Field Release of *Aroplectrus dimerus* Lin (Hymenoptera: Eulophidae) for Biological Control of the Nettle Caterpillar, *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae), in Hawaii

Draft Environmental Assessment
November 2007

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I. Proposed Action

An application was submitted by the Plant Pest Control Branch, Hawaii Department of Agriculture (HDOA), to the HDOA Plant Quarantine Branch, 1849 Auiki Street, Honolulu, HI 96819, for a permit to introduce *Aroplectrus dimerus* Lin (Hymenoptera: Eulophidae) into the State of Hawaii under the provisions of Hawaii Revised Statutes, Chapter 141, Department of Agriculture, and Chapter 150A, Plant and Non-Domestic Animal Quarantine. *Aroplectrus dimerus* will be used to control the nettle caterpillar (NC), *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae), an invasive species that has stinging spines and an appetite for a wide range of plants.

This Draft Environmental Assessment (DEA) was prepared by the applicant for the Office of Environmental Quality Control (OEQC), Department of Health, State of Hawaii, to comply with the provisions of Hawaii Revised Statutes, Chapter 343, Environmental Impact Statements.

II. Need for the Proposed Action

A. Detailed description of proposed action

<u>Purpose of the release</u>
The HDOA proposes to introduce the parasitic wasp *Aroplectrus dimerus* into Hawaii as a biological control agent of the nettle caterpillar (NC). Host specificity studies have been completed in the HDOA Insect Containment Facility and *A. dimerus* was found to attack only the NC and not any of 25 species of non-target Lepidoptera tested. The release of this parasitoid is expected to result in long-term control of this invasive pest.

<u>Need for release</u>
The medical importance of the NC has come to the forefront as it continues to disperse and comes into contact with human beings. The greatest numbers of complaints have come from homeowners who were stung while doing yard work. Symptoms include itching, welts, and blisters, sometimes requiring doctor or even hospital emergency room visits. NC infestations have recently been found in schoolyards and along hiking trails where stinging incidents may very likely occur. The movement of infested nursery plants on the Big Island has greatly contributed to the expansion of the NC range of infestation. Inter-island shipments of ornamental plants have resulted in the establishment of this pest on the islands of Oahu and Maui. Insecticide applications at plant nurseries are necessary to reduce NC numbers to low levels so that workers do not get stung and NC feeding damage to ornamental plants are minimized. NC populations will continue to increase unabated because of the year-round mild climatic conditions present in Hawaii and the abundance of suitable host plants found throughout the islands in residential and natural areas. The NC has a preference for a wide variety of monocot plants, including palms, irises, lilies, grasses, and ti plants, that are very commonly planted in yards and commercial landscapes. There is great potential for the NC to impact the visitor industry should the stinging caterpillars become well established in landscaped areas at hotels and along beaches.

Locations of rearing facilities and release sites

The HDOA Insect Containment Facility (ICF) is located at the HDOA Main Office Complex in the city of Honolulu, on the island of Oahu, in the State of Hawaii. The address of the property is 1428 South King Street, Honolulu, Hawaii 96814-2512. If *Aroplectrus dimerus* is approved for release from the ICF as a biological control agent, mass propagation of the wasp will be done in the HDOA Insect Rearing Facility at the same location. Release sites on all islands will be selected according to the area of highest infestation. *Aroplectrus dimerus* will be shipped as air cargo to the other Hawaiian islands for release where needed.

Number/quantity to be released

Inoculative releases of *Aroplectrus dimerus* adults will continue to be made until this parasitoid becomes well established statewide and NC population densities diminish. Numbers released cannot be predicted at this time.

Timing of release

No particular timing of releases is planned. Areas of high NC populations will have preference for releases of adult parasitoids.

Method of release

Adults of *Aroplectrus dimerus* will be released inoculatively in areas infested with the NC.

Common name and scientific classification

Aroplectrus dimerus Lin (Hymenoptera: Eulophidae). No common name.

Location of voucher specimens

Aroplectrus dimerus Lin (Hymenoptera: Eulophidae) was identified by Dr. Chao-dang Zhu on December 6, 2004. Zhu, a eulophid specialist at the Institute of Zoology, Chinese Academy of Sciences, Beijing, Peoples Republic of China, compared the Taiwan specimens with those at the Natural History Museum (London) and made the identification. Voucher specimens are deposited in the collections at the Natural History Museum (London), National Museum of Natural History (Washington D.C.), National Museum of Natural Science (Taichung, Taiwan), and Hawaii Department of Agriculture Taxonomy Unit (Honolulu, Hawaii).

B. Information on the target (host) organism(s)

Classification of target (host or pest) organism

The nettle caterpillar (NC), *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae), is a new immigrant pest in Hawaii that was first found during September 2001 at a plant nursery in Panaewa on the east side of the Big Island (island of Hawaii). It is believed to have arrived in Hawaii on *Rhapis* palm seedlings imported from Taiwan. Infestations are now found throughout the southeastern portion of the Big Island as well as in two or more localities on the west side. In mid-2007, NC infestations were discovered at a plant nursery on the island of Oahu at Kipapa and shortly thereafter at plant nurseries on Maui in Haiku. *D. pallivitta* occurs in China, Taiwan, Thailand, western Malaysia, and Indonesia (mainly Java). In these regions, the larvae have been reported to feed on the leaves of *Adenostemma* sp., *Areca* sp., *Breynia* sp., coconut, *Ficus* sp., grasses, maize, and oil palm. In its natural range, *D. pallivitta* is considered to be only a minor pest of coconut palms, probably due to the presence of natural enemies that do not occur in Hawaii (Cock et al. 1987).

Life history of the target organism

Chayopas (1982) (unpublished and from Cock et al. 1987) presented general life history data of *D. pallivitta* from Thailand. A detailed study on the biology was done in the HDOA Insect Containment Facility (see Appendix 2 - Nagamine and Epstein 2007) with the information summarized here.

Life stages: Eggs are elliptical, scale-like, and laid in masses, each egg measuring about 1.1 x 1.6 mm. Duration is seven days. There were variable numbers of larval instars, ranging from 8 to 11. Duration of all larval stages is summarized by the instar that pupated; they were 49d, 56d, 63d, and 73d for individuals that reached 8^{th}, 9^{th}, 10^{th}, and 11^{th} instars, respectively. The pupal (cocoon) stage averaged 19d with a range of 17-21d. Total duration of the life cycle, from egg to adult was 75d – 99d, depending on the number of larval instars. Adults of both sexes appear similar except that females are larger and have filiform antennae while males have bipectinate antennae. Adults do not feed.

Potential fecundity: Counts of ovarian eggs of one-day old females showed potential fecundity to be extremely high. Upon emergence, each female carried an average of 573 eggs, and, of these, 202 were mature.

Reproductive attributes: Females laid an average of 479 eggs (range = 306-676) over their lifetime, with a hatching rate of 55%. Females can begin mating on the day of emergence and can deposit their highest number of eggs on the second day after emergence, averaging 229 (range = 124-339). From then on, the number of eggs declined over the six-day oviposition period, while the post-oviposition period was 2.6 days. Female and male longevity averaged 9.7 and 11.0 days, respectively.

Pest status of pest organism

Caterpillar stings in Hawaii have become a human health problem as the NC becomes widespread and established in different habitats. Plant nursery workers, as well as homeowners, hikers, and school children, must now be aware of the hazard of making contact with the venomous spines of the caterpillar. Most commonly, people get stung while doing yard work and only realize it after having intense itching and the development of welts or blisters. Those that are hypersensitive seek treatment with a visit to the doctor or hospital emergency room. Reports of humans getting stung increase during the summer months when there is a natural NC population upsurge.

The polyphagous habit of the NC makes it a potentially damaging pest because of the wide variety and abundance of plants growing under mild year-round climatic conditions in Hawaii. Kishimoto (2006) observed feeding damage on 57 species of plants, representing 54 genera in 26 families. These include ornamental, crop, and indigenous plants, as well as some weeds. Some of the most favored ornamental plants include the golden-fruited palm (trade name “areca”;

Dypsis lutescens), rhapis palm (*Rhapis excelsa*), Phoenix palm (*Phoenix roebelenii*), Hawaiian ti (*Cordyline fruticosa*), mondo grass (*Ophiopogon japonicus*), and various species of lilies and irises. Insecticides to control NC infestations are costly to nurseries and homeowners. The tourist industry may also be impacted should the NC become established on the ubiquitous coconut palm used extensively in hotel landscaping. Caterpillars that sting visitors would negatively impact the image of Hawaii.

C. Biology of organism to be released

Aroplectrus dimerus life history (see Appendix 1 for details and pictures)
The general life cycle study for *A. dimerus* was done in the HDOA Insect Containment Facility (ICF). Complete biology studies were not possible due to the presence of a larval disease in the NC colonies. The disease, identified by Dr. Harry Kaya (University of California at Davis) as a cytoplasmic polyhedrosis virus (CPV), became entrenched in the ICF, such that the NC larvae could no longer be reared for more than one generation.

Aroplectrus dimerus is biparental. Both female and male adults are orange in color. It is a synovigenic species, i.e., females successively develop eggs to maturity throughout their reproductive life. *A. dimerus* is an ectoparasitoid and gregarious in habit. Typically 5-10 wasps develop from a single host larva, depending on the size of the host. The female first stings the host larva to paralyze it by inserting its ovipositor usually at the edges of the smooth ventral side (belly). The NC larva, when attacked by the wasp, may flail wildly and regurgitate a brownish liquid. The female wasp deposits single eggs externally on the host larva, most commonly on its lateral surfaces, embedded between segments. The host larva becomes totally immobilized within two days and remains adhered to the leaf substrate. The wasp larvae hatch from their eggs on the second day after oviposition and migrate to the belly of the host larva. They feed externally for six days and remain concealed under the host body. Dark fecal material is clearly seen in the gut as they reach maturity, and, about one day prior to pupation, the waste product (meconium) is discharged as brown, worm-like matter. The wasp pupae mature in five days and the adults emerge. The total life cycle is 13days; egg (2d), larva (6d), and pupa (5d).

Natural geographic range of *Aroplectrus dimerus*
Larry Nakahara (former HDOA Plant Pest Control Branch Manager) discovered *Aroplectrus dimerus* parasitizing *D. pallivitta* larvae at a plant nursery in Tien-wei, Taiwan on October 8, 2004. Tien-wei is located in central Taiwan and has a subtropical climate. In the scientific literature, this parasitoid is also recorded from China (Zhu et al. 2002), India (Singh et al. 1988), and the Philippines (Cock et al. 1987, Philippine Coconut Authority 1999).

Host range of *Aroplectrus dimerus* (see Appendix 1 for details)
There was no detailed host range information in the scientific literature for *Aroplectrus dimerus*. Host specificity tests were done in the HDOA Insect Containment Facility to determine if this parasitoid would attack any non-target insects. In Hawaii, there are no other species in the family Limacodidae other than the target pest *D. pallivitta,* as well as in the superfamily Zygaenoidea. Of the 25 Lepidoptera species tested, which represented 13 families, four are beneficial species (two weed biocontrol agents and two still under study in quarantine as weed biocontrol candidates), two are endemic Hawaiian species, and 19 are immigrant pests.

Host specificity evaluations were based on no-choice tests, with 20 larvae of each Lepidoptera species being exposed to *A. dimerus* females. The females did not deposit any eggs on any of the 500 total larvae exposed to them in the tests. Hence, there was no parasitoid emergence from the non-target Lepidoptera species.

Host range list

In the scientific literature, *Aroplectrus dimerus* has been recorded attacking six limacodid species in the Philippines (Cock et al. 1987,); *Darna mindanensis* Holloway, *Penthocrates albicapitata* Holloway, *P. rufa* Holloway, *P. rufofascia* Holloway, *P. styx* Holloway, and *P. zelaznyi* Holloway. In India, the limacodid *Parasa bicolor* Walker is also a recorded host (Singh 1988).

Parasites/hyperparasites

There are no records of parasites or hyperparasites attacking *Aroplectrus dimerus*.

Status as hyperparasite

There are no records of *Aroplectrus dimerus* attacking other parasitoids. This issue is not applicable because *A. dimerus* is strictly a parasitoid of limacodids and there are no parasitic Lepidoptera.

III. Alternatives to the Proposed Action

The actions being considered in this DEA are (1) No Action (not issuing a permit) or (2) issuing a permit for release of *Aroplectrus dimerus*. The no action alternative will allow the NC to continue to disperse unabated and become well established statewide. The issuance of a permit would result in releases of *A. dimerus*, which will eventually result in the suppression of NC infestations to manageable densities.

IV. Environmental Impacts of the Proposed Action and Alternatives

Expected environmental impacts of the proposed release

The release of the NC parasitoid *Aroplectrus dimerus* will have no negative impact on the natural environment. This parasitic wasp will not have any negative impact on the flora and fauna of Hawaii, both native and introduced. The only exception is that *A. dimerus* females will seek out NC larvae, sting and immobilize them, and lay eggs on them to perpetuate the existence of their species in Hawaii. The NC will be the only organism in the entire environment of Hawaii that will be impacted by the release of this parasitic wasp.

Environmental impacts associated with the no action alternative of not issuing permits for release will enable the NC to disperse throughout the Hawaiian Islands and multiply to high population densities that will cause significant damage to a wide variety of economically important plants, including native species. Large numbers of NC larvae on preferred host plants in gardens, landscaping, and natural areas will greatly increase contact with humans and result in stinging incidents. With no suppression of NC populations by natural enemies, problems caused by this pest will result in increased insecticide applications by residents, groundskeepers, nursery workers, and pest control operators to protect plants and to reduce stinging incidents.

Potential impacts on human environment

There will be no impact of the release of *Aroplectrus dimerus* on the human environment at all. This parasitoid is absolutely harmless to humans and all other animals except the NC.

Literature search for other host records

In the scientific literature, *Aroplectrus dimerus* has been recorded attacking six limacodid species in the Philippines (Cock et al. 1987, Philippine Coconut Authority 1999); *Darna mindanensis* Holloway, *Penthocrates albicapitata* Holloway, *P. rufa* Holloway, *P. rufofascia* Holloway, *P. styx* Holloway, and *P. zelaznyi* Holloway. In India, the limacodid *Parasa bicolor* Walker is also a recorded host (Singh 1988).

Host specificity in country of origin

No host specificity testing was done in the country of origin (Taiwan). Host specificity studies conducted in the HDOA Insect Containment Facility with 25 species of non-target Lepidoptera selected for testing clearly showed that *Aroplectrus dimerus* is highly specific. None of the test species was targeted by the parasitoid females and there was no sign of parasitization.

Interactions with established biocontrol agents

No biocontrol agents were previously released in Hawaii to suppress NC infestations. No natural enemies have been detected in association with NC infestations with the exception of incidental parasitization of *D. pallivitta* eggs by a *Trichogramma* species.

Potential impact on T&E species

Host specificity testing in the HDOA Insect Containment Facility showed that *Aroplectrus dimerus* will only have an impact on the target pest *D. pallivitta*. There will be no impact on any other insect or other fauna, or even flora in Hawaii.

Impact to related non-target potential hosts

In Hawaii, there are no other species in the family Limacodidae except the target pest *D. pallivitta*, as well as in the superfamily Zygaenoidea, in which the Limacodidae are classified taxonomically. Hence, there are no close relatives of the NC in Hawaii.

Potential of *Aroplectrus dimerus* to act as a hyperparasite

There are no records in the scientific literature of *Aroplectrus dimerus* acting as a hyperparasitoid. In literature, this parasitoid is only reported to attack limacodid species and since no Lepidoptera are parasitic, *A. dimerus* will definitely not behave as a hyperparasitic species.

Potential of *Aroplectrus dimerus* to attack non-targets

In Hawaii, there are no other species in the family Limacodidae and there are no species represented in the superfamily Zygaenoidea. Host specificity studies in quarantine and literature reports have shown that *A. dimerus* will only target limacodid Lepidoptera.

V. Listing of Agencies and Persons Consulted

Dr. Marc Epstein, an insect systematist and an authority on the family Limacodidae, formerly of the Smithsonian Institution, now with the California Department of Food and Agriculture, Sacramento, California.

Dr. Cheng-Shing Lin, Curator of Entomology at the National Museum of Natural Science, Taichung, Taiwan.

Dr. Chao-dang Zhu, a eulophid specialist at the Institute of Zoology, Chinese Academy of Sciences, Beijing, Peoples Republic of China.

VI. References

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VII. Appendices

Appendix 1 - Nagamine, W. T., L. M. Nakahara, J. Y. Yalemar, and M. M. Ramadan. 2007. Host specificity and biological studies for *Aroplectrus dimerus* Lin (Hymenoptera: Eurytomidae), a potential biological control agent of the stinging nettle caterpillar, *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae), in Hawaii. Report of the Hawaii Department of Agriculture, Plant Pest Control Branch.

Appendix 2 - Nagamine, W .T. and M. E. Epstein. 2007. Chronicles of *Darna pallivitta* (Moore 1877) (Lepidoptera: Limacodidae): biology and larval morphology of a new pest in Hawaii. Pan-Pacific Entomologist 83(2):120-135.

HAWAII DEPARTMENT OF AGRICULTURE
Honolulu, Hawaii

Host Specificity Testing for *Aroplectrus dimerus* Lin (Hymenoptera: Eulophidae), a biological control agent of the nettle caterpillar, *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae).

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Introduction

Nature of the problem

The nettle caterpillar, *Darna pallivitta* (Moore), is a new immigrant pest to Hawaii that was first noticed in September 2001 after workers at a nursery on the east side of the island of Hawaii (Big Island) were being "stung" by a caterpillar while handling rhapis palms (*Rhapis* sp.). It was suspected of having entered the state on palm seedlings legally imported from Taiwan. Immediately after its detection, an eradication attempt with pesticides was made but proved unsuccessful. In January 2002, surveys showed its establishment on three surrounding farms where the larvae were found feeding on coconut palm (*Cocos nucifera* L.), areca palm (*Chrysalidocarpus lutescens* Wendl), rhapis palm, Hawaiian ti (*Cordyline terminalis* Kunth), and *Dracaena* sp.

Darna pallivitta is now well established in the Hilo area on the east side of the Big Island and has slowly moved from the original infestation site southward into the Puna District. It was discovered in Kona on the west side of the island during September 2006 and at Kohala in the north side during February 2007, both of these infestations likely resulting from movement of infested plants. During June 2007, an infestation at a nursery on Oahu Island was discovered after workers were being stung while handling areca palm plants. The source of this infestation was believed to be the importation of palms about a year earlier from a nursery located on the east side of the Big Island, where *D. pallivitta* is firmly established. A similar scenario occurred on Maui island during July 2007, where a new infestation was found in an area nearby to plant nurseries.

The polyphagous habit of *D. pallivitta* increases its pest potential in Hawaii. Field observations of feeding damage include both weedy and ornamental plants commonly grown in residences and agriculture (Kishimoto 2006). Damage to ornamental plants, including the many palm species grown in Hawaii, could result in economic losses to the nursery industry and homeowners. Also potentially threatened by larval feeding are endemic plant species. Of medical importance are the stinging spines of the larva, which cause dermatitis on contact with the skin. Reports of people being stung by *D. pallivitta* larvae typically increase during the summer months due to population surges. Outbreaks in residential communities result in homeowners getting stung while working in the yard. Symptoms vary, but include itching, welts, and blisters, depending on a person's sensitivity. Doctor and emergency room visits for caterpillar stings have been recorded.

D. pallivitta occurs in China, Taiwan, Thailand, western Malaysia, Indonesia, and Java, and its host plants in those regions include *Adenostemma* sp., *Areca* sp., *Breynia* sp., coconut, *Ficus* sp., grasses, maize, and oil palm. It is considered only a minor pest of coconut palms in its natural range, probably due to the presence of natural enemies that do not occur in Hawaii (Holloway et al. 1987).

Biological control program

The Nettle Caterpillar Project was a joint project between the Hawaii Department of Agriculture (HDOA) and the University of Hawaii (Dr. Arnold Hara, UH-Manoa). The first effort to search for *D. pallivitta* natural enemies was collaboration with Dr. Dantje Sembel of Sam Ratulangi University located in Manado, North Sulawesi, Indonesia during 2003. Sembel, an entomologist and Professor in the Faculty of Agriculture, has vast experience in coconut pests at the Coconut Research Center in Manado. His collections of three limacodid species (*Pectinarosa alastor* Tams, *Thosea monoloncha* Meyrick, and *Darna catenatus* Snellen) from Sulawesi Island yielded a parasitoid, *Nesolynx* sp. (Hymenoptera: Eulophidae) attacking the cocoon stage of *Darna catenatus* Snellen. However, testing in the HDOA Insect Containment Facility showed this parasitoid to be a generalist as it developed on two species of fly pupae.

The second attempt to collect potential biological control agents was made by Kenneth Teramoto, Chief of the HDOA Biological Control Section, in collaboration with Dr. Banpot Napompeth of the National Biological Control Research Center in Thailand during June 2004. Teramoto and Napompeth searched central and southern Thailand and found the limacodid *Parasa lepida* Cramer being attacked by a braconid wasp, however, the parasitoids died before shipment to Hawaii.

Further exploration was conducted in Taiwan during October 2004 by Larry Nakahara, former manager of the HDOA Plant Pest Control Branch. Cooperation and critical field assistance in Taiwan was provided by Jai-Hsueh "Michelle" Lin, staff of the Taiwan Agricultural Research Institute (TARI), nurserymen from Tien-wei Village (central Taiwan) and Ping-tung County (southern Taiwan), and entomology students from Ping-tung University.

The major discovery of the *D. pallivitta* species was made by Nakahara at a Tien-wei nursery on October 8, 2004. There he also found parasitized larvae on ti plants (*Cordyline terminalis*), rhapis palms, and miniature coconut palms. Adult wasps began emerging from the parasitized caterpillars three days later. Collections of live, unparasitized *D. pallivitta* larvae were also made at two Ping-tung nurseries and these caterpillars were used for propagation of the parasitoid from Tien-wei. One shipment of parasitoids was sent by Nakahara to Hawaii for study in the HDOA Insect Containment Facility.

The parasitoid from Taiwan was identified as *Aroplectrus dimerus* Lin (Hymenoptera: Eulophidae) by Dr. Chao-dang Zhu on December 6, 2004. Zhu, a eulophid specialist at the Institute of Zoology, Chinese Academy of Sciences, Beijing, Peoples Republic of China, compared the Taiwan specimens with those at the Natural History Museum (London, UK) and made the identification. Specimens of *A. dimerus* are deposited there and also in the collections at the National Museum of Natural History (Washington D.C.), the National Museum of Natural Science (Taichung, Taiwan), and the Hawaii Department of Agriculture (Honolulu, Hawaii).

In the scientific literature, *A. dimerus* has been recorded attacking six limacodid species in the Philippines (Cock et al. 1987, Philippine Coconut Authority 1999); these are *Darna mindanensis* Holloway, *Penthocrates albicapitata* Holloway, *P. rufa* Holloway, *P. rufofascia* Holloway, *P. styx* Holloway, and *P. zelaznyi* Holloway. In India, the limacodid *Parasa bicolor* Walker is also a recorded host (Singh 1988).

There was no detailed biology in the scientific literature for *A. dimerus*, therefore, host specificity and life cycle studies were conducted in the HDOA Insect Containment Facility with the results presented in this report.

Materials and Methods

<u>Nettle caterpillar propagation</u>

Darna pallivitta larvae were reared in screened cages (42 x 42 x 62 cm) and fed leaves of Hawaiian ti or iris (*Tritonia crocosmiiflora*). After cocooning (pupating) and emergence of adults, about five female and five male moths were collected from the stock cage and placed in a wide-mouth, one-gallon glass jar for mating and egg-laying. A bouquet of ti or iris leaves, made with a strip of cotton wrapped around the petioles and snugly inserted into a small narrow-necked bottle or florist's vial, was placed into the jar. The mouth of the jar was covered with organdy cloth and secured with rubber bands. Moths usually laid eggs on the glass and not on the leaves. Newly hatched larvae crawled from the glass onto the leaves to feed. As they matured, the entire bouquet was transferred to a screened cage for continued feeding and development. A larval disease, identified by Dr. Harry Kaya (University of California at Davis) as a cytoplasmic polyhedrosis virus (CPV), later became entrenched in the HDOA Insect Containment Facility, such that *D. pallivitta* could no longer be reared for more than one generation. Despite decontamination efforts, the virus could not be eliminated from the lab. Instead, the HDOA Big Island Insectary, which was able to better manage the CPV disease, shipped *D. pallivitta* larvae to Oahu on a regular basis for propagation of the parasitoid.

<u>Parasitoid propagation</u>

Aroplectrus dimerus was reared in a one-gallon glass jar (previously described) containing 15 mid- to late-instar (ca. L6-L10) larvae and five mated female parasitoids. Honey was dotted inside the jar as a food source for the wasps. After a 7-day exposure period, the female wasps were removed. A new generation of adult wasps began emerging 13 days after initial exposure.

<u>Life cycle study</u>

The general life cycle for *A. dimerus* was determined, however, detailed studies were not possible due to the presence of the CPV disease in the *D. pallivitta* larval colonies.

Host specificity testing

In Hawaii, there are no other species in the family Limacodidae except *D. pallivitta*, and there are no species represented in its superfamily Zygaenoidea (Dalceridae, Epipyropidae, Lacturidae, Limacodidae, Megalopygidae, and Zygaenidae), hence, there were no Hawaiian species closely related taxonomically. Twenty-five Lepidoptera species (Table 1), representing 13 families, were tested to determine if the parasitoid *A. dimerus* would attack any non-target species. These included four beneficial species (two currently used for weed biological control and two still under study), two Hawaiian endemics, and 19 immigrant pests. For some species, field-collected larvae were used for testing if they were found in abundance. For others, field-collected eggs, larvae, or adults were then propagated in the lab for one or more generations to increase their numbers for testing. A few species were already being lab-reared for other projects and were readily available.

All host specificity testing for *A. dimerus* was conducted in the HDOA Insect Containment Facility. Host specificity evaluations were based on no-choice tests. Ten larvae of a Lepidoptera test species were placed in a one-gallon glass jar (previously described) with their food source and exposed to five *A. dimerus* females for a 24-hour period. The respective larval food sources were pods, flowers, or leaf bouquets, placed in the jar and replenished as necessary. The control replicate was done in the same way but with 10 *D. pallivitta* larvae and a bouquet of iris leaves as a food source. Honey was dotted in the jar for the wasps.

After the exposure period, each test larva was removed from the jar and the number of parasitoid eggs counted on its body using a dissecting microscope. The 10 test larvae were then placed in another jar with their respective food source to continue to feed until moth or parasitoid emergence occurred. The same procedure was followed with the 10 control (*D. pallivitta*) larvae, however, because of their long life cycle (ca. 10 weeks); the larvae were only held for parasitoid emergence (about 2-3 weeks). Parasitoids were used only once during testing, and their ages were the same for a test and control replicate, but may have varied among replicates of different species. Two replicates of 10 larvae each were conducted for each Lepidoptera species, for a total of 20 larvae tested per species.

Results

Life history

Aroplectrus dimerus is biparental, females and males both orange in color. It is a synovigenic species, i.e., females successively develop eggs to maturity throughout their reproductive life. It is an ectoparasitoid and gregarious in habit, typically 5-10 wasps developing from a single host larva, depending on its size. The female first stings the host larva to paralyze it, inserting its ovipositor usually at the edges of the smooth ventral side (belly) (Fig. 7). The *D. pallivitta* larva attacked by a wasp may flail wildly and regurgitate a brownish liquid (Fig. 8). The female wasp deposits single eggs externally on the host larva, most commonly laterally embedded between segments (Fig. 9). The host larva becomes totally immobilized within two days and remains adhered to the leaf substrate. The wasp larvae hatch from the eggs, also in two days, and migrate to the belly of the host larva (Fig. 10). They feed externally for six days and remain concealed under the host body (Fig. 11). The dark fecal material is clearly seen in the wasp larvae as they reach maturity, and about one day prior to pupation, the waste product (meconium) is discharged as a brown, worm-like matter (Fig. 12). The wasp pupae mature in five days (Fig. 13) and the adults then emerge. The total life cycle is 13 days; egg (2d), larva (6d), and pupa (5d).

Host specificity tests

Results of host specificity tests for *A. dimerus* are shown in Table 2. In no-choice tests, *A. dimerus* females did not deposit any eggs on any larvae of the 25 non-target Lepidoptera species tested, hence, there was no parasitoid emergence. All test larvae examined under a dissecting microscope also showed no evidence of injury due to ovipositional probing and there were no indications of larval regurgitation in the jar due to attack by an *A. dimerus* female. The female parasitoids also appeared to have no specific attraction to three larval species (*Agraulis vanillae*, *Nyctemera apicalis,* and *Secusio extensa*) that have long setal hairs somewhat similar to *D. pallivitta*.

Parasitism was recorded in all control (*D. pallivitta*) replicates for all Lepidoptera species tested. Analysis by One Way ANOVA showed a significant difference ($P \leq 0.05$) for parasitism among all non-target Lepidoptera species compared with their controls. The number of *D. pallivitta* larvae parasitized for a pair of replicates (N=20 larvae) ranged from 40-85%, with an average of 4.7 wasps emerging per parasitized larva.

Conclusion

The collection of *D. pallivitta* on palm plants at a Taiwan nursery by L. Nakahara in 2004 was a major finding in itself because of its minor pest status in its homeland. The second crucial discovery was of the eulophid wasp *A. dimerus* parasitizing *D. pallivitta* caterpillars. Natural enemies found in the native range of a pest are more likely to have evolved with its host and therefore have greater specificity. Our studies showed that *A. dimerus* did not attack any of the 25 Lepidoptera species tested. In total, 500 test larvae were exposed to the parasitoid with none being parasitized.

The development of *A. dimerus* is well-adapted to its host. The complete immobilization of the paralyzed *D. pallivitta* larva, timed with the hatching and migration of the wasp larvae to the host's belly, suggests a highly synchronized process. The maturing of the wasp larvae under the smooth belly of the host provides added protection. The specialized development of *A. dimerus* appears suited to the slug-like morphology of *D. pallivitta* larvae and would preclude its growth on any other type of caterpillars. Hawaii does not have any other species in the family Limacodidae nor in its superfamily Zygaenoidea. We conclude that the parasitoid *A. dimerus* is not a threat to non-target Lepidoptera species in Hawaii.

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Acknowledgements

We thank our HDOA colleagues Mohsen Ramadan for his technical advice and Clyde Hirayama and Patrick Conant for shipments of *D. pallivitta* larvae for parasitoid rearing. We also thank University of Hawaii students Leila Valdivia-Buitriago for providing endemic *Udea* caterpillars and Chris Kishimoto for his overall help on the project. We are grateful for the funding providing by UH researcher Arnold Hara and project assistance from Research Associate Ruth Niino-Duponte.

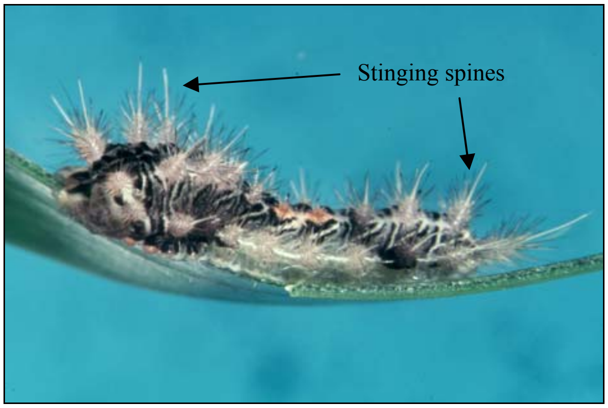

Fig.1. The nettle caterpillar, *Darna pallivitta*. Lateral view shows the venomous spines.

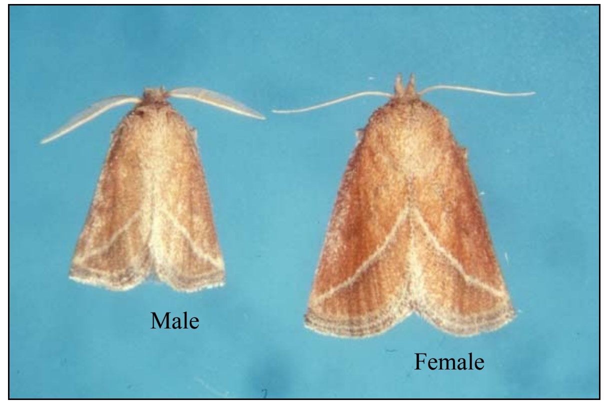

Fig.2. *Darna pallivitta* moths.

Fig. 3. Only midribs remain on a coconut leaf stripped by *D. pallivitta* larvae.

Fig. 4. Leaves of the Hawaiian ti plant are a favorite food of *D. pallivitta* larvae.

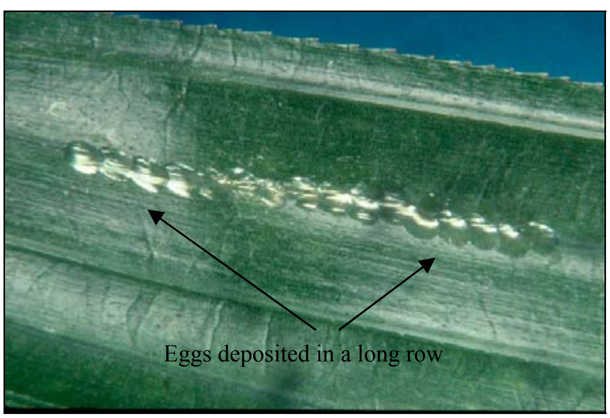

Fig. 5. *D. pallivitta* eggs appear as a silvery sheen on a leaf and are easily overlooked.

Fig. 6. *D. pallivitta* moth emerges from the cocoon (right) by popping open a cap.

Fig. 7. An *Aroplectrus* female "stings" a *Darna* larva on its fleshy underside "belly" to immobilize it before laying eggs on its host (ventral view, seen through glass jar).

Fig. 8. A *Darna* larva will sometimes flail and regurgitate a brownish liquid while being attacked by an *Aroplectrus* wasp.

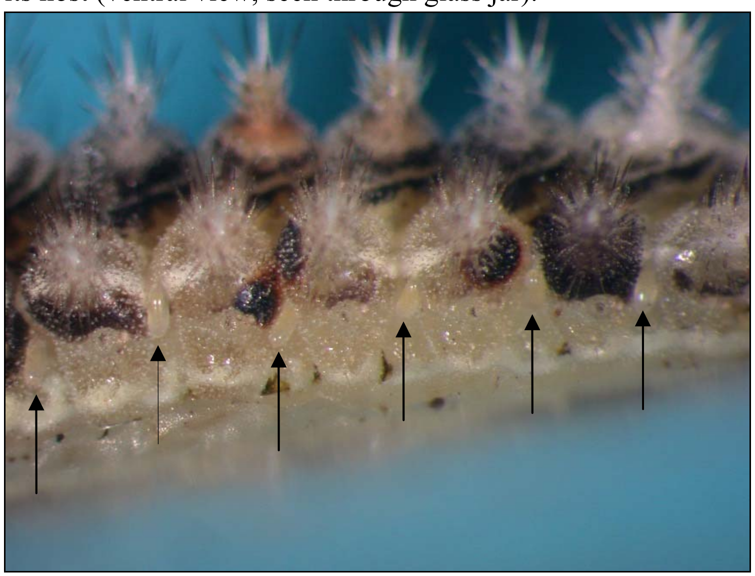

Fig. 9. *Aroplectrus* eggs are typically deposited on the sides of the *Darna* larva, in between the body segments.

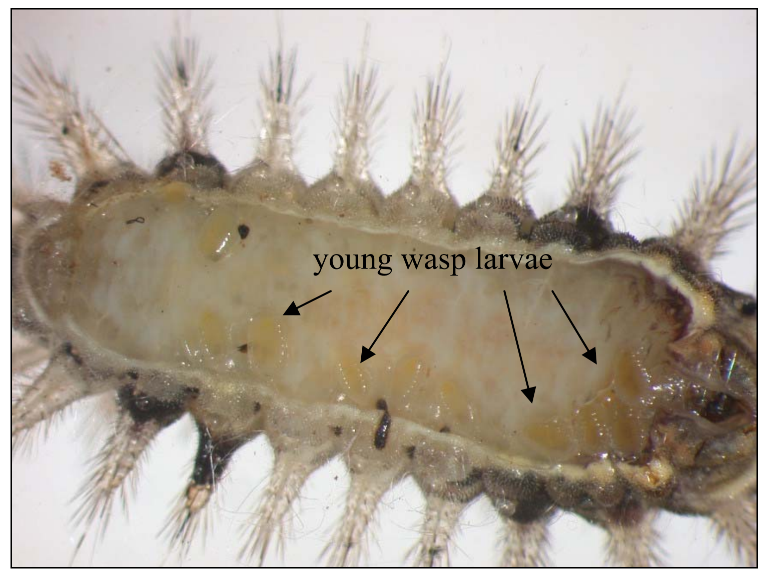

Fig. 10. Newly hatched *Aroplectrus* larvae migrate to the "belly" and begin feeding externally (ventral view, seen through glass jar).

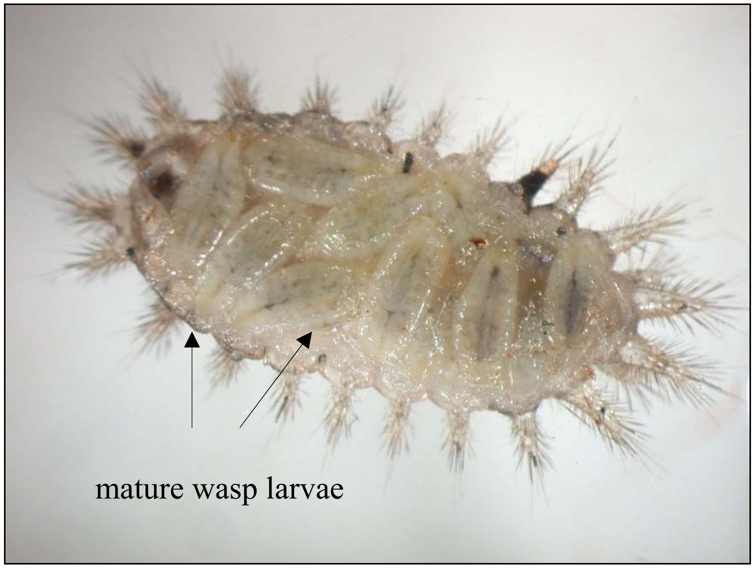

Fig. 11. Mature *Aroplectrus* larvae occupy the entire belly of the *Darna* larva (ventral view, seen through glass jar).

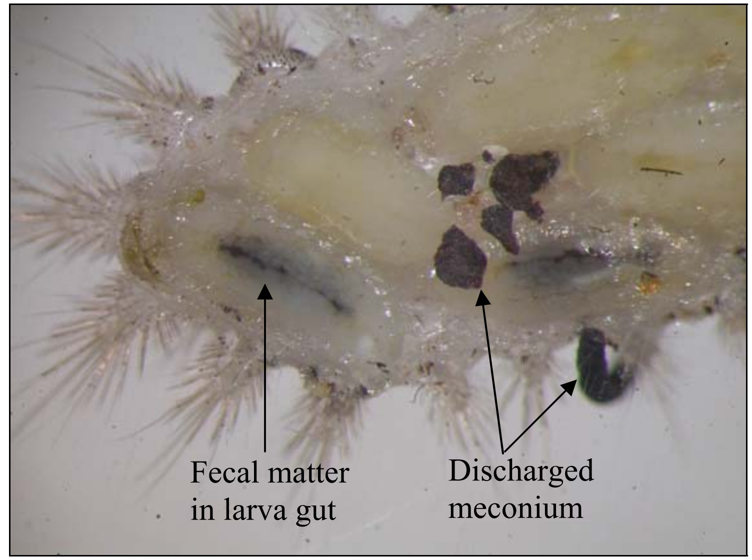

Fig. 12. Brownish meconium is discharged by *Aroplectrus* larvae prior to pupating (ventral view, seen through glass jar).

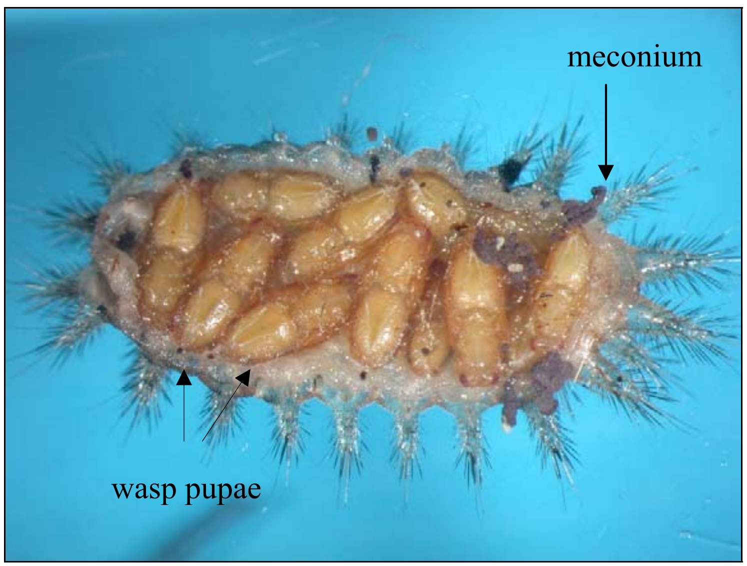

Fig. 13. *Aroplectrus* pupae are amber-colored prior to wasp emergence (ventral view, seen through glass jar).

Fig. 14. A parasitized larva is shriveled and normally stuck to a leaf with parasitoid meconium extruding from under the body.

Table 1. Non-target lepidopterous species used in host specificity tests for the parasitoid *Aroplectrus dimerus*.

Family, species name	Status	Source and host plant
Arctiidae		
***Nyctemera apicalis* Walker**[1] a leaf-feeder	Beneficial	Lab-reared, fireweed leaves, *Senecio madagascariensis*
***Secusio extensa* (Butler)**[1] a leaf-feeder	Beneficial	Lab-reared, fireweed leaves, *Senecio madagascariensis*
Choreutidae		
***Choreutis* sp.** a leaf-tier	Pest	Field-collected, weeping fig leaves, *Ficus benjamina*
Crambidae		
***Diaphania nitidalis* Cramer** pickleworm	Pest	Lab-reared, cucumber flowers/fruit, *Cucumis sativa*
***Omiodes blackburni* (Butler)** coconut leaf roller	Endemic	Lab-reared, coconut leaves, *Cocos nucifera*
***Udea stellata* (Butler)** a leaf-feeder	Endemic	Lab-reared, mamaki leaves, *Pipturis albidus*
Ethmiidae		
***Ethmia nigroapicella* (Sallmuller)**, kou leafworm	Pest	Field-collected, kou leaves, *Cordia subcordata*
Geometridae		
***Anacamptodes fragilaria* (Grossbeck)**, koa haole looper	Pest	Field-collected, koa-haole leaves, *Leucaena leucocephala*
***Macaria abydata* Guenee** koa haole moth	Pest	Field-collected, koa-haole leaves, *Leucaena leucocephala*
Lycaenidae		
***Lampides boeticus* (Linnaeus)** bean butterfly	Pest	Field-collected, rattlepod beans, *Crotalaria* sp.
Noctuidae		
***Achaea janata* (Linnaeus)** croton caterpillar	Pest	Field-collected, castor bean leaves, *Ricinus communis*
***Agrotis* sp.** a cutworm	Pest	Lab-reared, cotton leaves, *Gossypium hirsutum*
***Anomis flava* (Fabricius)** hibiscus caterpillar	Pest	Lab-reared, cotton leaves, *Gossypium hirsutum*
***Heliothis virescens* (Fabricius)** tobacco budworm	Pest	Field-collected, love-in-a-mist flowers, *Passiflora foetida*

Family, species name	**Status**	**Source and host plant**
Noctuidae, continued		
***Pandesma anysa* Guenee** a leaf-feeder	Pest	Field-collected, opiuma leaves, *Pithecellobium dulce*
***Spodoptera mauritia* (Boisduval)**, lawn armyworm	Pest	Lab-reared, undetermined grass species
Nymphalidae		
***Agraulis vanillae* (Linnaeus)** passion vine butterfly	Pest	Field-collected, passion vine leaves, *Passiflora edulis*
***Vanessa cardui* (Linnaeus)** painted lady	Pest	Field-collected, cheeseweed leaves, *Malva parviflora*
Pieridae		
***Pieris rapae* (Linnaeus)** imported cabbageworm	Pest	Field-collected, broccoli leaves, *Brassica oleracea*
Plutellidae		
***Plutella xylostella* (Linnaeus)**, diamondback moth	Pest	Field-collected, broccoli leaves, *Brassica oleracea*
Pyralidae		
***Hellula undalis* (Fabricius)** imported cabbage webworm	Pest	Field-collected, mustard cabbage leaves, *Brassica juncea*
Sphingidae		
***Daphnis nerii* (Linnaeus)** oleander hawk moth	Pest	Field-collected, oleander leaves, *Nerium oleander*
Tortricidae		
***Croesia zimmermani* Clarke** a biocontrol agent	Beneficial	Field-collected, blackberry leaves, *Rubus argutus*
***Cryptophlebia ombrodelta* (Lower)**, litchi fruit moth	Pest	Field-collected, undetermined legume species
***Episimus utilis* Zimmerman** a biocontrol agent	Beneficial	Field-collected, x-mas berry leaves, *Schinus terebinthifolius*

[1] Potential biological control agents being studied in HDOA Insect Quarantine Facility.

Table 2. Results of no-choice host specificity tests for the parasitoid *Aroplectrus dimerus* using 25 non-target Lepidoptera species and *Darna pallivitta* as the control. Two replicates of 10 Lepidoptera larvae each were conducted for each test species (N=20).

Family and species name	**No. parasitoid eggs deposited on larvae (mean ± SEM)**	**No. larvae parasitized**	**No. parasitoids emerging**	**No. moths of test sp. emerging**
Arctiidae				
Nyctemera apicalis	0	0	0	20 (100%)
D. pallivitta (control)	3.0 ± 0.9	10 (50%)	49 (42♀, 7♂)	-
ANOVA	F=11.9 DF=1,39 P=0.0014			
Secusio extensa	0	0	0	15 (75%)
D. pallivitta (control)	5.1 ± 1.1	13 (65%)	94 (61♀, 33♂)	-
ANOVA	F=19.7 DF=1,39 P=0.0001			
Choreutidae				
***Choreutis* sp.**	0	0	0	15 (75%)
D. pallivitta (control)	4.1 ± 0.9	12 (60%)	73 (52♀, 21♂)	-
ANOVA	F=21.0 DF=1,39 P=0.0000			
Crambidae				
Diaphania nitidalis	0	0	0	20 (100%)
D. pallivitta (control)	3.4 ± 0.7	14 (70%)	67 (52♀, 23♂)	-
ANOVA	F=21.0 DF=1,39 P = 0.0000			
Omiodes blackburni	0	0	0	14 (70%)
D. pallivitta (control)	4.5 ± 1.2	11 (55%)	87 (62♀, 25♂)	-
ANOVA	F=14.4 DF=1,39 P=0.0005			
Udea stellata	0	0	0	20 (100%)
D. pallivitta (control)	5.1 ± 1.1	12 (60%)	38 (26♀, 12♂)	-
ANOVA	F=19.5 DF=1,39 P=0.0001			

Family and species name	No. parasitoid eggs deposited on larvae (mean ± SEM)	No. larvae parasitized	No. parasitoids emerging	No. moths of test sp. emerging
Ethmiidae				
Ethmia nigroapicella	0	0	0	15 (75%)
D. pallivitta (control)	3.5 ± 0.8	13 (65%)	16 (10♀, 6♂)	-
ANOVA	F=18.5 DF=1,39 P=0.0001			
Geometridae				
Anacamptodes fragilaria	0	0	0	0 (0%)[1]
D. pallivitta (control)	4.9 ± 0.9	17 (85%)	36 (18♀, 18♂)	-
ANOVA	F=30.3 DF=1,39 P=0.0000			
Macaria abydata	0	0	0	4 (20%)
D. pallivitta (control)	3.6 ± 0.9	12 (60%)	27 (20♀, 7♂)	-
ANOVA	F=17.7 DF=1,39 P=0.0002			
Lycaenidae				
Lampides boeticus	0	0	0	20 (100%)
D. pallivitta (control)	5.5 ± 1.0	16 (80%)	45 (28♀, 17♂)	-
ANOVA	F=32.4 DF=1,39 P=0.0000			
Noctuidae				
Achaea janata	0	0	0	19 (95%)
D. pallivitta (control)	4.7 ± 1.0	14 (70%)	88 (54♀, 34♂)	-
ANOVA	F=21.9 DF=1,39 P = 0.0000			
***Agrotis* sp.**	0	0	0	1 (5%) [1]
D. pallivitta (control)	3.7 ± 1.2	11 (55%)	49 (22♀, 29♂)	-
ANOVA	F=8.66 DF=1,39 P=0.0055			

Family and species name	**No. parasitoid eggs deposited on larvae (mean ± SEM)**	**No. larvae parasitized**	**No. parasitoids emerging**	**No. moths of test sp. emerging**
Anomis flava	0	0	0	20 (100%)
D. pallivitta (control)	4.8 ± 0.9	13 (65%)	91 (61♀, 30♂)	-
ANOVA	F=30.1 DF=1,39 P=0.0000			
Heliothis virescens	0	0	0	13 (65%)
D. pallivitta (control)	5.1 ± 1.4	9 (45%)	60 (38♀, 22♂)	-
ANOVA	F=12.6 DF=1,39 P=0.0010			
Pandesma anysa	0	0	0	18 (90%)
D. pallivitta (control)	5.3 ± 1.1	14 (70%)	101 (70♀, 31♂)	-
ANOVA	F=24.6 DF=1,39 P=0.0000			
Spodoptera mauritia	0	0	0	19 (95%)
D. pallivitta (control)	4.2 ± 0.9	14 (70%)	80 (67♀, 13♂)	-
ANOVA	F=23.7 DF=1,39 P=0.0000			
Nymphalidae				
Agraulis vanillae	0	0	0	1 (5%) [1]
D. pallivitta (control)	4.5 ± 0.9	14 (70%)	76 (46♀, 30♂)	-
ANOVA	F=26.0 DF=1,39 P=0.0000			
Vanessa cardui	0	0	0	19 (95%)
D. pallivitta (control)	4.4 ± 1.0	12 (60%)	68 (45♀, 23♂)	-
ANOVA	F=19.0 DF=1,39 P=0.0001			
Pieridae				
Pieris rapae	0	0	0	19 (95%)
D. pallivitta (control)	4.4 ± 1.0	13 (65%)	68 (39♀, 29♂)	-
ANOVA	F=17.7 DF=1,39 P=0.0002			

Family and species name	**No. parasitoid eggs deposited on larvae (mean ± SEM)**	**No. larvae parasitized**	**No. parasitoids emerging**	**No. moths of test sp. emerging**
Plutellidae				
Plutella xylostella	0	0	0	20 (100%)
D. pallivitta (control)	3.5 ± 1.3	8 (40%)	44 (28♀, 16♂)	-
ANOVA	F=7.67 DF=1,39 P=0.0086			
Pyralidae				
Hellula undalis	0	0	0	10 (50%)
D. pallivitta (control)	4.8 ± 1.0	12 (60%)	56 (33♀, 23♂)	-
ANOVA	F=21.6 DF=1,39 P=0.0000			
Sphingidae				
Daphnis nerii	0	0	0	18 (90%)
D. pallivitta (control)	4.6 ± 0.9	15 (75%)	67 (6♀, 61♂)	-
ANOVA	F=28.2 DF=1,39 P=0.0000			
Tortricidae				
Croesia zimmermani	0	0	0	19 (95%)
D. pallivitta (control)	2.6 ± 0.8	8 (40%)	13 (9♀, 4♂)	-
ANOVA	F=9.63 DF=1,39 P=0.0036			
Cryptophlebia ombrodelta	0	0	0	20 (100%)
D. pallivitta (control)	3.0 ± 0.9	11 (55%)	33 (24♀, 9♂)	-
ANOVA	F=10.6 DF=1,39 P=0.0024			
Episimus utilis	0	0	0	20 (100%)
D. pallivitta (control)	3.9 ± 0.5	17 (85%)	42 (23♀, 19♂)	-
ANOVA	F=51.0 DF=1,39 P=0.0000			

[1] Disease or undetermined cause prevented larvae from completing development.