* and *D. rapae* on Guam and Saipan.

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Materials and Methods

Aphids were collected on Guam (497 collections), Saipan (188 collections) and Rota (21 collections) from a variety of plant hosts in agricultural and natural habitats from 2016 through 2018. Parasitoids were reared from aphids held in the laboratory as described by Miller *et al*. (2002). The number of aphids per sample varied widely: ranging from 25 to >200 aphids per site. Collected aphids, along with parts of their host plant, were placed in screen-covered semitransparent plastic containers (300 ml) and held at 24□5° C for 20-35 days. Emerged parasitoids were collected and stored in 95% ethanol in 2 ml cryogenic vials (Corning, One Riverfront Plaza, Corning, NY). Sample locations were recorded using a handheld GPS receiver (Garmin Foretrex 401, Olathe KS).

Aphids and parasitoids were identified by the author and support staff at the University of Guam. Voucher specimens of aphids and parasitoids collected in this study are maintained at the Western Pacific Tropical Research Center, University of Guam. Aphid nomenclature follows Remaudière and Remaudière (1997) while aphidiid nomenclature follows Starý (1975).

Results

Tables 1 and 2 show the parasitoid-aphid-host plant relationships documented in this study. Aphidiid parasitoids were collected only from aphids on Guam. None were collected in any samples from Rota. Aphid mummies were observed on *A. gossypii* on cucumber and pumpkin on Saipan, but the samples were accidentally destroyed before parasitoids could be reared and identified, and no further parasitized samples were collected. On Guam a total of 387 *L. testaceipes* were collected from 53 parasitized aphid samples while of a total of ten parasitized aphid samples yielded only 15 *L. oregmae*. Of the 706 aphid samples collected on Guam, Saipan, and Rota, only 59 (8%) contained parasitized aphids, and these occurred only on Guam. On Guam, *A. gossypii* was the most frequently collected aphid and *L. testaceipes* the most commonly collected aphidiid of parasitoids reared from aphids, followed by *L. oregmae*. *Lysiphlebus testaceipes* was collected most frequently on *A. gossypii* found on chili pepper (45%), *Capsicum annuum* L., followed by eggplant (28%), *Abelmoschus melongena* L. Fewer samples of cucumber (11%), *Cucumis sativa* L., taro (8%), *Colocasia escuelenta* L., and coat buttons (8%), *Tridax procumbens* L. yielded *L. testaceipes*. *Lysiphlebus testaceipes* also was collected from *Aphis craccivora* Koch exclusively collected from long bean, *Vigna unguiculata* (L.) Walp., and from a single collection of *Hysteroneura setariae* (Thomas) on fingergrass, *Chloris barbata* Swingle, and two collections of *Aphis (Toxoptera) citricidus* (Kirkaldy) on calamondin, *Fortunella japonica* (Thunb.) Swingle.

Lipolexis oregmae was collected from only two samples each of *A. gossypii* on *C. annuum* and *C. sativa*, and from two collections of *A. craccivora* on long bean, *V. unguiculata*. This parasitoid also was reared from a single collection of *Cerataphis* sp. on swordgrass, *Miscanthus floridus* (Labill.) Warb. ex Schum. and Lauterb.

Table 1. Documentation of Guam aphid-parasitoid associations on various host plants. Data shown are latitude-longitude in decimal degrees, sample date (day-month-year), and sample reference number in ().

Parasitoid X Aphid index
Lipolexis oregmae **(Gahan)**
Aphis craccivora Koch on *Vigna unguiculata* (L.): Yigo 13.53236 N 144.87314 E 12-IV-16 (145, 146, 147); 13.53249 N 144.87318 E 8-VI-16 (211);
Aphis gossypii Glover on *Cucumis sativa* L.: Inarajan 13.28266 N 144.75650 E 19-II-16 (64); on *Capsicum annum* L.: Yigo E 13.532344 N 144.873368 E 6-IV-18, 13-IV-18 (306, 327); 13.529842 N 144.873863 29-V-18 (529).
Cerataphis sp. on *Miscanthus floridulus* (Labill.) Warb. ex. Schum. and Laterb.: Inarajan 13.28259 N 144.75648 E 9-II-16 (48).
Lysiphlebus testaceipes **(Cresson)**
Aphis craccivora Koch on *Vigna unguiculate* (L.): Yigo 13.53233 N 144,87309 E 25-II-16 (67); 13.53236 N 144.87308 E 12-IV-16 (143, 144,146); 13.53241 N 144.87232 E 12-IV-16 (148); 13.529679 N 144.873835 E 17-VII-18 (495, 496); Mangilao 13.44899 N 144.79953 E 19-V-16 (208); 13.44900 N 144.79950 E (209); 13.44989 N 144.79951 E 19-V-16 (210).
Aphis gossypii Glover on *Alpinia purpurata* (Viell.) K. Schum.: Agana Heights 13.470383 N 144.745242 E 18-I-18 (242); Yona 13.41801 N 144.745333 29-I-18 (259); on *Capsicum annuum* L.: Yigo 13.532344 N 144.873368 E 29-III-18 (281) 6-IV-18 (306, 307), 13-IV-18 (327, 328, 332, 334, 335, 336), 30-IV-18 (370); 13. 532361 N 144.873432 E 14-VI-18 (437); 13.529842 N 144. 873863 E 17-VIII-18 (528, 530, 539, 549, 552, 554, 556); on *Colocasia esculenta* (L.) Schott: 13.529761 N 144. 873926 E 30-IV-18 (356, 357, 359); on *Cucumis sativa* L.: Inarajan 13.28269 N 144.75655 E 2-IV-16 (44); 13.28262 N 144.75661 E 19-II-16 (61); Yigo 13.53240 N 144.87318 E 8-VI-16 (212, 213); on *Abelmoschus esculentus* L.: Mangilao 12.44886 N 144.79967 E 19-V-16 (204); on *Solanum melogena* L.: Yigo 13.31472 N 144.52260 E 22-V-18 (385, 386); 13.529729 N 144.873879 E 22-VI-18 (459); 13.314720 144.52260 E 21-VIII-18 (559, 566, 568, 575, 577, 581); on *Tridax procumbens* L. Agana Heights 13.47063 N 144.745075 E 26-I-18 (255, 256, 257)
Hysteroneura setariae (Thomas) on *Chloris barbata* Sw.: Agana Heights 13.47063 N 144.745075 E 26-I-18 (251).
Aphis (Toxoptera) citricidus (Kirkaldy) on *Fortunella japonica* (Thunb.) Swingle: Yigo 13.53247 N

Table 2. Aphid species and parasitoid host associations on Guam.

Aphis craccivora **Koch**
Lipolexis oregmae (Gahan)
Lysiphlebus testaceipes (Cresson)
Aphis gossypii **Glover**
Lipolexis oregmae (Gahan)
Lysiphlebus testaceipes (Cresson)
Cerataphis sp.
Lipolexis oregmae (Gahan)
Hysteroneura setariae **(Thomas)**
Lysiphlebus testaceipes (Cresson)
Aphis (Toxoptera) citricidus **(Kirkaldy)**
Lysiphlebus testaceipes (Cresson)

Discussion

Miller *et al*. (2002) and Pike et al. (2000) documented 21 exotic aphid pests and their associated aphidiid parasitoids that have become established on the Micronesian islands of Guam, Saipan, Tinian, and Rota. Some of these aphid pests, specifically *A. gossypii*, *A. craccivora*, *Pentalonia nigronervosa* Coquerel, and *A.* (*T.*) *citricidus*, cause serious damage and yield loss to several crop plants while other less common aphids cause damage to ornamental plantings. No aphids or aphidiid parasitoids indigenous to the Mariana Islands have been documented. *Aphidius matricariae* has been documented on Guam in the past (Pike *et al*. 2000) but was rare, and was not observed in this study. Past efforts to introduce *A. colemani* and *D. rapae* for biological control of aphids had temporary and minimal success with *A. colemani* being found on *A.* (*T.*) *citricidus* in lemon, *Citrus limon* (L.), one year after its release at a single site in 1998 at the Northern Mariana College's Kagman Experimental Farm, which is occupied presently by the CNMI Department of Lands and Natural Resources. These trees are still present on the property and are infested with *A.* (*T.*) *citricidus* but no parasitoids have been observed since the original release of *A. colemani* in 1998.

The question of why the extensive releases of *A. colemani* and *D. rapae* did not result in the establishment of one or both parasitoids species is perplexing and may rest on the identity of the diverse genetic strains of aphids found in the islands and those of the parasitoids released. In the initial parasitoid releases, *A. colemani* was obtained from a commercial insectary in the United Kingdom (Koppert UK, Ltd., Haverhill, Suffolk, UK), imported to Guam, and screened in a bio-secure insectary for hyperparasitoids prior to release. *Diaeretialla rapae* was obtained from aphids collected in the field by K.S. Pike, IAREC-Prosser, Washington State University, in eastern Washington state, was hand carried to Guam, and was screened for hyperparasitoids prior to release in the field. Both parasitoids were released into sleeve cages secured over populations of the target aphids *A. gossypii*, *A*. (*T.*) *citricidus*, *A. craccivora* and *P. nigronervosa*. Genetic analyses of *A. gossypii* and *P. nigronervosa* in Micronesia revealed significant genetic variation among aphid populations in Micronesia that was primarily correlated to the species of host plant the aphid was collected from (Foottit *et al*. 2010, Miller *et al.* 2019). Similar genetic studies have not been conducted for aphidiid parasitoids. Matching parasitoid genetic strain to a particular aphid genetic strain is not done routinely, if ever. Rather, aphid and parasitoid identification and prey-parasitoid matching is accomplished based on morphological similarities and previously described host and host plant associations. Given the range of genetic variation in aphids described by Foottit *et al*. (2010) and Miller *et al*. (2019), genetic matching of parasitoid and host may increase the chances of successful establishment of the parasitoid and control of the target pest.

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