THE STINGING NETTLE CATERPILLAR, *DARNA PALLIVITTA* (MOORE) (LEPIDOPTERA: LIMACODIDAE) IN HAWAII

ITS CURRENT HOST RANGE, BIOLOGY AND POPULATION DYNAMICS

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

ENTOMOLOGY

AUGUST 2006

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ACKNOWLEDGMENTS

To my major advisor, Dr. Arnold Hara and to my committee members Drs. Mark Wright, and Daniel Rubinoff, and Walter Nagamine, thank you very much for all of your help and guidance. Thank you to Ruth Niino-DuPonte, and Christopher Jacobsen and especially to Stacey Chun of the Beaumont Research Center. Thanks to all the Entomology graduate students for all of the assistance during this study. I would like to Patrick Conant and Clyde Hirayama of the Hawaii State Department of Agriculture – Hilo Branch for their assistance and advice. I am also grateful to the Hawaii State Department of Agriculture for allowing me to work inside of their quarantine facility, the Hawaii State Department of Health, H. Eunice Nursery, and USDA T-STAR. I would especially like to thank my parents, family, and friends for all of their help, support, and encouragement.

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CHAPTER 1

Literature Review

1.1. Lepidoptera: Limacodidae

The family Limacodidae is most diverse in tropical areas but can be found in other climates and locations. Limacodids can be found on every continent except Antarctica (Ivinskis, 1992) and have been documented in North America (Epstein, 1997), Central and South America (Stephens, 1975 and Mariau, 1976), Africa (Igbinosa, 1985 and Heppner, 1991), Australia (Reader and Hochuli, 2003), Asia (Wood, et al.1976). So far approximately 1000 species have been described (Holloway, 1986 and Epstein, 1996) though doubtless the total is higher since most attention is given to the economically important members of this family.

Limacodid larvae are characterized by bright colors and ornate designs, as well as being external-feeding with retractile heads (Epstein, 1996). The larvae have reduced or no abdominal prolegs (Cock, et al. 1987 and Epstein, 1996) which result in peristaltic movement, hence the nickname "slug caterpillars". According to Cock, et al. (1987), limacodids can be separated into two groups – the gelatine caterpillars which have no stinging spines and the nettle caterpillars which possess stinging spines mounted on scoli. Limacodids that have no spines are thought to have been derived from the nettle caterpillars (Cock, et al. 1987). The venom that causes skin irritation is primarily from a mixture of histamines (Balit, et al. 2003 and Kawamoto, 1978).

In general, limacodids are polyphagous (Cock, et. al, 1987 and Epstein, 1996). Holloway (1986) lists limacodids in Borneo feeding on plants in the following families: Palmae, Rubiacceae, Leguminosae, Euphorbiaceae, Anacardiaceae, Musaceae, Rutaceae,

Musaceae, Myrtaceae, Theaceae, Sterculiaceae, Sapindaceae, Combretaceae, Barringtoniaceae, Annonaceae, Bombacaceae, Lauraceae, Lythraceae, Dipterocaceae, Compositae, Ebenaceae, Ehretiaceae, Flacourtiaceae, Gramineaeceae, Punicaceae, Rhizophoraceae, Rosaceae, Salicaeceae, Solanaceae,and Stilaginaceae.
Braza (1988) reported a limacodid attacking *Acacia mangium* (Fabaceae) seedlings in the Phiippines and Dyar (1895) reported feeding on oak (Fagaceae) and hickory (Juglandaceae) in North America.

The polyphagous nature of limacodids makes some species pests of many economically important crops (Mariau, 1982), especially members of the palm family (Arecaceae). *Parasa lepida* is recorded as a pest of coconuts in Indonesia (de Chenon, 1982). *Parasa viridissima* has been a pest of palms in Africa (Mariau, 1976a and Igbinosa, 1985). *Setothosea asigna* is a pest of oil palm in Southeast Asia (Sasaerila, et al. 1997). Syed and Shah (1976) list *Setora nitens*, *Thosea bisura*, *Darna trima*, *T. vetusta*, *Ploneta diducta*, *Cania robusta*, *Berthamula chara*, *Cheromettia sumatrensis* and *Trichogyia* sp. as pests of oil palms in Sabah, Malaysia. *Sibine apicalis* was described as a pest of bananas and *S. horrida* as a pest of plantains in Central America (Stephens, 1975).

The majority of literature on limacodid control comes from Asia. Control measures for many limacodid pests have been chemical or biological with an emphasis being placed on the use of biological control as it is cheaper and considered to be environmentally safer. Biological control agents include parasitoids, predators, and pathogens. Cock, et al (1987) document a number of wasps, tachinids and bombyliids (Dipterans) that parasitize the eggs, larvae, and pupae of limacodids. Epstein (1997) and

Adams and Yanega (1991) also recorded bombyliids parasitizing limacodids in New Jersey, USA and Mexico. It is interesting to note that records of limacodid eggs being parasitized by *Trichogramma* species are almost nonexistent. This is unusual considering the polyphagous nature of this genus of parasitoid and its wide use and distribution as one of the most important biological control agents in the world (Knutson, 1998). Only Wei (1985) and and Hoong and Hoh (1992) describe *Trichogramma* species parasitizing limacodid eggs although the exact species of *Trichogramma* is unknown.

Some hemipterans (Pentatomidae, Pyrrhocoridae, and Reduviidae) are known predators of limacodids and may exert a significant amount of control on low density populations (Cock, et al. 1987). Although not listed as a primary predator, de Chenon (1982) made an unusual observation of a Pyralid (*Phycita dentilinella*) feeding on the pupae of *Parasa lepida*. Cock, et al. (1987) further list another Pyralid, *Ectomyelois ceratoniae*, as a possible predator of the cocoons of *Parasa philepida* in the Philippines. Birds and monkeys have occasionally been observed feeding on larvae as well (Cock, et al. 1987).

Pathogens used for control of limacodids include several viruses (Syed and Shah, 1977; Tiong, 1982; de Chenon, et al. 1988) and fungi (Mackenzie, 1977; Cock, et al. 1987; Lay, 1996; Siburat and Mojiun, 1998).

Chemical control has been achieved with the use of organophosphates (monocrotophos and metamidophos) and synthetic pyrethroids (alpha cypermethrin and Lamba-cyhalothrin) (Siburat and Mojiun, 1998), spinosyns (Spinosad "Conserve") and carbamates (carbaryl "Sevin") (Hara, et al. 2003), although in the past DDT was also used (Wood, et al. 1976). A surprising find is that applications of *Bacillus thuringiensis* did not provide satisfactory control of limacodid populations (Mariau, 1976b and Basri, et al. 1994) unless used at much higher levels than recommended by the manufactures (Basri, et al. 1994). The reasons for this lower susceptibility to Bt are unclear at this time.

All limacodid eggs are dorsoventrally flattened (Cock, et al. 1987 and Epstein, 1996). Eggs may be laid singly, in groups where the eggs may overlap each other (Epstein, 1996), or in rows (Cock, et al. 1987).

Larvae of limacodids utilize two different feeding habits. Early instar larvae (up to fourth instar) feed on the parenchyma on only one side of the leaf (Dyar, 1895). Cock, et al. (1987) describe this type of feeding as "window feeding" since the cuticle on the other side of the leaf is not eaten through. As the larvae mature, they are able to eat through the entire leaf. Larvae of limacodids may have up to 11 instars before moulting (Chayopas 1982). Many limacodid larvae are gregarious for at least part of their lives. De Chenon (1982) reports that *Latoia* (*Parasa*) *lepida* (Cramer) was gregarious throughout its larval life, while Reader and Hochuli (2003) observed that the larvae of *Doratifera casta* are gregarious after emergence from the egg but became solitary as the larvae matured.

Cocoons of most limacodids are usually oval-shaped and brown in color (Epstein, 1996) but some species may construct white or patterned cocoons with vertical stripes visible on the cocoon (Cock, et. al. 1987 and Ishii, 1984) (Fig. 1.1). Limacodids are known to construct cocoons that have a hardened outer shell, usually with a smooth texture which may be from an unidentified fluid excreted by the larvae (Common, 1990). Ishii et al. (1984) found that the hardness of the cocoon of *Monema flavescens* was the result of a silk protein produced by the silk glands, being combined with a sclerotized protein produced by the salivary glands, compacted into the netting of fine silk the prepupa had previously spun around itself. Some species may have their cocoons surrounded by a webbing of fine silk and others may have stinging spines or tubercles incorporated into the cocoon to help prevent attack from natural enemies. The anterior end of the cocoon has a circular area that is made weaker during construction, the point where the moth will emerge from the cocoon. This weak point is not visible on the outside of the cocoon (Dyar, 1895 and Epstein, 1996). This construction and emergence behavior by the moth has given rise to the nickname "cup moth" in some areas, since the empty cocoon looks like a cup, in many cases with the circular lid still attached in the open position. Pupation may take place in a variety of locations (on the host plant or on the ground) depending on species. Dyar (1896) found *Sisyrosea nasoni* pupating on the ground. De Chenon (1982) found *Latoia lepida* pupating at the leaf bases and occasionally at the collar of young coconuts in the crowns of *Cocos nucifera.* Cock, et al. (1987) also list tree trunks and leaves as pupation sites.

Cock, et al. (1987) gives the most detailed (and general) taxonomic descriptions of many species (both larval and adult) but explain that wing venation and genetalia of adults are of primary importance in identification. Adult limacodids were thought to be incapable of feeding due to the degenerate nature of their mouthparts (Cock, et al. 1987) but Epstein (1996) was able to observe species feeding on sugar water in the lab. The introduction of sugar water for lab reared moths may increase longevity. All species are apparently nocturnal. Adults also assume an uncharacteristic stance while in a resting position in which the front part of the body is pushed up in an angle away from its resting substrate (Fig. 1.2).

1.2. Focus on *Darna*

The genus *Darna* is native to Asia and has been recorded from Japan and China to as far south as Indonesia. Outbreaks of numerous species have been recorded on economically important crops (Cock, et al. 1987) and some species of *Darna* are listed as pests. *Darna catenatus* is listed as a serious pest of coconut (*Cocos nucifera*) during the dry season in Indonesia (Woodroof, 1970 and Davis and Sudasrip, 1982). *Darna bradleyi* is an occasional pest on oil palms (Ang, et al. 1997) and *Darna metaleuca* is considered the most important pest on oil palm in South America (Mariau, 1976b). Cock, et al. 1987 further list *Darna diducta* as a sporadic pest in coconut near Jakarta, *Darna pallivitta* as a minor pest (probably on coconut, oil palm and *Areca* species), *Darna sordida* as a pest on oil palm and coconut in South Sumatra, *Darna furva* as a key pest on oil palm in south Thailand, and *Darna mindanenesis* as an occasional pest of coconut and cocoa in the Philippines.

*Darna trim*a is the most important pest in the genus, being a serious pest of oil palms in Malaysia (Hoong and Hoh, 1992; Lay, 1996 and Siburat and Mojiun, 1998). *D. trima* is the most common limacodid to have outbreaks in Borneo (Cock, et al. 1987). Kimura (1979) also lists *D. trima* as a pest of sago palm. *Darna trima* outbreaks have also occurred on cocoa in Java and West Malaysia where the damaged trees suffer dieback of their lateral branches and excessive development of lateral buds (Entwistle, 1972). Severe infestations may have up to 2000 larvae per frond (Tiong and Munroe

1977) and may be related to dry conditions or droughts or insecticide applications which decrease populations of natural enemies allowing *Darna* and other Lepidopteran pest populations to dramatically increase (Mackenzie, 1977, Syed and Shah, 1976, and Cock, et al. 1987).

Sex pheromones (decadeinoates) for *D. trima* and *D. bradleyi* (Sasaerila, et al. 2000a) have already been discovered and synthesized. The synthetic sex pheromones were more attractive to adult males than unmated adult females producing the natural pheromone. The synthetic compounds were developed for monitoring populations of *D. trima* and *D. bradleyi* in oil palm plantations throughout Asia. The use of synthetic pheromones in traps resulted in the elucidation of activity periods when *D. trima* and *D. bradleyi* search for mates and the height at which the adults are most active (Sasaerila, et al. 2000b). *D. trima* and *D. bradleyi* males were active from 5:30 – 6:45 p.m. and flew at heights that were less than 5 meters tall.

Control of pest populations of *Darna* species has been achieved by using chemical, biological, and cultural control methods. Wood, et al. (1976), working with *D. trima* and other limacodid species experimented with contact and systemic chemical insecticides applied in foliar sprays, and systemic insecticide applications which included trunk injections and as granules at the base of test trees. Sprays of organochlorines (endosulfan) and carbamates (aminocarb and methomyl) were all successful and systemic application of the organophosphate monocrotophos was also effective. However, organochlorines (DDT and endosulfan) in particular, were discouraged from being used because of their low specificity and long residual effects. The use of organochlorines were also blamed for causing outbreaks of caterpillars since their use also killed off the natural enemies of these insects. An insect growth regulator (IGR) called triflumuron (Alsystin) was also effective (Ng, et al. 1986).

Several Hymenopteran natural enemies for the genus *Darna* have been found including ichneumonids, braconids, eulophids, chalcids, and even chrysidids (Cock, et al. 1987). In addition, tachinids and bombyliids (Diptera) have also been found to parasitize the larvae of certain *Darna* species (Cock, et. al. 1987). *Trichogramma* species have not been recorded parasitizing the eggs of *Darna* despite being highly polyphagous in nature (Knutson, 1998). Predators of *Darna* species include a clerid, a pentatomid, and a reduviid attacking *D. trima* (Cock, et al. 1987). Several viral pathogens have also been discovered infecting *Darna* species including a granulosis virus infecting *D. trima* in Sarawak, Malaysia (Tiong, 1982) and a small non-occluded virus infecting *D. catenatus* in Sulawesi, Indonesia (De Chenon, et al. 1988). Cock, et al. (1987) also found a densovirus infecting *D. sordida* and *D. trima* and a fungal pathogen, *Cordyceps*, infecting *D. mindanensis* in Mindanao, Philippines. Microbial control of *Darna* species is achieved by using the above mentioned viral pathogens collected from infected larvae, mixed with water and the solution sprayed onto outbreaks of larvae, with excellent results (Tiong and Munroe, 1977). Microbial control may also be more economical than chemical controls while offering highly successful control. Tiong and Munroe (1977) found that application of a granulosis virus suspension made from crushed diseased *D. trima* and mixed with water, successfully controlled an outbreak of *D. trima* at 40% of the cost of using a chemical control.

Cultural control methods were advocated for their safety to the environment and minimal costs to the growers. Controlled weeding and encouraged growth of plants (particularly in the genus *Euphorbia*) that provided nectar for parasitoids was suggested by several researchers to control a variety of caterpillar pests including *Darna* species (Syed and Shah, 1977; Mackenzie, 1977; and Lay, 1996).

There are reports of *Darna* being introduced to three other areas with subsequent establishment (Genty, 1976, Yoshimoto, 1997, and Conant, et al. 2002). In two cases the introduced species has become an invasive pest. Genty (1976) described *Darna metaleuca* (originally from Southeast Asia) as one of the worst pests defoliating oil palms in South America. Although *D. pallivitta* has been discovered in Okinawa (Yoshimoto, 1997), its status as a pest is yet unknown. However, *D. pallivitta* has recently become an invasive pest in the State of Hawaii (Conant, et al. 2002).

1.3. *Darna pallivitta* (Moore), The Nettle Caterpillar, In Hawaii

Darna pallivitta was first described by Moore in 1877 and is a native of China, Taiwan, Thailand, Malaysia, Java, and Borneo (Cock, et al. 1987). *Darna pallivitta* was first found in Japan on the island of Okinawa in 1996 (Yoshimoto, 1997). Cock, et al. (1987) list palms (*Areca* sp., coconut, and oil palm), *Adenostemma* sp., *Breynia* sp. *Ficus* sp., grasses and *Zea mays* as food plants and Tominaga (1999) recorded larvae feeding on the grasses *Panicum repens* and *Paspalum urvillei* in Okinawa. *Darna pallivitta* is listed as a minor pest by Cock, et al. (1987). Yoshimoto (1997) predicted that *D. pallivitta's* status as a harmful insect in Japan would remain low due to a slow range expansion.

Darna pallivitta was first discovered in Hawaii in September 2001 (Conant, et al. 2002). The first observations were at a nursery in the Panaewa area of Hilo, Hawaii. It is likely that *D. pallivitta* likely arrived as pupae on a shipment of rhapis palms that originated in Taiwan (L. Nakahara, personal communication). *Darna pallivitta* escaped eradication efforts and has established itself on the Big Island with occasional outbreaks occurring (Fig. 1.3). Nagamine (unpublished data) has found that each developmental stage of *D. pallivitta* in Hawaii is longer in duration than that listed in Cock, et al. (1987). It was also found that some larvae in Hawaii take on a pink to reddish hue prior to reaching maturity (C. Kishimoto, personal observation) (Fig. 1.4). This coloration has not been noted elsewhere in *D. pallivitta* though Cock, et al. (1987) state that this trait does occur in other Limacodid species.

In Hawaii, larvae of *D. pallivitta* have been observed feeding on at least 45 different plant species in 22 families (Conant, et al. 2005 and California Plant Pest and Disease Report, 2005), thus becoming a quarantine pest to the export foliage and cut flower industries in Hawaii which are worth approximately US $35 million annually (Hawaii Agricultural Statistics Service, 2003). *Darna pallivitta* also poses a health concern to local residents as it possesses stinging spines which induce an immediate burning sensation as soon as it contacts the skin (Chun, et al. 2005)

Since its arrival to the state of Hawaii, *D. pallivitta*, as of May 2006 has not expanded its range from the island of Hawaii (Chun, et al. 2005) and the known expansion of its range has been approximately 16.1 km (10 miles) from the initial site of infestation. The Hawaii Department of Agriculture intercepted larvae and pupae on the island of Maui in 2004 but subsequent surveys have found no establishment (D. Oishi, personal communication). However as with other invasive insect species, it is probable that *D. palliivitta* will eventually spread to the other Hawaiian Islands (State of Hawaii Department of Agriculture, 2000). Five interceptions of *D. pallivitta* have also been made in California (California Plant Pest and Disease Report, 2005). Interceptions in California have occurred on fishtail palms (*Caryota mitis*) and on a lei and flowers of ilima (*Sida fallax*) (Epstein and Kinnee, 2003)

Recent studies by the United States Department of Agricutlture (USDA) Pacific Basin Research Center in Hawaii has isolated a pheromone (7,9-decadeinoates) from *D. pallivitta* females in an effort to discover a successful method for detection of *D. pallivitta* on the other islands in the State of Hawaii (M. Siderhurst, personal communication). Initial results have shown some promise in this research.

1.4. Thesis Structure:

An understanding of *Darna pallivitta*'s ecology in Hawaii is the central aim of this thesis. *D. pallivitta*'s food-plant range in Hawaii is investigated and descriptions on field biology are reported. The effect of abiotic factors on *D. pallivitta* populations will be determined and natural enemies of *D. pallivitta* that currently reside in Hawaii will be documented.

Host Range in Hawaii

The family Limacodidae is highly polyphagous in nature and the host range of *Darna pallivitta* has not been studied in Hawaii. Investigation of a wide range of plants is necessary to determine risks to economically important species, indigenous species, and other refugia species that are potential sources of infestation (e.g. weeds). Both commercially important and weed species of plants will be tested and field observations carried out to further determine food plants of *D. pallivitta*.

Field Biology of *D. pallivitta*

Currently, the basic biology of *D. pallivitta* is known from laboratory observations while little is known about its behavior under field conditions. Spatial determination of larval feeding sites and temporal periods of adult activity will be studied.

Weather Factors Affecting Population Dynamics

Understanding abiotic causes of population increases of a pest is important in formulating pest control strategies. Both juvenile and adult populations of *D. pallivitta* will be monitored to determine fluctuations and patterns in abundance. Results will be correlated with temperature, humidity, and rainfall data to determine their effects on field populations.

Existing Mortality Factors In Hawaii

D. pallivitta may come under attack from a variety of natural enemies that may act individually or in concert with each other to offer a successful level of control. Sentinel surveys and field collections will determine the presence of natural mortality factors of *D. pallivitta* that currently reside in Hawaii and their potential contribution to control will be determined.

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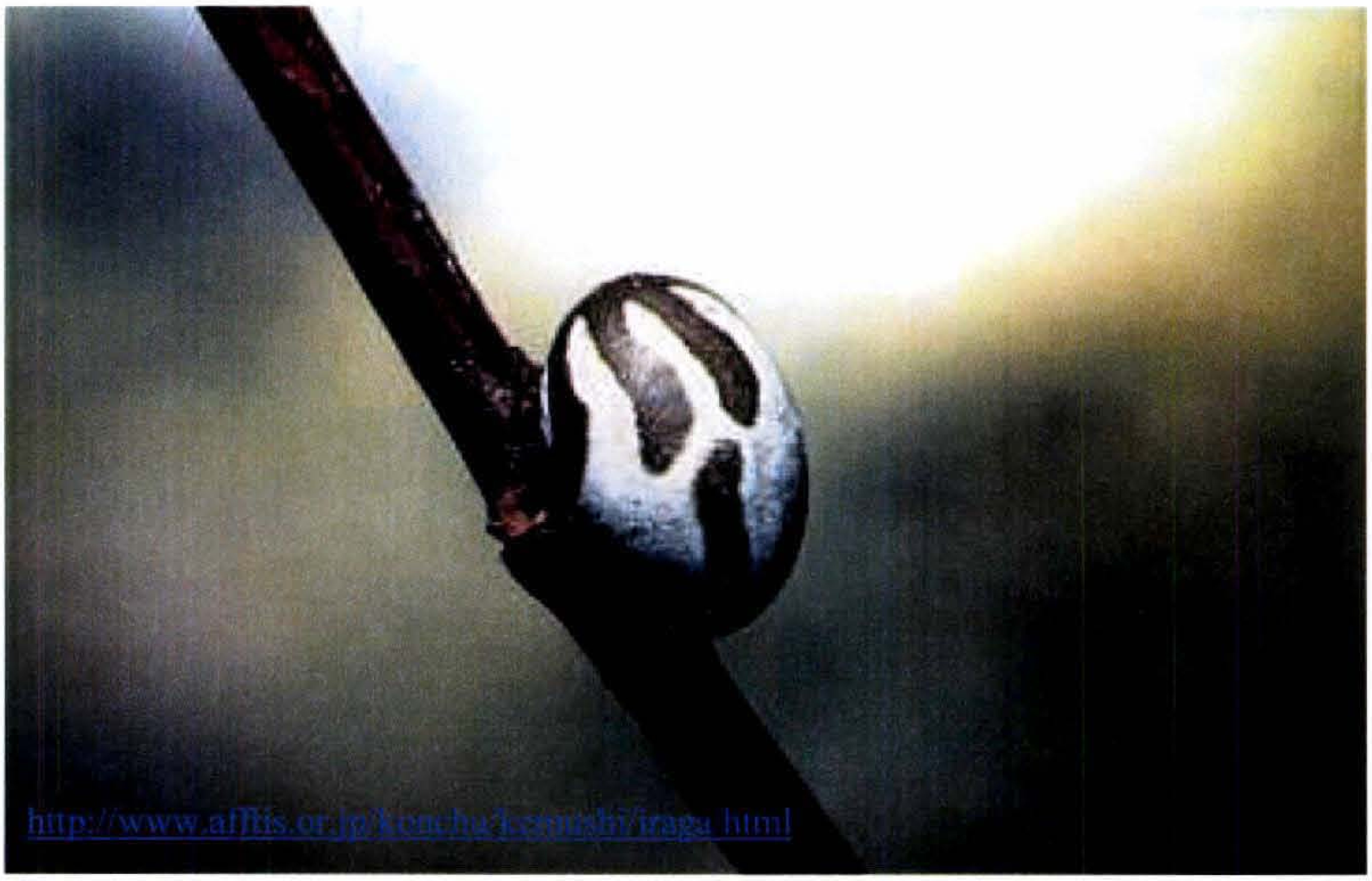

Fig. 1.1. Cocoon of *Monema flavecsens* showing striped coloration. Photo courtesy of http://www.afftis.or.jp/konchu/kemushi/iraga.html.

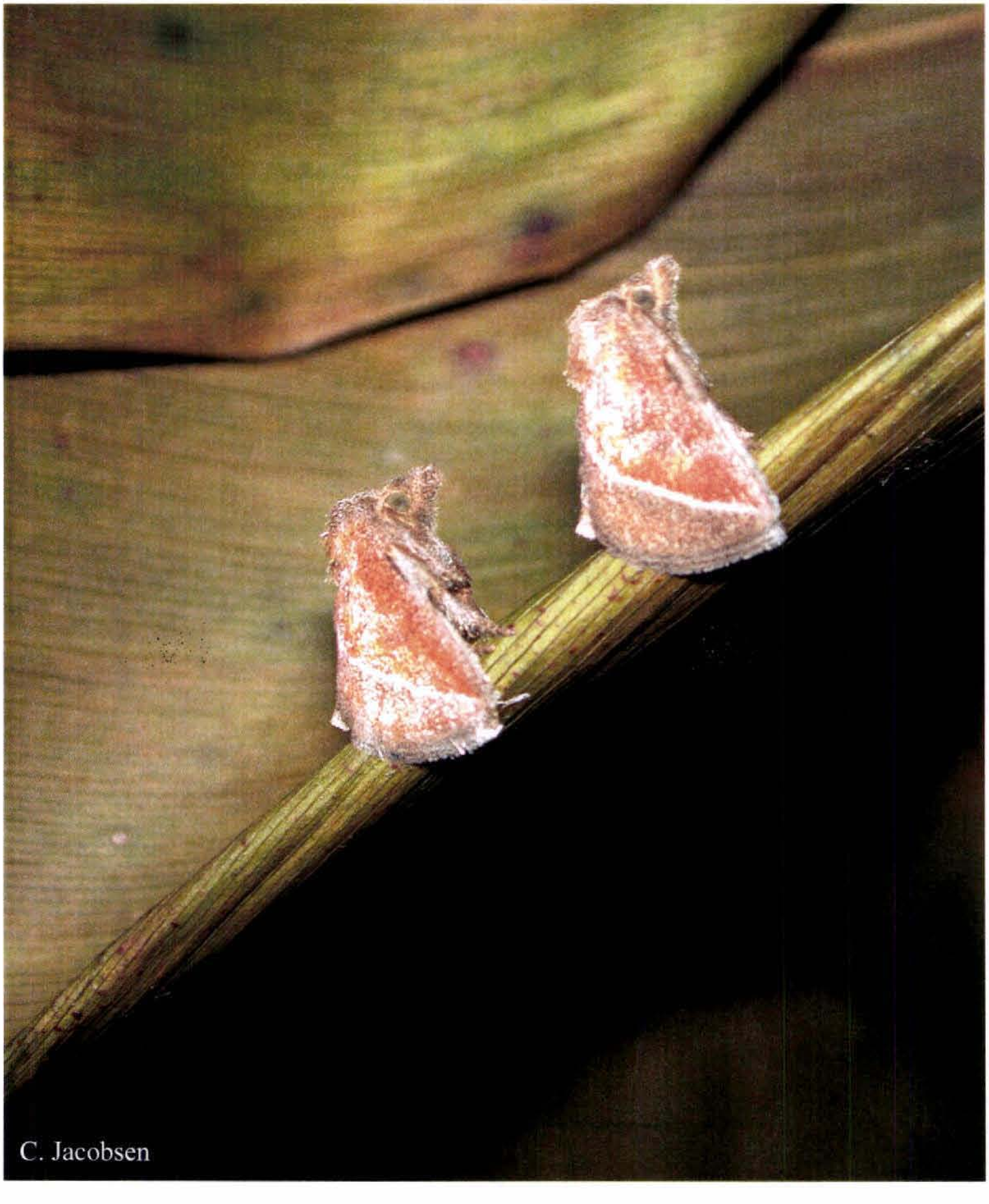

Fig. 1.2. Adult *D. pallivitta* at rest. Note the unusual posture – resting on wings and hind legs.

Fig.1.3. Severe *D. pallivitta* infestation on mondo grass (*Ophiopogon japonicus*).

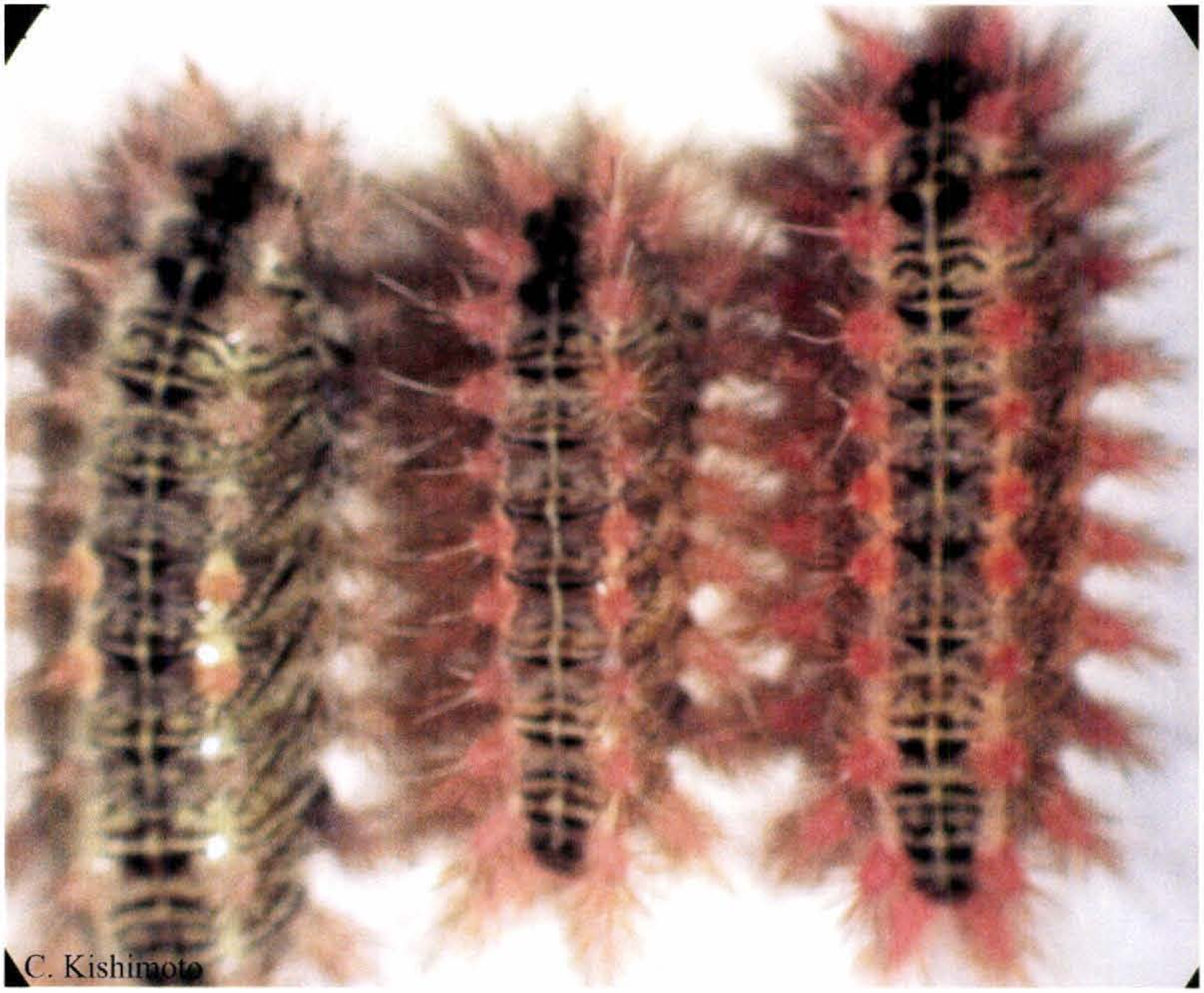

Fig. 1.4. *D. pallivitta* color variations. Larva at left is characteristically colored. Larvae at center and right have pink and red coloration respectively.

CHAPTER 2

Host Range of *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae) in Hawaii

2.1. Abstract

The nettle caterpillar, *Darna pallivitta* Moore, was first discovered on the island of Hawaii in September 2001. Thought to have arrived as hitchhiking pupae on a shipment of Rhapis palm seedlings from Taiwan, *D. pallivitta* evaded eradication efforts and has become an agricultural pest as well as a health concern due to its painful sting. Potential host plants were determined by their ability to support development of larvae into adults. In addition to this, a feeding list of plant species was compiled based on field observations of *D. pallivitta* feeding. Of the twenty-three plant species tested, eleven species encompassing eight families were found to support larval development to adulthood. Four species of native plants were found to be hosts and 63.6% of host plants were monocots. A generalized linear model showed that host plant species was a significant variable in larval development time. Observations of larvae in the field indicate that *D. pallivitta* feeds on 57 different species of plants representing 54 genera in 26 families. Monocots appear to be the preferred food plant with 61.8% of observed food plants being monocots. Palms and grasses have the highest number of species on the feeding list. *D. pallivitta* is highly polyphagous with many common landscape plants serving as hosts.

2.2. Introduction

The nettle caterpillar, *Darna pallivitta* (Moore), was discovered in the Panaewa area (119° 39 min. 13 sec. N / 155° 3 min. 32 sec. W) on the island of Hawaii in September

2001 and was thought to have arrived from Taiwan on a shipment of rhapis palm seedlings (Conant, et al. 2002). *Darna pallivitta* quickly became established and has become both an agricultural pest and a health concern. *Darna pallivitta* is also a pest of quarantine concern, causing shipments of palms and other floral products from Hawaii to be intercepted and rejected in California (Epstein and Kinee, 2003 and California Plant Pest and Disease Report, 2005) and intercepted and treated by the Hawaii State Department of Agriculture (HDOA) on Maui (D. Oishi, personal communication). Larvae of *D. pallivitta* inflict a painful sting when their urticating spines or setae release venom upon contact with the skin (Epstein, 1996).

Larvae in the family Limacodidae are polyphagous (Holloway, 1986) and have been recorded feeding on a variety of different plant species including coffee (Holloway, 1986), bananas (Stephens, 1975), and sugarcane (Pawar, et al. 1981). Holloway (1986) also listed *Psidium*, *Eugenia*, *Imperata*, *Theobroma*, *Camellia* and *Citrus* as food plants and Cock, et al. (1987) listed *Adenostemma* sp., *Areca* sp., *Breynia* sp., *Cocos nucifera*, *Ficus* sp., grasses, maize, and *Elaeis guineensis* as food plants.

Heavy infestations may result in total defoliation of leaves to the midrib (Igbinosa, 1985). Cock, et al. (1987) consider *D. pallivitta* as a minor pest, though to what crop(s) is unknown. Whether *D. pallivitta* would be limited to the above mentioned plant species in Hawaii is also unknown. It was important to determine the host range and potential impacts of *D. pallivitta* on Hawaii's plants of agricultural and cultural value.

I conducted no-choice host range tests to identify plant species in Hawaii that could support the larval development of *D. pallivitta* to adulthood. This overall host range is termed the physiological host range (Onstad, et al. 1996 and Schaffner, 2001). No-choice host range tests are a type of host range test that can be used to determine host plant range. Another type of host range test may include choice tests in which the insect is given a choice between a normal host plant (control) and a test plant species (Cullen, 1988). Although no-choice host range tests have been criticized for only revealing an organism's physiological host range and not it's ecological or realized host range, they can be important for eliminating many plant species from further consideration in more precise tests such as choice tests or ovipositional preference tests in which the adult female is allowed to oviposit on a variety of different plant species to determine preferences for potential host plants (Cullen, 1988).

Observations in the field were also employed to compile a list of plant species that *D. pallivitta* was found feeding upon. Determining host plant species and preferred food sources of *D. pallivitta* will enable plant nurseries, plant exporters, quarantine inspectors, landscapers and homeowners to detect and/or deter potential *D. pallivitta* infestations.

2.3. Materials and Methods

Insects

A colony of *D. pallivitta* was established at the Hawaii State Department of Agriculture's (HDOA) quarantine facility on Oahu. The colony was started with pupae received from the HDOA's Hilo insectary on the Island of Hawaii in 2002. The cocoons were placed in a 42 x 42 x 62 cm cage. Emerging adults were allowed to mate and oviposit onto ti leaf (*Cordyline fruticosa*) or Iris (*Tritonia crocosmiiflora*) bouquets. The *C. fruticosa* and *T. crocosmiiflora* bouquets also served as a food source for the newly hatched larvae. *Darna pallivitta* colonies were reared under 24.6 ± 1.5° C and 70.6 ± 5.2% RH with a photoperiod of 12:12 (L:D).

Host Range Testing

No-choice host range tests were conducted at the HDOA quarantine facility in Honolulu, Hawaii. Twenty-three plant species representing 15 families (Table 2.1) were selected based on their economic value and prevalence in Hawaii's landscape (including both indigenous and weed species). Ten of the 23 plants tested were native to Hawaii (Table 2.1). Experiments were conducted in an environment-controlled room at 24.6 ± 1.5° C with 70.6 ± 5.2% relative humidity and a photoperiod of 12:12h (L:D). Bouquets of plant terminals containing up to 4 leaves (depending on leaf size of each plant species used) were placed in 1 dram (3.7 ml) glass vials filled with tap water. Only mature leaves were used for *D. pallivitta* diet since Cock, et al. (1987) suggest that larvae prefer to feed on mature foliage. As larvae matured, leaf bouquet size was tripled to accommodate the larger larvae and bouquets were placed into 4 dram (14.79 ml) glass vials. Leaf bouquets were replaced when they had been totally eaten or were starting to show signs of desiccation (usually after 2 -3 days). Five first-instar larvae were placed onto each leaf bouquet using a fine-tipped natural hair paintbrush (Royal Crafter's Choice Set CC 205, Royal Brush Mfg., Hammond, Ind.). Bouquets with first instar larvae were placed into quart sized plastic containers with a circular portion of the lid cut out and replaced with Lumite screen (amber 52 X 52 mesh, SI Corporation, Gainseville, GA) which was glued to the remaining lid with Shoe Goo® (International Technical Trading Inc., Tokyo, Japan). When larvae began to outgrow their plastic containers, they were transferred to glass jars (vol. 3.79 L) where they were kept until pupation (Fig. 2.1).

Number of days until pupation and moth emergence were recorded. Due to the length of *D. pallivitta*'s larval period (45 – 72 days) (Nagamine, unpublished data), the duration of each test was limited to three months. Any larvae that were not close to pupation at the end of this period were classified as unable to complete development (would not survive to pupation). Each test consisted of five replicates per plant species. Upon pupation, cocoons were isolated in individual 4 dram (14.79 ml) glass vials and observed until adult emergence. Emerging adult males and females from the same host plant were introduced into glass jars (3.79 L) to mate and oviposit to determine emerging adult fertility. Depending on adult availability, up to three pairings of males and females per test plant were completed and qualitative observations of presence or absence of eggs and emerging larvae were noted. Due to its lengthy lifecycle, offspring of this second generation were not reared to adulthood. After determining fertility of the adults, the larvae were discarded.

Field Observations

Sampling of food plant species in the field was conducted during September 2001 to the present. While conducting random field surveys, we recorded all plant species on which *D. pallivitta* larvae were observed feeding on.

Data Analysis

Analysis of Variance (ANOVA) run on a single generalized linear model (GLM) (SAS Institute, 1998) was run to detect the effect of host plant species on development of *D. pallivitta* larvae. Mean times to pupation on different host plants were compared by Tukey pairwise comparisons. Test plants where no pupation occurred were excluded from the GLM to allow a meaningful analysis of variance.

2.4. Results

Host Range Test

Of the 23 plant species tested, *D. pallivitta* was able to successfully complete larval development and pupation on 11 species (Table 2.1), from 11 genera in 8 families. Seven of the 11 host plants (63.6%) were monocots. Four of the 11 confirmed host plants are native to Hawaii, the monocots *Vigna marina* and *Pritchardia hillebrandei* and dicots *Acacia koa* and *Pipturus albidus*. Of the 12 plants that *D. pallivitta* did not complete its lifecycle on, the mean days of survival per plant species ranged from 5.56 days to 28.72 days (Table 2.1). However, in the case of *Dianella sandwicensis*, *Psidium guajava*, *Clidemia hirta*, and *Murraya paniculata*, some larvae were able to survive for up to 2–3 months. None of the larvae had pupated at the end of the 3 month experiment deadline.

Vigna marina was the optimal host plant of the four native species tested (Table 2.1), with the highest rate of larval pupation and adult emergence and the lowest duration of time to pupation and adult emergence. *Pritchardia hillebrandei* was the poorest of the native host plants having the lowest rate of larval pupation and adult emergence and having the longest duration of time from pupation to adult emergence (Table 2.1).

Darna pallivitta appears better adapted to mature on monocots than on dicots. Mean time to pupation showed that larvae reared on monocots tended to pupate sooner than larvae reared on dicots with the exception of *P. hillebrandei* and *D. massangiana*, both of which had very low rates of successful pupation and adult emergence (Table 2.1). Mean time to pupation varied from 40.57 ± 5.43 days to 79.50 ± 3.50 days (Table 2.1) and was dependent on the plant species used ($F_{10;41} = 42.84$, $P < 0.0001$).

Tukey's pairwise comparisons showed the mean times until pupation for *Tritonia crocosmiiflora*, *Vigna marina*, *Cordyline fruticosa*, and *Commelina diffusa* differed significantly ($P > 0.050$). Mean times to pupation of larvae for *C. diffusa* and *Veitchia merrillii*, were not significantly different, nor were the mean times until pupation for *Averrhoa carambola* and *Acacia koa*, *Coffea arbica* and *Pritchardia hillebrandei*, and *Dracaena massangiana* and *Pipturus albidus* (Fig. 2.2).

Mean times for adult emergence ranged from 19.46 ± 1.54 days to 23.36 ± 6.64 days though some larvae took up to 31 days before emergence. Emergence times depended on the plant species on which the larvae were reared ($F_{10; 41} = 5.54$; $P < 0.0001$). Tukey's pairwise comparison performed for the duration of pupation to successful emergence showed there were significant differences in times for emergence (Fig. 2.3). The mean rate of successful adult emergence for all host plants varied from 8% to 96%. *Darna pallivitta* adults from all successful test plants were able to mate and produce offspring, except those adults reared on *P. hillebrandei* and *D. massangiana*. No offspring were produced from larvae reared on *P. hillebrandei* and *D. massangiana* because very few larvae survived to adulthood and those that did were the same sex. Six larvae survived to adulthood on *P. hillebrandei* with six males produced. Only two larvae survived to adulthood on *D. massangiana*, with both adults being female. Due to differences in emergence times of adult *D. pallivitta* reared on *P. albidus*, only two tests for fertility were conducted on this plant species. One of these replicates produced offspring.

Field Observations

Larvae of *D. pallivitta* were observed feeding on 57 species of plants representing 54 genera in 26 families (Table 2.2). Field observations indicate that monocots are favored over dicots as food plants in roughly a 2:1 ratio with plants in the family Arecaceae (palms) having the most recorded food plants (9 species). The family Fabaceae (legumes) has the second highest recorded number of food plants (7 species) but is dicotyledonous. Six Poaceae (grasses) were recorded as food plants. Five plant species (*Pritchardia hillebrandei*, *Vigna marina*, *Acacia koa*, *Alyxia oliviformis*, and *Pipturus albidus*) that *D. pallivitta* larvae have been observed feeding on in the field are native to Hawaii.

2.5. Discussion

D. pallivitta is highly polyphagous, recorded feeding on many plants in its native East Asia (Holloway, 1986 and Cock, et. al 1987) and in Hawaii (California Plant Pest and Disease Report, 2005). In this study *D. pallivitta* was recorded feeding on 57 plant species in 26 families preferring monocots, which laboratory studies confirmed. Many species of limacodids are listed as pests of monocotyledonous crops (Stephens, 1975, Igbinosa, 1985, and Cock, et. al, 1987).

Results of host range testing supported field observations. Host plants that were monocots outnumbered host plants that were dicots. Seven of the eleven laboratory host plants were monocots. A little less than half of the plants tested were native to Hawaii, and four native test plants are hosts. Larvae that were reared on some of these native plant species had relatively high levels of survivability as well, which was evidenced by the number of emerging adults from *V. marina* and *A. koa*.

Since its introduction to Hawaii, the known diet breadth of *D. pallivitta* has expanded considerably. Plants in the family Fabaceae have never been previously recorded as hosts but we observed *D. pallivitta* feeding on a variety of legumes in the field. It is unknown how many species are suitable host plants of the 55 plant species recorded but given *D. pallivitta*'s polyphagous nature, it is highly likely that more plants listed on the feeding and host list (Table 2.2) are hosts upon which complete development can occur. Larger, more mature larvae may also have an expanded host range which would enable them to consume plants they would be unable to survive off of as early instar larvae. In the field, middle to late instar larvae of *D. pallivitta* have been observed wandering to different plant species presumably to feed.

Twenty-three plant species tested as hosts is a relatively small number. Testing more plants was not possible during this experiment for a number of reasons. Long larval development periods of *D. pallivitta* made the time period of each experiment correspondingly long and further experiments had to be abandoned due to a cytoplasmic polyhedrosis virus which infected lab colonies both on Oahu and Hilo, making it impossible to obtain healthy larvae for use in experiments.

No-choice host range tests were carried out to determine *D. pallivitta*'s physiological host range in order to get an approximation of the number of plant species which could become potential hosts and therefore, potential sources of infestation. However, no-choice test results were not always an indication of preferred hosts in the field. During no-choice host range tests, starfruit (*Averrhoa carambola*) was found to be a good host plant (68% adult emergence) but observations of starfruit trees in the field never recorded a single sighting of *D. pallivitta*. Conversely, *D. massangiana* was shown to be a suboptimal host plant in the laboratory. However, numerous field observations show a number of middle and late instar *D. pallivitta* larvae feeding on *D. massangiana* in the field indicating that *D. massangiana* may be a better host plant than lab results would suggest.

Much more in-depth work needs to be completed before *D. pallivitta*'s apparent or ecological host range in Hawaii is known with certainty. Indeed, the no-choice host range tests and the observed feeding list are just the first steps. An ovipositional preference test described by Van Driesche and Bellows (1996) could perhaps give a definitive answer to this question of expansion of *D. pallivitta*'s host range. A small preliminary ovipositional preference test using *T. crocosmiiflora* and *Cordyline fruticosa* as the control showed that adult *D. pallivitta* overwhelmingly preferred to oviposit on the cage corners but also found that more eggs were oviposited on *T. crocosmiiflosra* than the control indicating that ovipositional preference tests may be successfully completed. Further casual observations have noted that adults also prefer to oviposit on the glass of cages similar to other lepidopterous species (Cai, et al. 2002). In conclusion, the results of this study confirm *D. pallivitta*'s wide host range and underscore the need for effective management due to its potential to expand and impact Hawaii's native and introduced flora.

2.6. Acknowledgements:

I would like to thank Stacey Chun, Christopher Jacobsen and Ruth Niino-Duponte at the Beaumont Agricultural Research Center, Pat Conant and Clyde Hirayama at the Hawaii Department of Agriculture – Hilo Branch, and Ray Baker and Karen Shigematsu of Lyon Arboretum for all of their help and advice and the United States Department of Agriculture under CSREES Special Grant No. 2005-34135-1640, managed by the Tropical/Subtropical Agricultural Research (T-STAR) Pacific Basin Administrative Group (PBAG) for funding this research.

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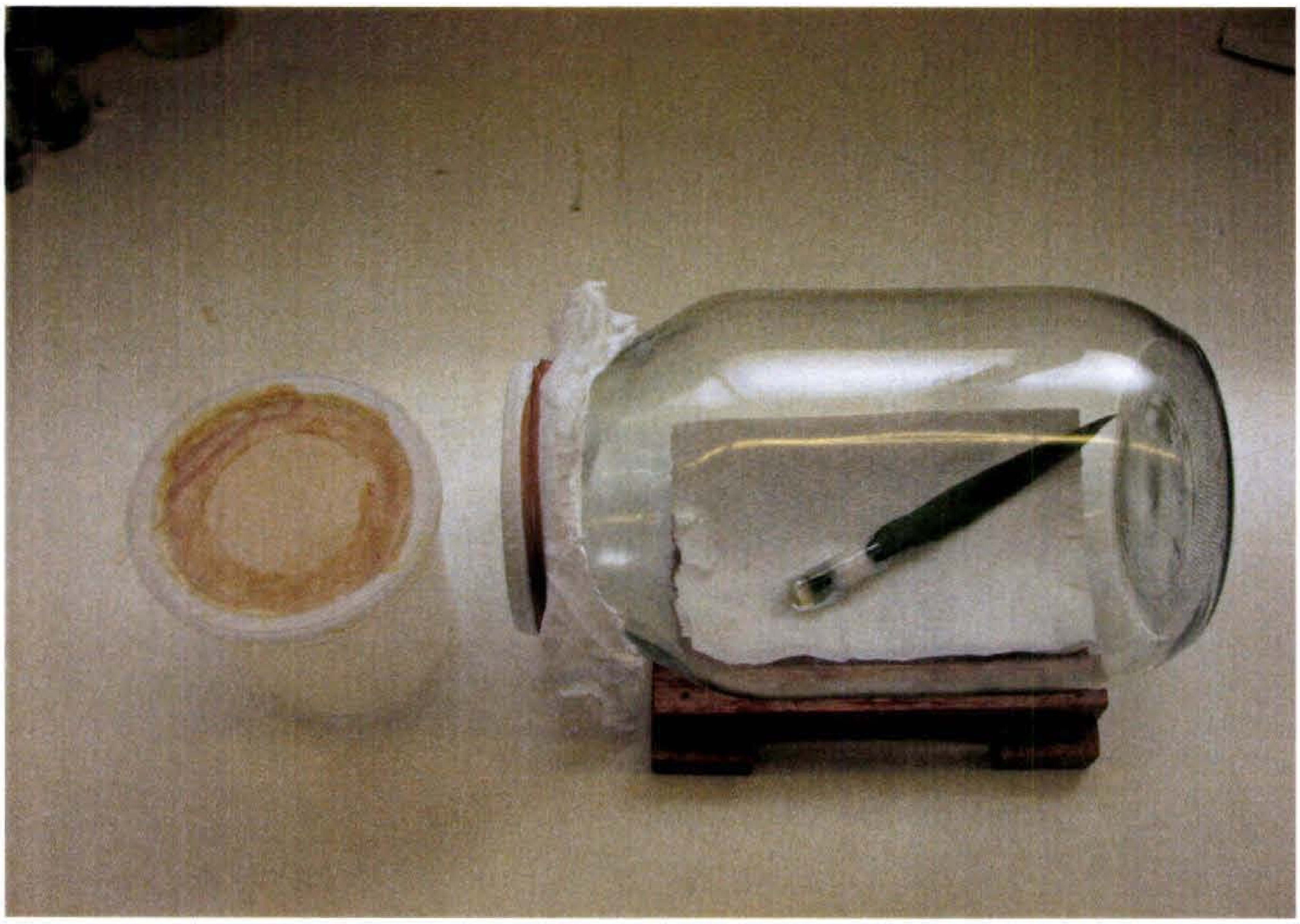

Fig. 2.1. Containers used to rear *D. pallivitta* in host range testing experiments.

Table 2.1. Rates of successful pupation and adult emergence with mean number of days for pupation and emergence to occur.

Plant Species	Family	Status	Mean Rate of Pupation	Mean Time To Pupation or death (days) (**)	Mean Rate of Emergence	Mean Time To Emergence (days)
Commelina diffusa	Commelinaceae	Weed sp.	96%	46.96 ± 11.04	96%	19.63 ± 3.57
Tritonia crocosmiiflora	Iridaceae	Foliage sp.	96%	40.57 ± 5.43	92%	23.26 ± 3.74
Veitchia merrillii	Arecaceae	Foliage sp.	92%	50.17 ± 9.17	92%	22.30 ± 8.70
Vigna marina	Fabaceae	Indigenous sp.	84%	41.67 ± 7.67	84%	19.71 ± 1.71
Coffea arabica	Rubiaceae	Agricultural sp.	80%	71.40 ± 24.60	78%	21.11 ± 2.11
Cordyline fruticosa	Agavaceae	Foliage sp.	78%	46.42 ± 10.58	78%	21.02 ± 5.98
Averrhoa carambola	Oxalidaceae	Agricultural sp.	68%	55.72 ± 13.28	68%	21.00 ± 4.00
Acacia koa	Fabaceae	Indigenous sp.	60%	57.73 ± 16.27	60%	23.28 ± 2.80
Pipturus albidus	Urticaceae	Indigenous sp.	48%	79.23 ± 19.23	48%	21.83 ± 2.83
Pritchardia hillebrandei	Arecaceae	Indigenous sp.	16%	74.50 ± 17.50	12%	23.33 ± 2.67
Dracaena massangiana	Agavaceae	Foliage sp.	8%	79.50 ± 3.50	8%	19.50 ± 1.50
Pritchardia martii *	Arecaceae	Foliage sp.	-	5.56 ± 3.56 **	-	-
Spathyphylum cleviandii *	Araceae	Foliage sp.	-	5.96 ± 6.04 **	-	-
Cibotium chamissoi *	Dicksoniaceae	Foliage sp.	-	7.64 ± 3.64 **	-	-
Tillandsia cyanea *	Bromeliaceae	Indigenous sp.	-	7.68 ± 3.68 **	-	-
Freycinetia arborea *	Pandanaceae	Indigenous sp.	-	8.28 ± 5.28 **	-	-
Pandanus tectorius *	Pandanaceae	Indigenous sp.	-	9.40 ± 7.60 **	-	-
Metrosideros polymorpha *	Myrtaceae	Indigenous sp.	-	9.84 ± 3.16 **	-	-
Monstera deliciosa *	Araceae	Foliage sp.	-	11.88 ± 31.20 **	-	-
Dianella sandwicensis *	Liliaceae	Indigenous sp.	-	23.76 ± 49.24 **	-	-
Psidium guajava *	Myrtaceae	Agricultural sp.	-	23.80 ± 43.20 **	-	-
Clidemia hirta *	Melastomataceae	Weed sp.	-	25.92 ± 68.08 **	-	-
Murraya paniculata *	Rutaceae	Foliage sp.	-	28.72 ± 34.28 **	-	-

* Species indigenous to Hawaii

** Plant species with 0% pupation

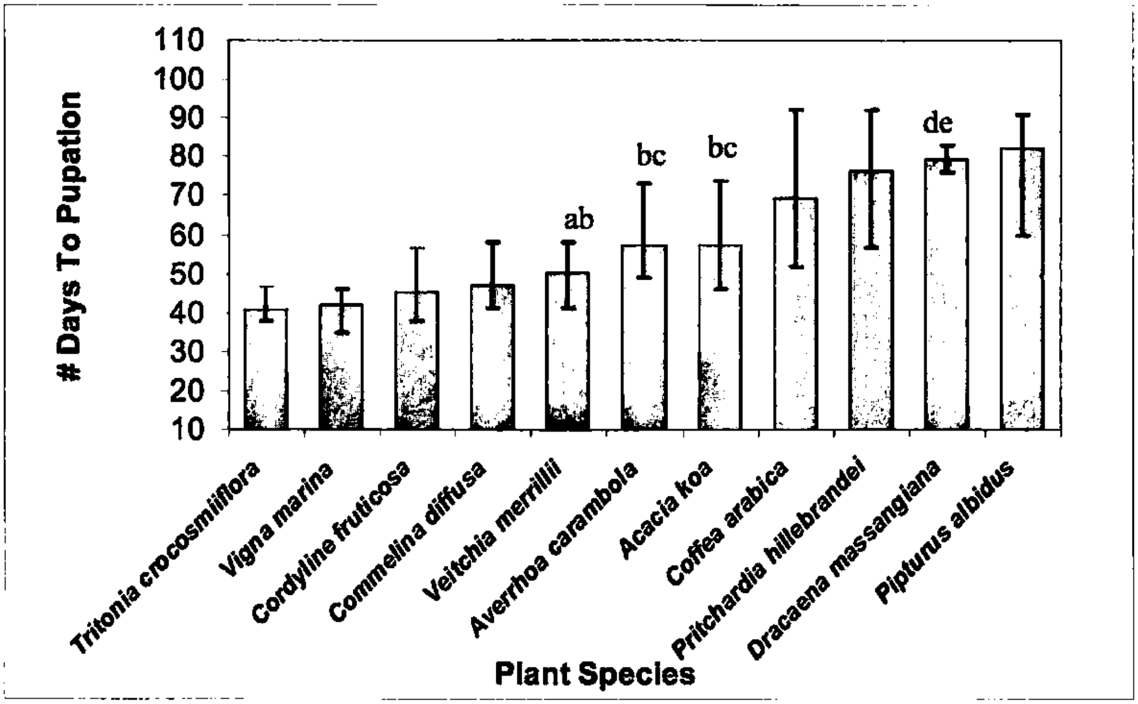

Fig. 2.2. Mean days for duration of time until pupation occurs with Tukey's pairwise comparisons. Means with the same letter are not significantly different.

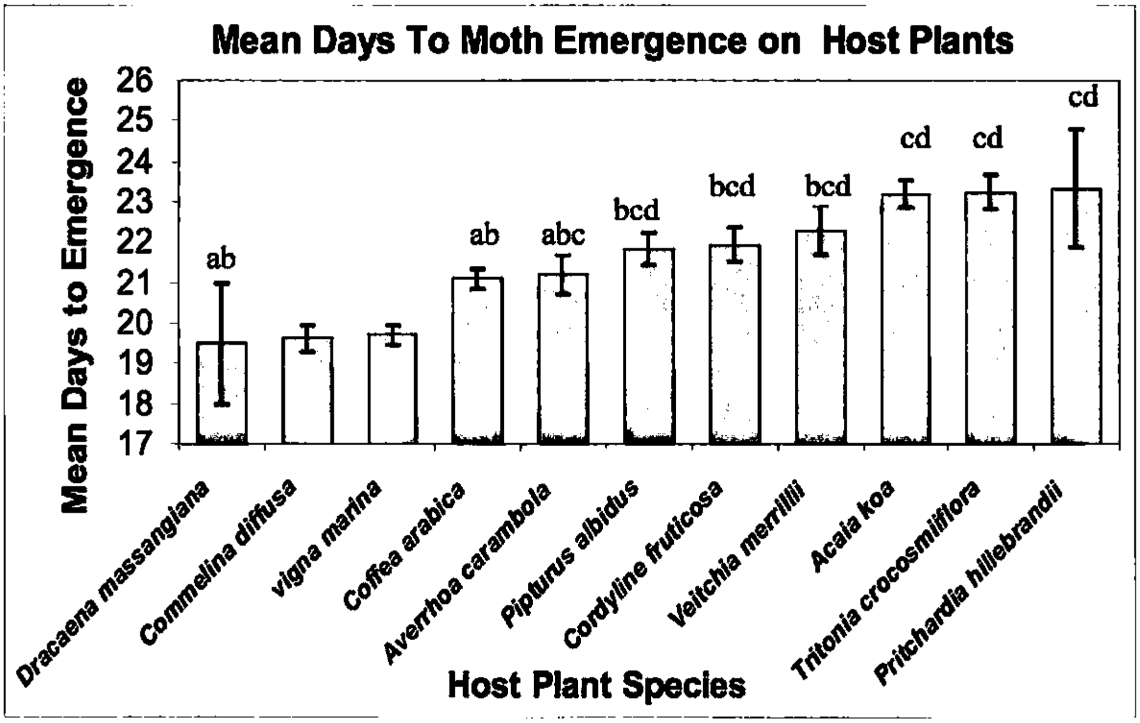

Fig. 2.3. Mean days for successful emergence of pupae on each host plant with Tukey's pairwise comparisons. Means with the same letter are not significantly different.

Table 2.2. Recorded feeding list of *Darna pallivitta*. List compiled by P. Conant, C. Hirayama, S. Chun, and C. Kishimoto

Common Name	**Scientific Name**	**Family**	**Moncot / Dicot**
Fish Tail Palm	*Caryota mitis*	Arecaceae	Monocot
Areca Palm	*Dypsis lutescens*	Arecaceae	Monocot
Phoenix Palm	*Phoenix roebelenii*	Arecaceae	Monocot
Rhapis Palm	*Rhapis excelsa*	Arecaceae	Monocot
Coconut Palm	*Cocos nucifera*	Arecaceae	Monocot
Pony Tail Palm	*Nolina recurvata*	Arecaceae	Monocot
Manila Palm	*Veitchia merrillii*	Arecaceae	Monocot
Loulu Palm	*Pritchardia hillebrandei*	Arecaceae	Monocot
Cat Palm	*Chamaedorea cataractum*	Arecaceae	Monocot
Perennial Peanut	*Arachis pintoi*	Fabaceae	Dicot
Manualoa Vine	*Canavalia cathartica*	Fabaceae	Dicot
Silverlieaf Desmodium	*Desmodium uncinatum*	Fabaceae	Dicot
Wiliwili	*Erythrina sandwicensis*	Fabaceae	Dicot
Koa*	*Acacia koa*	Fabaceae	Dicot
Sleeping Grass	*Mimosa pudica*	Fabaceae	Dicot
Vigna (Beach Pea)*	*Vigna marina*	Fabaceae	Dicot
Vasey's Grass	*Paspalum urvillei*	Poaceae	Monocot
Hilo Grass	*Paspalum conjugatum* Berg	Poaceae	Monocot
Napier Grass	*Pennisetum purpureum*	Poaceae	Monocot
Sorghum	*Sorghum bicolor*	Poaceae	Monocot
Wainaku Grass	*Panicum repens*	Poaceae	Monocot
California Grass	*Brachiaria mutica*	Poaceae	Monocot
Dracaena 'Lisa', 'Compacta'	*Dracaena deremensis*	Agavaceae	Monocot
Dracaena	*Dracaena massangiana*	Agavaceae	Monocot
Ti	*Cordyline fruticosa*	Agavaceae	Monocot
Walking Iris	*Neomarica gracilis*	Iridaceae	Monocot
Iris	*Tritonia croscosmiiflora*	Iridaceae	Monocot
Gladiolus	*Gladiolus* sp,	Iridaceae	Monocot
Coffee	*Coffea arabica*	Rubiaceae	Dicot
Gardenia	*Gardenia jasminoides*	Rubiaceae	Dicot
Koster's Curse / Clidemia	*Clidemia hirta*	Melastomataceae	Dicot
Glory Bush	*Tibouchina urvilleana*	Melastomataceae	Dicot
Maile*	*Alyxia oliviformis*	Apocynaceae	Dicot
Chinese Star Jasmine	*Trachelospermum jasminoides*	Apocynaceae	Dicot
Chick Weed	*Drymaria cordata*	Caryophyllaceae	Dicot
Pineapple	*Annanas sativus*	Bromeliaceae	Monocot
Pink Quill	*Tillandsia cyanea*	Bromeliaceae	Monocot
Banana	*Musa sp.*	Musaceae	Monocot
Honohono Grass	*Commelina diffusa*	Commelinaceae	Monocot
Red Ginger	*Alpinia purpurata*	Zingiberaceae	Monocot
Rabbit Foot Fern	*Phlebodium aureum*	Polypodiaceae	Monocot
Whale Back	*Curculigo capitulata*	Hypoxidaceae	Monocot

Table 2.2. (Continued) Recorded feeding list of *Darna pallivitta*.

Common Name	**Scientific Name**	**Family**	**Moncot / Dicot**
Whale Back	*Curculigo capitulata*	Hypoxidaceae	Monocot
Monstera	*Monstera deliciosa*	Araceae	Monocot
Mamaki*	*Pipturus albidus*	Urticaceae	Dicot
Strawberry Guava	*Psidium cattleianum*	Myrtaceae	Dicot
Sweet Guava (waiawi)	*Psidium guajava*	Myrtaceae	Dicot
Shampoo Ginger	*Costus spicatus*	Costaceae	Monocot
Wedalia	*Wedalia trilobata*	Asteraceae	Dicot
Starfruit	*Averrhoa carambola*	Oxalidaceae	Dicot
Mondo, Monkey Grass	*Ophiopogon japonicus*	Liliaceae	Monocot
Lillyturf	*Liriope muscari*	Liliaceae	Monocot
Pointsettia	*Euphorbia pulcherrima*	Euphorbiaceae	Dicot
Strawberry	*Fragaria ananassa*	Rosaceae	Dicot
Rose	*Rosa sp.*	Rosaceae	Dicot
Bamboo Orchid	*Arundiana graminifolia*	Orchidaceae	Monocot
Rhododendron	*Rhododendron* sp.	Ericaceae	Dicot
Razor Grass (Hawaii Nutrush)	*Scleria testacea*	Cyperaceae	Monocot

CHAPTER 3

The Biology of *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae), A Recently Introduced Invasive Species In Hawaii

3.1. Abstract

The biology of *Darna pallivitta*, a recent invasive species in Hawaii, was investigated. Adults were collected in light traps during three intervals throughout the night to determine when they were most active. Light trapping was also conducted during the first ninety minutes after sunset to determine when adult activity began and if there were any differences in activity times between males and females. The height of larvae observed in the field at five different plots was also recorded to determine any preference for a certain height above the ground. Adult females were found to be most active just after sunset while males were most active during the second half of the night. Larvae had a preference for the first meter of vegetation above ground. Results from this research may form the basis for future control measures.

3.2. Introduction

The family Limacodidae is a diverse group, comprising approximately 1,000 species (Holloway, 1986 and Epstein, 1996). Although limacodids are found primarily in tropical areas, Cock, et al. (1987) state that they can be found in all of the zoogeographic regions of the world as well.

The limacodid, *Darna pallivitta* Moore, is a recent arrival to Hawaii (Chun et. al, 2005) and is the only member of the family in the state (Nishida, 2002). In the years since its arrival, *D. pallivitta* has been classified as an agricultural pest of quarantine importance and a new pest alert was issued by the Hawaii State Department of Agriculture (Conant, et al. 2002). *Darna pallivitta* has also become a health concern because of the stinging spines it possesses. When *D. pallivitta* first arrived in Hawaii, very little was known about *D. pallivitta*. Since then, Nagamine and Epstein (unpublished) have covered much of the basic biology of this species under laboratory conditions, but little is known about *D. pallivitta's* habits under field conditions. The results of these field experiments and observations will help to create a basis for possible control measures for *D. pallivitta*. Larval preferences of a specific height as well as adult activity will be examined in this study.

3.3. Materials and Methods

Moth Activity

Adult *D. pallivitta* activity was determined by using three universal black light traps (BioQuip model no. 2851U BioQuip, Rancho Dominguez, CA). The light was mounted on a 5 gallon bucket that had no drainage system instead of the 3.5 gallon bucket from the manufacturer. The BioQuip buckets' drainage system was not adequate and made it possible for insects to be damaged or lost during heavy rains as well as allowing lizards and centipedes to enter the bucket and consume trapped insects. A 1/4 inch (6.35 mm) hole was drilled in the center of the 5 gallon bucket's bottom and a long stem funnel with its top covered with plastic screen was inserted into the hole. The bucket traps were then placed on cinder blocks to maximize drainage during rain and to help prevent scavengers such as lizards or centipedes from consuming any insects. The bucket traps were placed side by side with about a one foot (30.50 cm) distance between each trap. The total height of a trap placed on a cinder block was approximately one meter. The black light in each trap was connected to a Flexcharge PRGTMR 12 V Programmable Timer (SES Flexcharge USA, Charlevoix, MI) powered by a 12 V lead-acid battery. The timer in each trap was set for one of three 3.5 hour intervals with each timer set for consecutive intervals giving a 10.5 hour duration for each experiment. Light trapping was from 1930h until 0600h. Trapping took place four times at three locations during the period of June – September 2005. Traps were set out for three consecutive days before being cleared. After collection, the number of adult *D. pallivitta* caught were sexed and counted for each time interval. Light traps were placed at three different locations in Hilo, Hawaii; H. Eunice Nursery (19° 38 min. 55 sec. N / 155° 4 min. 47 sec. W), Ainalako (19° 39 min. 44 sec. N / 155° 5 min. 33 sec. W), and the University of Hawaii College of Tropical Agriculture and Human Resources' Waiakea Research Station (19° 38 min. 38 sec. N / 155° 4 min. 47 sec. W).

Male / Female Activity

Black-lighting using an ultraviolet light against a white sheet was conducted after sunset for approximately 1.5 hours to determine when *D. pallivitta* adults became active and to determine any temporal differences between male and female activity. Trapping took place at 3 different locations in the Panaewa area on the island of Hawaii: Panaewa Zoo (19° 19 min. 38 sec. N / 155° 3 min. 27 sec. W), Gervais Orchids, and H. Eunice Nursery. An 18 inch (45.7 cm) 15 watt Sylvania model F15 T8 black light (Osram Sylvania, Danvers, MA, USA) powered by a 12 V lead-acid battery was hung against a white sheet measuring 1.5 x 1.2 m (5 x 4 ft) which was suspended from a rope approximately 1.5 m (5 ft) above the ground. All *D. pallivitta* adults that were attracted to the sheet were collected and sexed. None of the adults were released after collection. This method of moth trapping was conducted on 27 nights from September 2003 until October 2005 although the majority of the light trapping (25 nights) was conducted from July 2004 through May 2005. Black lighting and staggered light trapping never took place at the same location on the same night.

Larval Height Preference In Plant Canopy

Initial larval surveys (Chapter 4) revealed that the majority of larvae were observed in the lower parts of plants. Therefore we sought to determine a height preference for height above ground within the plant canopy. From June 2004 through May 2005 we surveyed five sites - two plots at the H. Eunice Nursery (containing *Dypsis lutescens* or *Phoenix roebellini*), two plots at the Panaewa Zoo (*D. lutescens* or *Cordyline fruticosa*), and one plot at the Waiakea Research Station (*C. fruticosa*). During surveys to determine larval phenology at the five plots, the height of each larva observed was classified into one of three categories –less than approximately 0.9 m (below 3 feet), approximately 0.9 – 1.8 m (3 – 6 feet) and above 1.8 m (above 6 feet). Observed larvae were separated into 3 classes based on maturity in order to determine if height preference changed with larval maturity. Class 1 larvae were made up of early instar larvae that had a "windowing" mode of feeding on the leaf and usually consisted of larvae from instars 1 – 4 (Cock, et al. 1987). Class 2 larvae consisted of middle instar larvae that were able to eat through the entire leaf but were not yet ready to pupate. Class 2 larvae usually consisted of larvae in instars 5-7. Class 3 larvae were made up of late instar larvae that were nearing pupation (Nagamine and Epstein, unpublished data). Class 3 larvae were in instars 8 –

11. Only sites where surveyed vegetation grew taller than 2 meters were considered to decrease bias toward larval preference to any particular height category.

Data Analysis

Analysis of Variance (ANOVA) using a Generalized Linear Model (GLM) Procedure (SAS, 1997) was conducted to determine significant differences in preferences of larvae for different heights within the plant canopy. Chi-square analysis was used to determine differences between the number of moths caught during staggered black light trapping.

3.4. Results

Moth Activity

Almost all adult *D. pallivitta* that were captured by black light traps were males. A total of 1,628 adults were collected, of which only two were female. The staggered timing technique of light trapping showed that adult male *D. pallivitta* were most active later in the evening up to the predawn hours. There were more than twice as many moths caught during the second interval (11:00 p.m. – 2:30 a.m.) as the first interval (7:30 p.m. – 11:00 p.m.) with the third interval (2:30 a.m. – 6:00 a.m.) contributing the highest moth counts (Fig. 3.1). Chi-square analysis of cumulative collections at each site revealed significant differences in the number of moths trapped during the three time periods (H. Eunice Nursery: $\chi^2 = 17.54$; $P < 0.0005$ df = 2; Ainalako: $\chi^2 = 19.03$; $P < 0.0005$ df = 2; Waiakea Experiment Station: $\chi^2 = 52.90$; $P < 0.0005$ df = 2). Pooled collections for this study showed an increase in the number of adults collected as the night hours progressed. The two females that were captured were collected during the first time interval (between

7:30 p.m. – 11:00 p.m.) of the evening. No other females were collected during the other time intervals.

Male / Female Activity

A total of 91 adult *D. pallivitta* were collected – all female - during the 26 evenings in which black light trapping with a sheet was conducted. Nightly catches ranged between 0 to 18 moths. This indicates that female *D. pallivitta* are active earlier in the evening than adult males and that the sexes are not active at the same time. Adult females were collected in the highest densities between 40 to 70 minutes after sunset (Fig. 3.2) which coincided with the approximate times that the staggered light trapping began. Only 3 females were collected within the first thirty minutes after sunset (none within the first 20 minutes) and only one female was collected more than 80 minutes post sunset. The flight behavior of adult females was very erratic. Most females did not land on the sheet for an extended period of time and if disturbed by other insects landing around them, would frequently fly away. Many would continue to fly around the sheet in a very unpredictable manner usually "bouncing" off the sheet many times making collection a challenge. Occasionally, after contacting the sheet, females would also land on the ground directly under the sheet. Whenever possible, these adults were also collected and counted toward the number of adults collected from the sheet since they had also been attracted to the light.

Larval Height Preference in Plant Canopy

There was a large difference between the number of larvae observed at each height category above ground (Fig. 3.3). In particular, there was a highly significant difference between the number of larvae that were observed within the first meter of the plant canopy and the number of larvae observed at other heights ($\chi^2 = 314.83$, $P<0.0001$; df = 1). Larvae found at heights above 0.9m were combined for chi-square analysis since the value of the last category (higher than 6 feet (1.8 m)) was too small for analysis (n = 2). Of the 516 larvae that were surveyed, approximately 90% (463) of the larvae were observed less than one meter from the ground; 94% (128 of 136 larvae) of class 1 larvae were found less than a meter above ground with none being found above 1.8 m. Eighty-five percent (245 of 289 larvae) of all class 2 larvae were found less than a 0.9 m from the ground. Eighty-five percent of class 3 larvae were found less than 0.9 m above ground. Only two larvae were observed above 2 meters (one class 2 larva and one class 3 larva). Analysis of Variance using a Generalized Linear Model (GLM) also showed a significant preference for a specific height within the plant canopy ($F_{8;\,126} = 7.01$; $P < 0.0001$) with a highly significant variation in the number of larvae found by height ($F_{2;\,126} = 21.32$ $P < 0.0001$). No significant interaction was found between total number of larvae observed and height ($F_{4;\,126} = 1.94$, $P > 0.1$).

3.5. Discussion

Most of the adults that were collected during staggered light trapping were collected later in the evening (11:30 p.m. – 6 a.m.). Almost all adults that were collected were males. These findings are also supported by Hawaii State Department of Health (DOH) ‘New Jersey’ light trap samples which operated from 6:00 p.m. through 6:00 a.m. Only 2% of all *D. pallivitta* adults collected were female (n = 3,269 moths). Although no similar data focusing on other limacodid species could be found, Reed, et al. (1975), working with pink bollworm (*Pectinophora gossypiella*) also found that bucket style light trapping consistently caught more males than females. It is unknown why only a small number of adult female *D. pallivitta* were collected in light traps but it is probable that females are not as attracted to the light traps during the night because they may be more focused on mating and oviposition. A similar conclusion was reached by Persson (1976) while working with noctuid moths. Many more males could be trapped because males were actively flying for longer periods of time while searching for calling females. Thus, they had a higher chance of coming into a light trap's attraction zone (Baker and Sadovy, 1978).

Female *D. pallivitta* become active during the early evening similar to other limacodid species (Dyar, 1899 and Sasaerila, 2000). Female *D. pallivitta* became active earlier in the evening than males. This was evidenced by the number of females caught shortly after sunset while not a single male was observed at the sheet during this time period. These results are contrary to the findings of other researchers who observed that adult male limacodids are usually active before females with the females attracted to the sheet traps soon after the males (J. Lill, personal communication). However, these differences may vary from species to species within the family Limacodidae. High numbers could be the result of high population density within the areas surveyed (M. Epstein, personal communication). The reason for the differences in periods of activity between the sexes is unclear. Perhaps females need higher minimum temperatures to become active than males (Persson, 1976 and Howell, 1979). Thus, females may be active earlier in the evening when it is warmer. However, temperature was not recorded during light trapping in this study. Another possible explanation for males becoming active later in the evening is this may be a time when females start releasing pheromones to attract mates and when mating takes place (Reed, et al. 1975)

The larvae of *D. pallivitta* showed a very strong preference for lower strata of plants. This preference likely varies from species to species within the family (Dyar, 1895 and 1896). Larvae were observed feeding on the mature leaves of plants which is common among other limacodid species (Chander, 1983, Cock, et al. 1987, Pook, et al. 1998). The occurrence of mature vegetation lower in the plant canopy could be a reason why *D. pallivitta* prefers lower strata on plants. However, mature vegetation is also found in abundance higher in the canopy where larvae were rarely observed. An exception to this may be during heavy outbreaks where high numbers of larvae may lead to competition for food, resulting in more larvae being found higher in the canopy. Larvae were also observed feeding on the new growth of *Cordyline fruticosa* (ti), *Phoenix roebellini* (phoenix palm), *Dypsis* lutescens, (areca palm), and *Commelina diffusa* (honohono grass) in the field as well as young fronds of *Cocos nucifera* (coconut) under laboratory conditions. Larvae also move "sluggishly", most likely due to their small thoracic legs and absence of abdominal prolegs (Epstein, 1989) which probably results in a restricted range for most larvae in relation to location of emergence from the egg (Cock, et al. 1987). This may be a reason why few larvae were found higher in the canopy. *D. pallivitta* larvae were often observed on leaves in close proximity to the ground as well as near the interior of the plants, especially in *Dracaena* spp. and clumping varieties of palms such as *Dypsis lutescens* and *Phoenix roebellini*. A preference for lower strata within the plant canopy may also be a behavior of the larvae to help avoid detection from natural enemies who search visually for their prey (Stamp and Bowers, 1990a).

Gregarious behavior is a trait that a number of limacodids share (Cock et. al, 1987). Gregariousness may benefit early instar larvae by facilitating growth by modification of the local microclimate around the larvae (Stamp and Bowers, 1990b), helping to initiate feeding, and may even help deter natural enemies from attacking the larvae (Denno and Benrey, 1997). Denno and Benrey (1997) and Reader and Hochuli (2003) found that aggregated behavior may help in feeding by initiating feeding sites more easily and promotes increased larval growth. Stamp and Bowers (1988) working with a gregarious Saturnid (*Hemileuca lucina*), suggested that smaller less mobile larvae grouped together may deter attacks from predators.

These findings, in particular female activity during the early evening, and larval preference for lower strata within the plant canopy, will be helpful in detecting movements of *D. pallivitta*, formulating and implementing chemical and physical control strategies as well as providing quarantine information for this invasive stinging caterpillar.

3.6. Acknowledgements

We would like to thank Dr. Marc Epstein and Dr. John Lill for their knowledge and suggestions. We would also like to thank Dr. Daniel Rubinoff and Dr. Mark Wright for their help in editing this article and Christopher Jacobsen and Ruth Niino-DuPonte of the Beaumont Agricultural Research Center for their assistance in this research.

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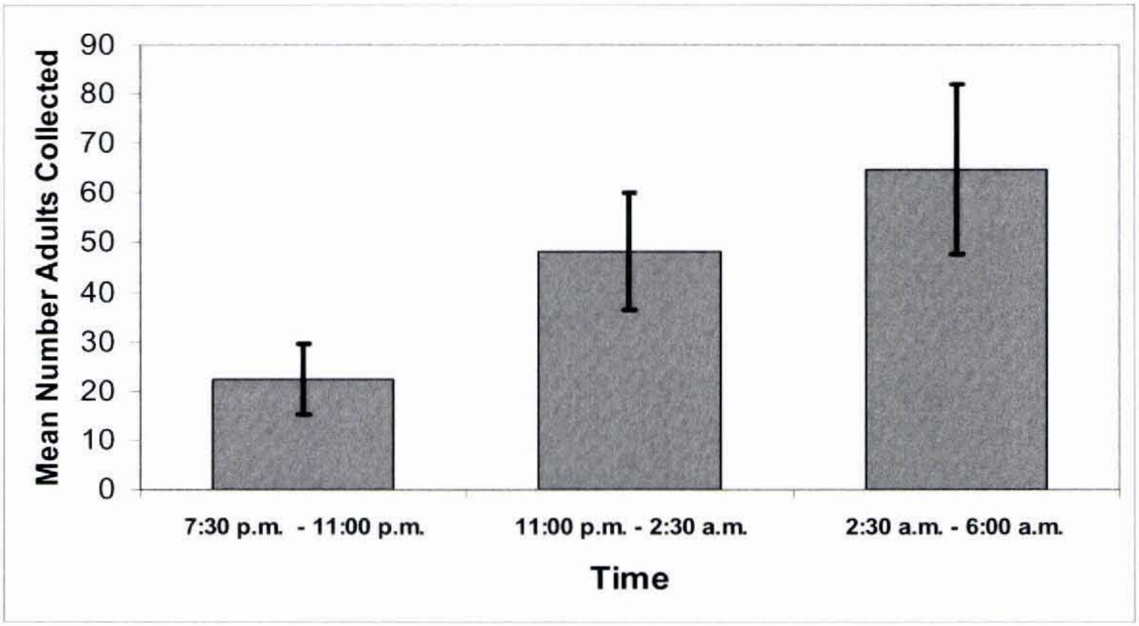

Fig. 3.1. Pooled adult *D. pallivitta* collections from all traps and sites during staggered light trapping surveys.

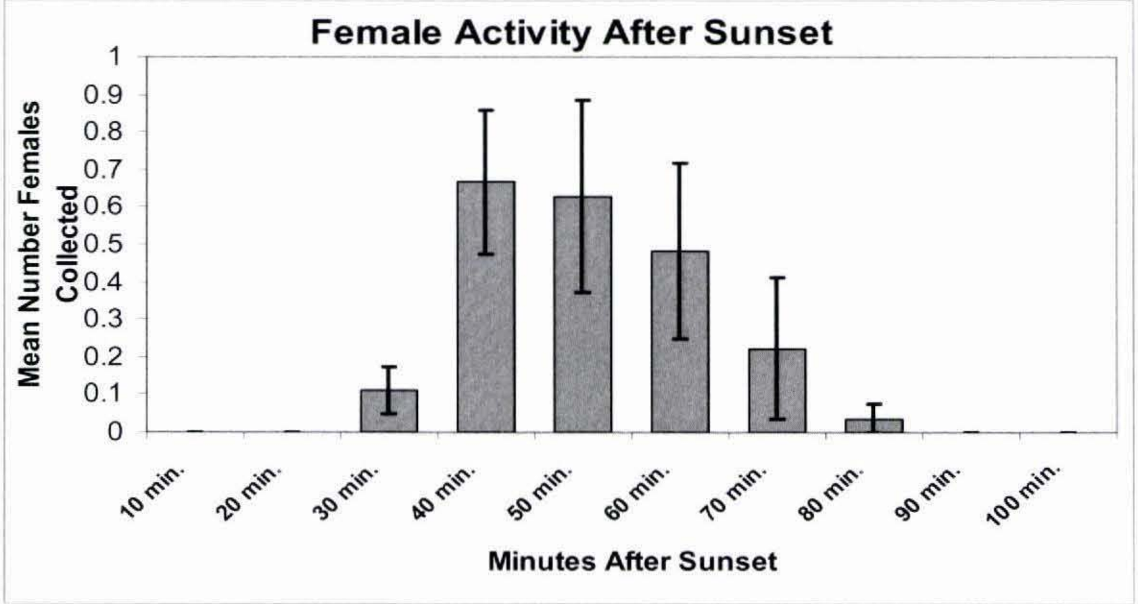

Fig. 3.2. Black lighting results showing *D. pallivitta* female collections after sunset.

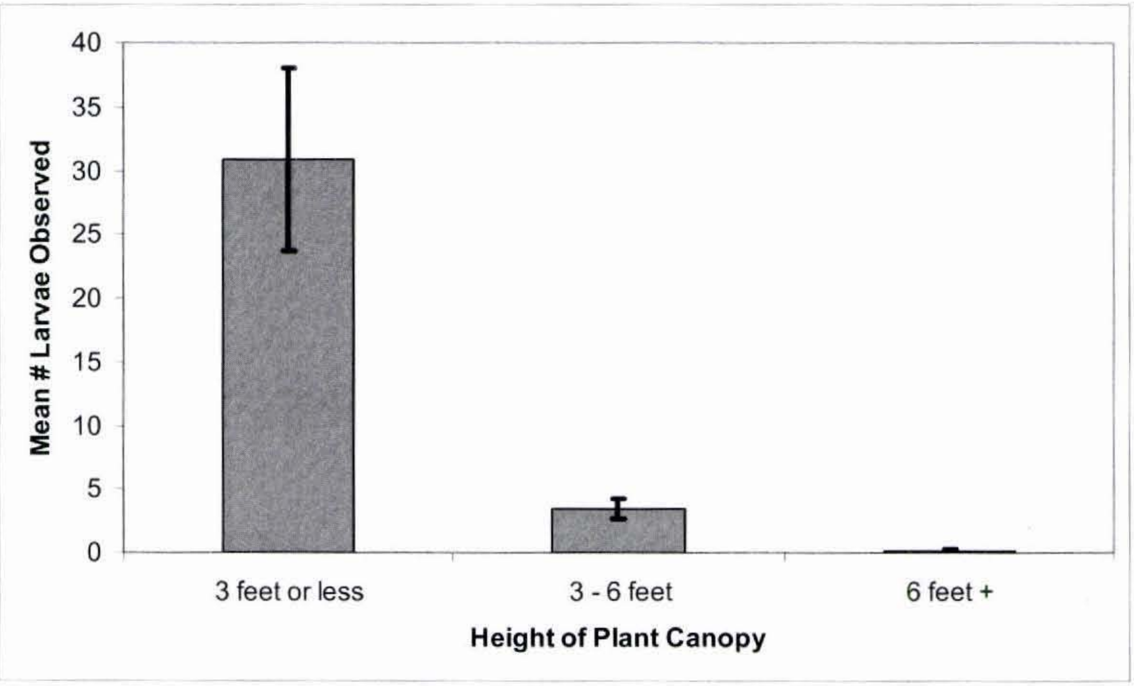

Fig. 3.3. Bar graph showing pooled data on the number of larvae observed for each height category.

CHAPTER 4

Population Dynamics of the Nettle Caterpillar, *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae) In Hawaii

4.1. Abstract

This study focuses on the abiotic factors affecting the population dynamics of a newly arrived invasive species in Hawaii, the nettle caterpillar, *Darna pallivitta* (Moore), which is a quarantine pest and a health concern due to its painful sting. Field surveys and light trapping were conducted to correlate larval and adult populations with weather data (rainfall, relative humidity, maximum and minimum temperatures). No significant correlations between weather factors and larval and adult observations were found. However, generational synchrony existed in *D. pallivitta* populations for most of the year. This may be useful in formulating control strategies since populations may be controlled if new generations can be accurately predicted.

4.2. Introduction

Darna pallivitta (Moore) is a relatively new invasive species to the state of Hawaii, having been discovered in the Panaewa area (119° 39 min. 13 sec. N / 155° 3 min. 32 sec. W) on the island of Hawaii in September 2001 (Conant, et al. 2002). Upon its arrival, *D. pallivitta* established and has become both a quarantine concern because of its broad host range (Epstein and Kinnee, 2003 and California Plant Pest and Disease Report, 2005) and a health concern because of its painful sting (Chun, et al. 2005). Young larvae of *D. pallivitta* are gregarious with heavy infestations causing severe defoliation. Occasional outbreaks of the larvae have also been reported (Hara and Conant, personal communication). It has been noted for several species of Limacodidae that weather conditions as well as natural enemies may play a large role in affecting their population dynamics (Syed and Shah, 1977, Siburat, Cock, et al. 1987, and Mojiun, 1998).

Population dynamics is a term commonly used to include both the measurement of population changes from generation to generation and the study of factors, both biotic and abiotic, that are responsible for the changes (Morris, 1957). One of the factors potentially responsible for changes within a population is weather conditions (Uvarov, 1931, Moran, 1953 and Kingsolver, 1989). Climate plays a vital role especially in abundance, distribution and population build up of insect pests and their hosts (Padhi and Saha, 2004). In an insect population, weather may affect oviposition (Courtney and Duggan, 1983), larval survival (Garg and Sethi, 1980), rate of development (Solbreck, 1976), and adult activity (Courtney and Duggan, 1983). For example rainfall has been implicated as a factor in outbreaks of *Spodoptera exempta* (Tucker, 1994). Relative humidity is a factor in adult activity of *Agrotis ipsilon* (Nasr, et al. 1980).

Very little is known about the abiotic factors affecting the population dynamics of *D. pallivitta*. Therefore, the focus of this research was to quantify the effects, if any, that weather conditions exert on populations of *D. pallivitta*.

4.3. Materials and Methods

Field Surveys

Larval surveys were conducted at ten plots throughout the Panaewa area and into the Waiakea area approximately five to six miles (8.0 – 9.7 km) south of Hilo, Hawaii. Seven plots were originally surveyed – 3 plots at H. Eunice Nursery (19° 38 min. 55 sec.

N / 155° 4 min. 47 sec. W) and 2 plots each at the Waiakea Research Station (WRS) (19° 38 min. 38 sec. N / 155° 4 min. 47 sec. W) and the Panaewa Zoo (19° 38 min. 51 sec. N / 155° 3 min. 24 sec. W) beginning June 2003. Three more plots were added (two at WRS and one in Ainalako) by the beginning of 2004. Sites were surveyed at two week intervals from June 2003 through August 2004 and on a monthly basis from September 2004 through May 2005, after 14 months of biweekly surveys. Thirty-four surveys were completed in total.

The host species sampled was restricted to the known host vegetation at each plot except for plot number ten (WRS) which was surveyed with mixed hosts. All host vegetation was examined for the presence of larvae in each plot. Observations of any eggs and pupae at the survey plots were also noted. The duration of each survey was thirty person minutes. Survey sites are listed on Table 1, together with the plant species sampled at each site.

Since *D. pallivitta* has a long larval stage with up to eleven instars (Nagamine, unpublished data), larvae were grouped into three classes based on maturity. Class 1 larvae were grouped together based on their feeding style (window feeding) and consisted of larvae that ranged from first to fourth instar. Class 2 larvae (fifth – seventh instar) were grouped together based on having a different mode of feeding (feeding on the entire leaf) from the larvae in class 1 but are still not mature enough to pupate. Class 3 larvae (eighth – eleventh instar) consisted of mature larvae that were close to pupating. By grouping larvae this way, information about each generation of larvae could be accurately gathered and the cycle of each generation could be monitored clearly.

Light Trapping

Adult *D. pallivitta* populations were monitored by sorting through Hawaii Department of Health (DOH) mosquito light trap samples. Light traps were of the 'New Jersey' design (BioQuip Products Incorporated, Rancho Dominguez, CA) connected to a timer (Model 4001-00 TD-1744-00, Paragon Electric Company Incorporated, Two Rivers, WI, USA or Model 1101 120 VAC 60Hz, Tork, Mount Vernon, NY, USA) set to activate from 6 p.m. through 6 a.m. on a daily basis. Each trap used 40 watt incandescent bulbs and was suspended 1.8 – 2 meters above the ground. The top of each trap was also fitted with a ¼ inch (6.35 mm) wire mesh covering to keep large moth species (e.g. sphingids) from flying into the trap. Contents of traps were sorted weekly. Numbers of adults caught and the location of the trap were noted.

Influence of Weather

Since WRS was the only known location that collected daily weather data in the Panaewa/Keaau area, daily temperature, rainfall, and relative humidity (RH) data from WRS were correlated with counts from all larval surveys and the light trap data to determine if there was a seasonal and/or weather effect on larval and adult populations of *D. pallivitta*. Daily maximum and minimum temperature, rainfall, relative humidity and adult trap data were reported as the daily average over a 2-3 week period corresponding with days in which larval and adult counts were made. Collections of adult *D. pallivitta* from the DOH light trap located at the Hilo International Airport were analyzed using weather data from Hilo International Airport provided by the National Oceanic and Atmospheric Administration (NOAA).

Data Analysis

Darna pallivitta larval and adult counts were correlated with corresponding weather data using Pearson's correlation analysis to determine the effects of weather conditions on *D. pallivitta* populations. All analyses were performed using Minitab statistical software (Minitab, 2004).

4.4. Results

Field Surveys

Field surveys showed that *D. pallivitta* has 3 to 4 generations per year in Hawaii (Fig. 4.1). Class 1 larvae were found in highest densities during the months of May, July, and November. Class 2 larvae were most abundant in the months of August, February and late May/early June. Class 3 larvae were most abundant during the months of September, late February/early March and late May/ early June. *Darna pallivitta* generations appear to be synchronous from Fall through Spring. Generational synchrony can be defined as all individuals of a generation being in the same developmental phase at the same time (Cock, et al. 1987). However, during the late spring and summer months generations tend to overlap, so it is not uncommon to see larvae of different developmental phases in the same area at the same time. The summer months are also the time of year when larval populations tended to be at their highest densities (Fig. 4.1). The months of March, April, and October appear to be the times when the larval populations of *D. pallivitta* are lowest (Fig. 4.1).

There were three outbreaks that occurred during the survey period. Outbreaks were defined as large and sudden increases of larvae observed at any single survey site.

During the survey period, outbreaks occurred during June/August 2003 and May 2005 at plot 1 and during February/March 2004 at plot 10. Outbreaks tended to skew larval abundance graphs by increasing the total number of larvae observed. The outbreaks during July - September 2003, February/March 2004 and May 2005 all show a sudden increase in the amount of larvae observed. During outbreaks, the vast majority of larvae observed during surveys were from a single plot suggesting fairly localized outbreaks.

Presence of eggs was recorded during 12 of the 33 surveys. Eggs were most abundant during late September and October 2003 and 2004 (when eggs were observed at 4 and 3 sites respectively) and July 2004 (5 sites). Periods of egg abundance coincided with increased numbers of adults collected in DOH light traps and lower numbers of Class 2 and 3 larvae observed in the field (Figs. 4.1 & 4.6).

Cocoons were rarely observed during surveys. They were observed during only 5 surveys and at never more than one location per survey. There was only one instance of more than one cocoon being found during a survey (Site 10 on Feb. 13, 2004). *Darna pallivitta* tends to pupate in the leaf litter on the ground or in the leaf axils of their host plant (Fig. 4.2). Most cocoons were observed during larval surveys which focused mainly on the foliar portions of the plant.

Although larval populations tended to increase and decrease about the same time each year, levels of increase and decrease varied widely (Figs. 4.3 – 4.5). All larval classes did not show significant correlations from year 1 to year 2 (class 1: $r = 0.454$; $P = 0.161$) (class 2: $r = 0.528$; $P = 0.095$) (class 3: $r = 0.117$; $P = 0.733$).

Influence of Weather on Larval Populations

All correlations between weather factors (rainfall, maximum temperature, minimum temperature, and relative humidity) and *D. pallivitta* larval observations are shown in Table 4.2. Overall, there was no correlation between the total number of larvae observed and surrounding weather conditions. Multiple regression analysis showed relative humidity explained most of the variation in larval populations but overall, weather had no significant impact on larval populations ($r = 20.8$; $P = 0.136$). However, there was a strong correlation between daily minimum temperature and early instar larvae (instars 1-4) (Table 4.2) which indicates that early instar larvae may prefer cooler temperatures in which to become active.

Adults

During this study period, 3,269 adults were collected by DOH light traps, 3212 (90%) were males, 100 moths (2.84%) were females, and 210 moths (5.96%) were unable to be sexed due to loss of bodyparts. Adult *D. pallivitta* were collected in highest abundances from the months of July through October with increases in adults collected also found in May (Fig. 4.1). The months of December through March yielded very low numbers. August also showed a decrease in the number of adults collected. There was a period from June through November 2004 when there was a great fluctuation in adult numbers with the only prolonged decrease coming in August. Moth numbers at this time appear to be influenced by the overlapping of generations that happens during the summer months. Generation overlap is characterized by the simultaneous presence of all lifecycle stages at the same location. There was a significant positive correlation in overall number of adults collected per week from year to year ($r = 0.438$; $P = 0.001$).

There were 13 sites where DOH light traps collected adults. However, there were only four sites - Hilo Municipal Golf Course (HMGC), University of Hawaii at Hilo, Agricultural Farm Laboratory (UHAFL), Keaau Elementary School (KS), and Hilo International Airport (HA) - where adults were collected with enough frequency to subject collections to statistical analysis. Frequency of adults collected at weekly intervals at these four sites ranged from 37.74% (KS) to 81.13% (HMGC). Adults were not collected for more than four weeks at any of the other nine sites, thus not yielding data that would be amenable to rigorous analysis.

Influence of Weather on Adult Populations

Temperature was more closely correlated with activity than rainfall and relative humidity (Table 4.2). Both maximum and minimum temperatures produced positive correlations with the number of moths collected while rainfall and RH produced small negative correlations. Multiple regression analysis determined that the mean daily high temperature explained most of the variation in the DOH moth collections ($r = 21.3\%$; $P = 0.002$). The effect of relative humidity on moth collections at HA could not be determined since no data on humidity was available for HA. Rainfall and relative humidity showed no significant correlations with moth activity at all four locations.

Daily maximum temperature had the strongest effect on adult activity while the daily minimum temperature had a smaller positive effect on the number of adults collected (Table 4.2). These findings indicate that moth activity increases with an increase in temperature.

4.5. Discussion

Few significant correlations could be made between larval observations or adult light trap collections. *Darna pallivitta* populations were synchronous for most of the year meaning most individuals of a population are at the same developmental stage at the same time (Auslander, et al. 1974) - except during late spring and summer when populations overlap (Fig.4.1). This overlapping of generations was most likely caused by increased maximum and minimum temperatures coupled with lower levels of rainfall. Lower rainfall is thought to be correlated with increases in other limacodid populations (Syed and Shah, 1976, Igbinosa, 1985, and Young, 1971). Though larvae were more numerous during the summer, they were not necessarily in an outbreak stage where hundreds of larvae may be present in a specific area or location causing potential economic damage.

Reasons for the three outbreaks that occurred during the survey period are unclear. Seasonality does not appear to be a factor since the largest outbreak occurred during February – March 2004 – a time of year when larval populations are usually low. However reports of large numbers of adults collected from nearby light traps during the previous October undoubtedly helped introduce large numbers of progeny to the area. Outbreaks of *D. pallivitta* may indeed be confined to small isolated areas (Cock, et al. 1987). This was evidenced from all outbreaks found during this study in which high larval densities were observed at only one plot while surveys at other plots showed much lower numbers of larvae present though populations at all sites followed the same trend of generational synchrony. DOH light traps also did not show a large increase of adults collected in other locations during these isolated outbreaks.

Outbreaks are also typified by generational synchrony in which almost all members of that generation are in the same developmental stage at the same time (Cock, et al. 1987). In the field, it was also common to see large middle (6th -7th instar) and late instar larvae together.

The overall data suggest that only temperature had a significant impact on the number of larvae and adults observed in this study. Only early instar larvae were affected by minimum daily temperatures while both minimum and maximum daily temperatures positively affected adult activity.

Elkinton and Liebhold (1990) suggest: "Weather affects all of the dynamical interactions of forest insect populations, although the mechanisms are often obscure and complex." The same is probably true for *D. pallivitta*. Weather almost certainly plays a role its population dynamics (Padhi and Saha, 2004) but the mechanism is unclear. It is most likely the compounded effects of multiple factors that influence the abiotic impact on *D. pallivitta* populations. High rainfall, temperature and/or high humidity may create suitable conditions for infections by pathogens and temperature and humidity may affect moth activity. We were unable to determine the cause of larval *D. pallivitta* outbreaks in Hawaii. Cock, et al (1987) and Igbinosa (1985) cite prolonged periods of low rainfall or drought and insecticide applications as possible causes of outbreaks in limacodid larval populations. Low rainfall may contribute to an outbreak by decreasing risk of disease. Rain may also displace young larvae from their host plants, dropping them where they may be unable to feed or in puddles where they may drown. Insecticide applications may decrease populations of natural enemies. With the exception of short instances of low rainfall 2-3 months prior to each *D. pallivitta* outbreak, there has been no prolonged period with low rainfall prior to any outbreak (Fig. 4.7). There were also no pesticides sprayed at any of the sites where outbreaks occurred until after the outbreak had subsided (only at Site #10).

Darna pallivitta adults also followed a synchronous generational pattern with moth catches usually increasing just after an increase in Class 2 and 3 larval observations. Although adults do not favor a certain temperature to initiate activity it is interesting to note that adults may be negatively affected by the daily average relative humidity in Hawaii. Many studies have found increase in relative humidity plays a positive role in the activity of many moth species (Ismail, et al. 1992, Geethalakshmi, et al. 2004). It is possible that the Hilo area of the Big Island where *D. pallivitta* is established may be too moist for *D. pallivitta*'s preference for most of the year.

The finding of consistent generational synchrony (discrete generation mode) throughout most of the year should be helpful when developing and implementing control measures which would also decrease *D. pallivitta* numbers during the summer months when populations are usually at their highest. Being able to accurately predict when a new generation will occur will be useful in planning when to implement chemical control measures which could severely impact the population of both the new and future generations. Our findings may also imply that multiple insecticide applications may have to be used during the summer months when multiple generations of *D. pallivitta* may exist at the same time.

4.6. Acknowledgements

I would like to thank Dr. Daniel Rubinoff, and Dr. Mark Wright of the University of Hawaii, Stacey Chun, Ruth Niino-DuPone, and Christopher Jacobsen of the Beaumont Research Center in Hilo and Pat Conant and Clyde Hirayama of the Hawaii State Department of Agriculture – Hilo for all of their assistance and advice on this project.

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Table 4.1. Location of larval survey sites and known host plant species surveyed.

Plot #	Location	Vegetation Surveyed
1	H. Eunice Nursery	*Phoenix roebelenii*
2	H. Eunice Nursery	*Dypsis lutescens*
3	H. Eunice Nursery	*Paspalum conjugatum*
4	Waiakea Experiment Station	*Desmodium uncinatum*
5	Waiakea Experiment Station	*Commelina diffusa*
6	Waiakea Experiment Station	*Cordyline fruticosa*
7	Panaewa Zoo	*Dypsis lutescens*
8	Panaewa Zoo	*Cordyline fruticosa*
9	Ainalakou	*Panicum repens*
10	Waiakea Experiment Station	*Commelina diffusa, Desmodium uncinatum Wedalia trilobata, Paspalum conjugatum*

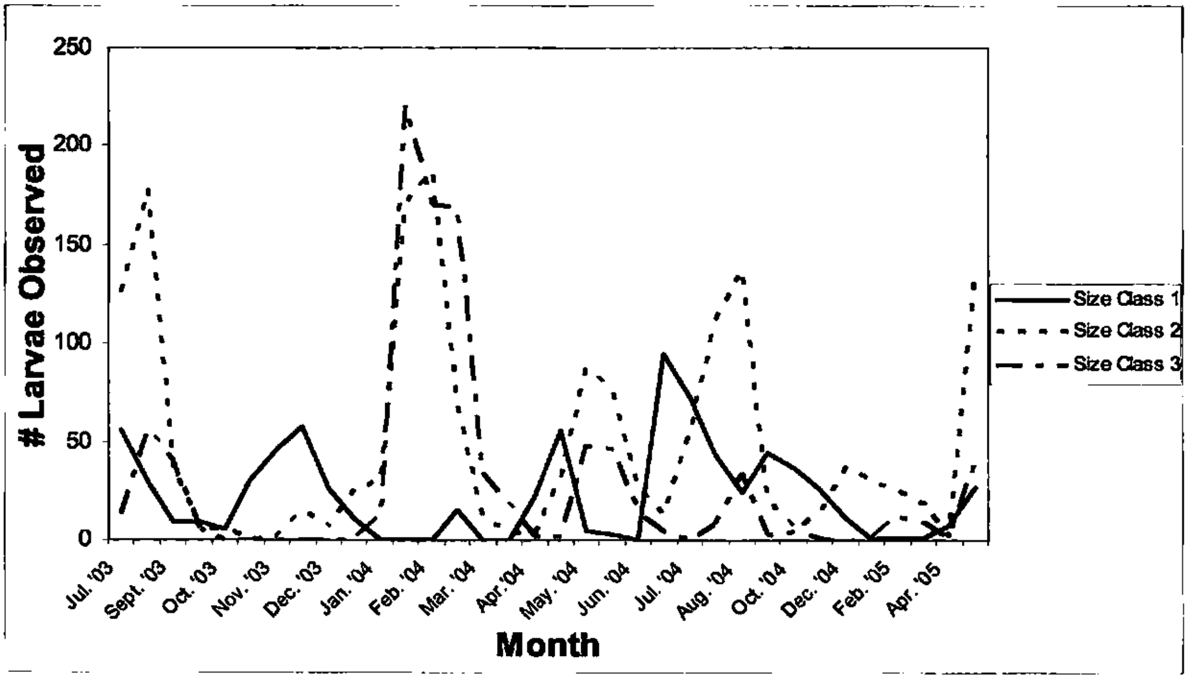

Fig.4.1. Line graph showing total number of larvae observed at 10 survey sites from July 2003 – May 2005. Note: high larval numbers during Feb.-Mar. 2004 are due to a large outbreak at plot #10 (WRS).

Fig. 4.2. Observed pupation sites for *Darna pallivitta* – Leaf litter (A) and between palm axils (B).

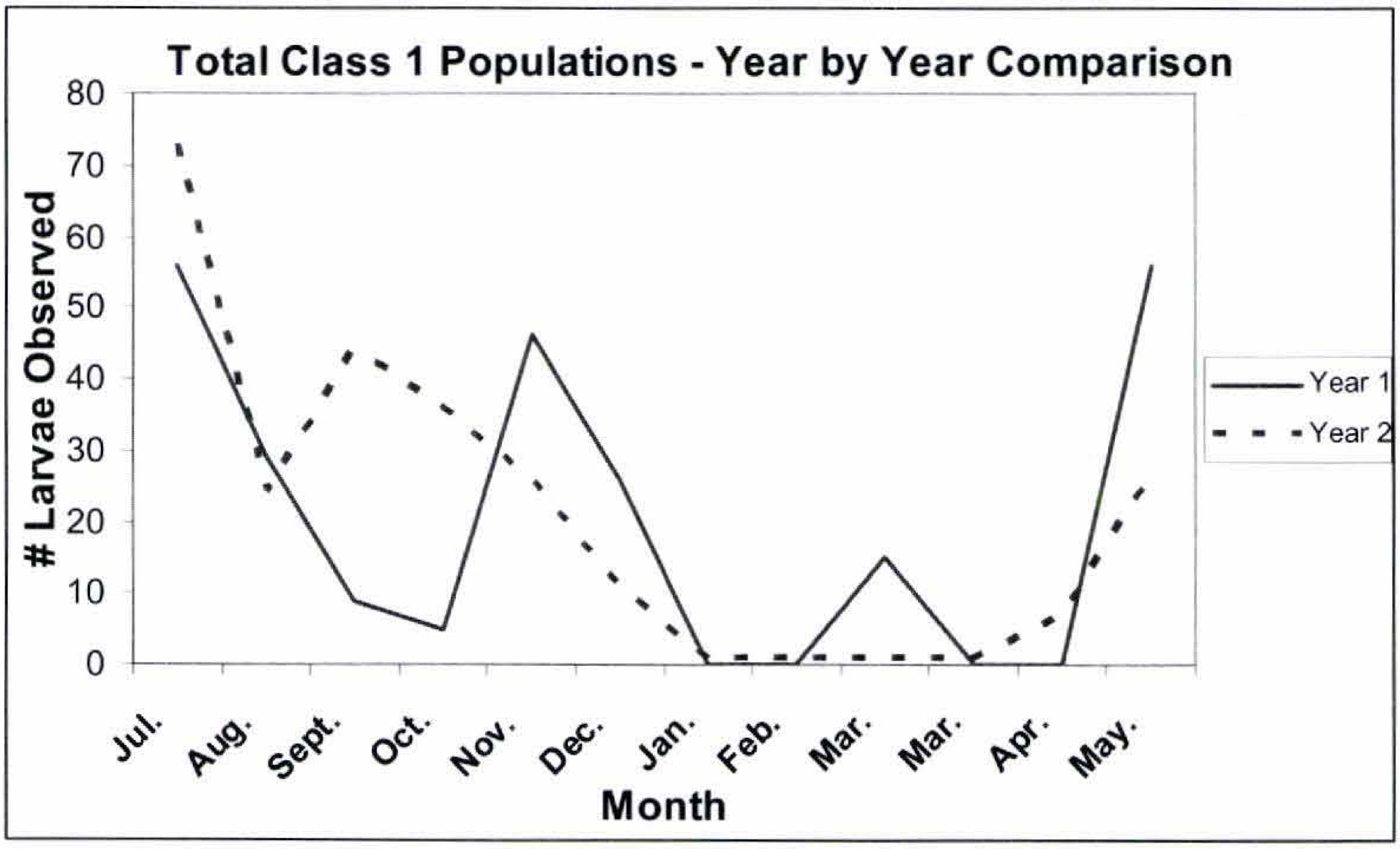

Fig. 4.3. Year by year comparison of Class 1 larval populations.

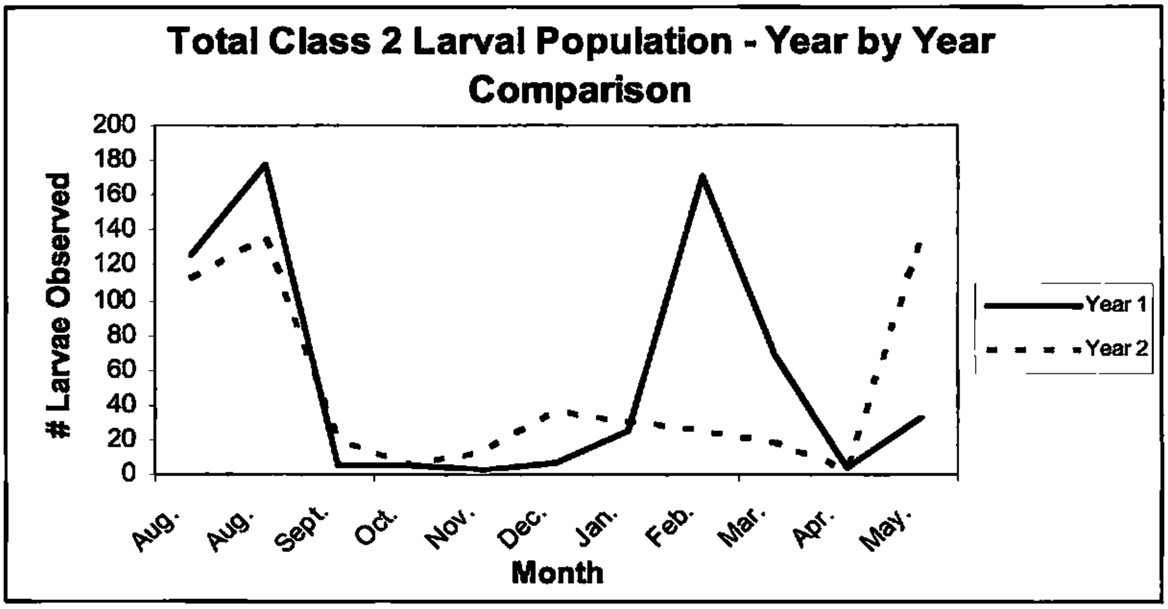

Fig. 4.4 Year by year Comparison of Class 2 larval populations.

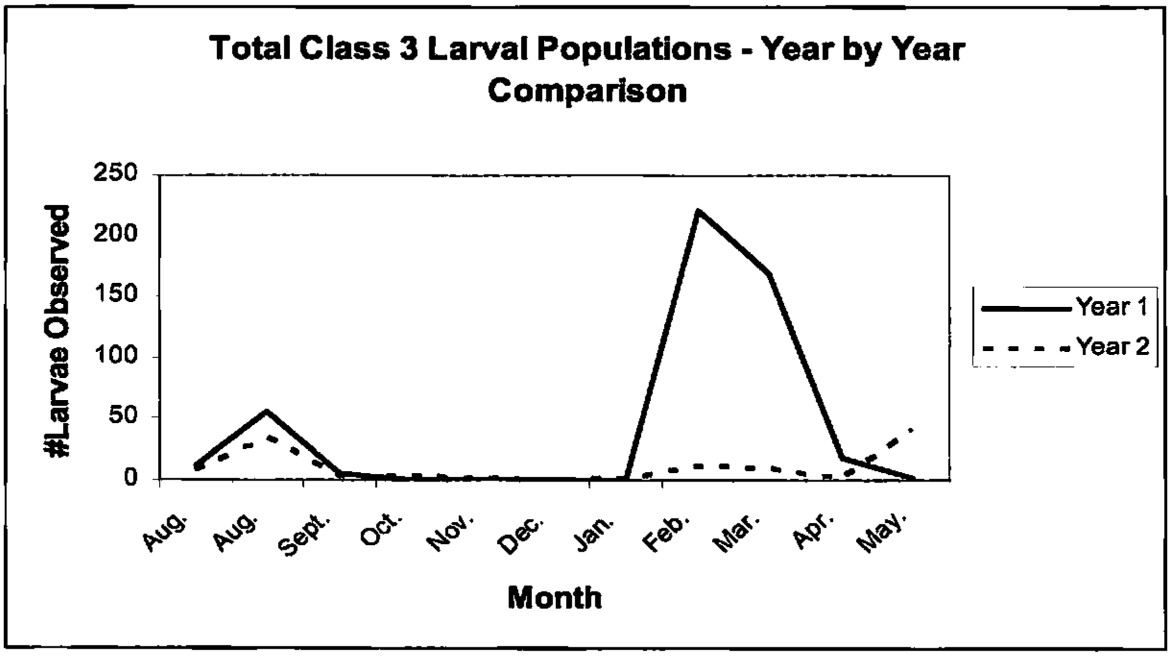

Fig. 4.5. Year by year Comparison of Class 3 larval populations.

Table 4.2. Waiakea Research Station weather data correlated with *D. pallivitta* larval surveys and DOH moth collections. Hilo Airport moth collection data is correlated with NOAA weather data collected on location.

Environmental Factors	Correlation Coefficient	P Values
Total Larvae Observed		
Rainfall	-0.075	0.675
Max. Temp.	-0.014	0.938
Min. Temp.	0.147	0.407
% RH	-0.26	0.137
Class 1 Larvae Observed		
Rainfall	-0.129	0.468
Max. Temp.	0.264	0.131
Min. Temp.	0.628	0.021
% RH	0.056	0.752
Class 2 Larvae Observed		
Rainfall	-0.158	0.373
Max. Temp.	0.052	0.769
Min. Temp.	0.224	0.204
% RH	-0.26	0.138
Class 3 Larvae Observed		
Rainfall	0.089	0.617
Max. Temp.	-0.203	0.25
Min. Temp.	-0.147	0.406
% RH	-0.232	0.187

Table 4.2. (Continued) Waiakea Research Station weather data correlated with *D. pallivitta* larval surveys and DOH moth collections. Hilo Airport moth collection data is correlated with NOAA weather data collected on location.

Environmental Factors	**Correlation Coefficient**	**P Values**
DOH Adult Collections		
Rainfall		
Hilo Muni. Golf Course	-0.257	0.074
U.H.H Ag. Farm	-0.289	0.044
Hilo Airport	-0.128	0.379
Keaau School	-0.148	0.31
Maximum Temp. (C°)		
Hilo Muni. Golf Course	0.475	0.001
U.H.H Ag. Farm	0.475	0.001
Hilo Airport	0.112	0.442
Keaau School	0.423	0.002
Minimum Temp. (C°)		
Hilo Muni. Golf Course	0.397	0.005
U.H.H Ag. Farm	0.406	0.004
Hilo Airport	0.044	0.765
Keaau School	0.267	0.064
RH (%)		
Hilo Muni. Golf Course	-0.082	0.577
U.H.H Ag. Farm	-0.124	0.394
Keaau School	-0.047	0.751

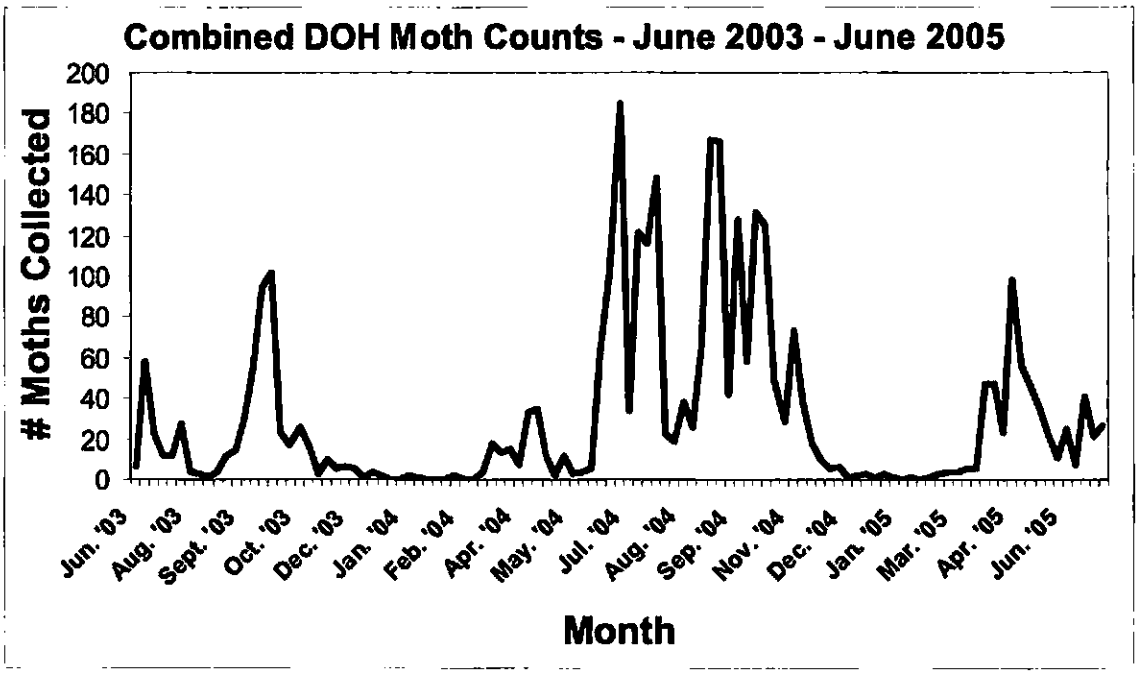

Fig. 4.6. *D. pallivitta* adults collected from DOH light traps from June 2003 – June 2005. Numbers are based on total number of adults collected at all light trap stations per week.

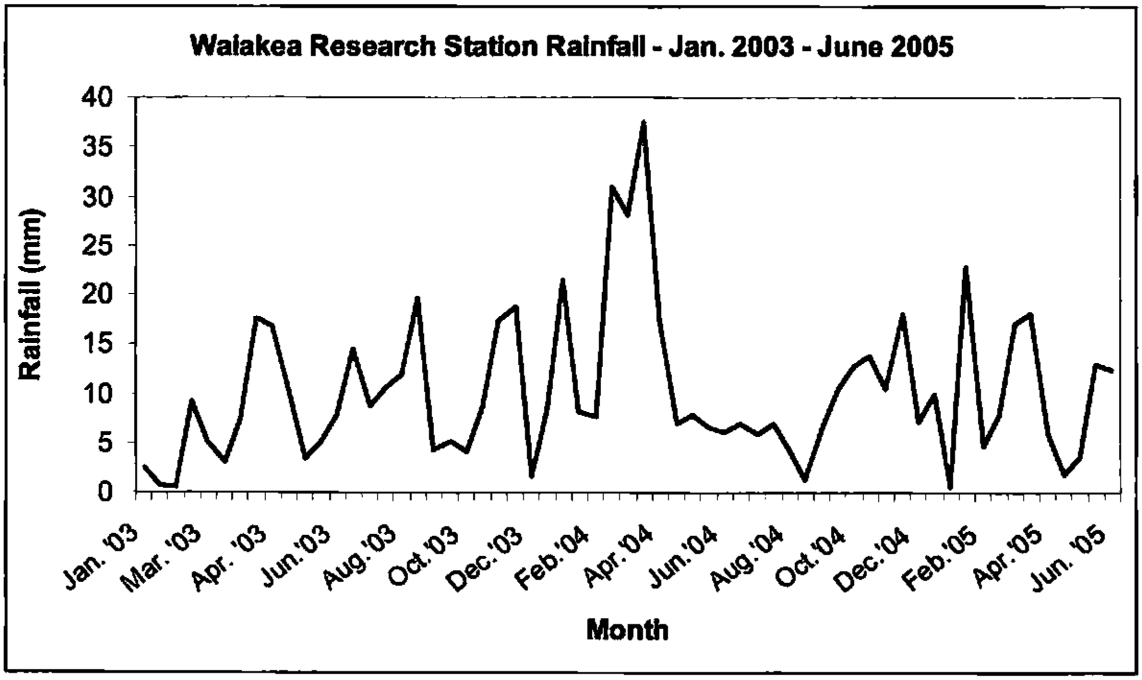

Fig. 4.7. Waiakea Research Station rainfall data. Data based on two to three week average daily rainfall totals.

CHAPTER 5

Occurrence of Natural Enemies of *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae) In Hawaii

5.1. Abstract

The occurrence of natural enemies of an invasive stinging lepidopteran, *Darna pallivitta* (Moore) were observed in Panaewa and its surrounding areas on the island of Hawaii. Laboratory reared *D. pallivitta* eggs on host plants were placed in the field to observe for egg parasitoids during September 2003 through December 2004. Larval field collections were also conducted from September 2004 until May 2005. Sentinel egg surveys revealed the parasitoid *Trichogramma papilionis* attacking the eggs of *D. pallivitta*. Parasitism rates were as high as 100% though parasitism by *T. papilionis* was only recorded during a two month period from September to November 2003. A virus found infecting larvae in the field was identified as a cytoplasmic polyhedrosis virus (CPV) and virulence was confirmed by Koch's Postulates. CPV appears to be endemic in a number of areas and displayed density dependent properties of infection, but did not always successfully control populations of *D. pallivitta*. We conclude that at this time, the state of Hawaii does not have any naturally occurring biocontrol agents for *D. pallivitta* that will suppress populations to an acceptable level.

5.2. Introduction

Biological control is the use of parasitoid, predator, pathogen, antagonist, or competitor populations to suppress a pest population, making the pest less abundant and thus less damaging than it would be otherwise (Van Driesche and Bellows, 1996).

Biological control agents play an important role in the suppression of insect pests. Past reliance on pesticides has led to environmental contamination, risks to human health, and failure to control the pests themselves - due to pesticide suppression of natural enemies and resistance of the pest to chemical controls - thereby making biocontrol an attractive alternative (Van Driesche and Bellows, 1996).

Fortuitous biocontrol describes the accidental immigration of an introduced pest's natural enemy to the new area where the pest has become established, or the control of an exotic pest by an indigenous biocontrol agent (DeBach 1974).

Darna pallivitta Moore is a relatively new invasive insect to the island of Hawaii (Conant, et al. 2002) that has rapidly become a polyphagous agricultural pest with stinging spines that cause health concerns. Fortuitous biocontrol may contribute to suppressing *D. pallivitta* populations which can reach outbreak proportions.

Fortuitous biocontrol has already occurred in the State of Hawaii on such insects as *Phyllocnistis citrella* (Nagamine and Heu, 2003), *Aleurodicus dugesii* (Heu, et al. 2004), and *Homalodisca coagulata* (Heu, et al. 2004).

Although the Hawaii State Department of Agriculture (HDOA) is currently conducting a classical biocontrol program for *D. pallivitta*, the release of any natural enemies is years away. Therefore, the purpose of this research is to discover any natural enemies already fortuitously attacking *D. pallivitta* on the island of Hawaii and if found, to determine whether or not they provide successful suppression of *D. pallivitta* populations.

5.3. Materials and Methods

Sentinel Egg Surveys

Sentinel egg surveys are designed to detect egg parasitism in the field by exposing lab reared eggs to parasitoids in the field for set periods of time, after which, the eggs are returned to the lab and any parasitoids are reared. In collaboration with the the Hawaii State Department of Agriculture – Hilo (HDOA - Hilo), 70 sentinel egg surveys were carried out from September 2003 until December 2004. Surveys were completed in and around the Panaewa area (119° 39 min. 13 sec. N, 155° 3 min. 19 sec. W) located approximately 5 miles (8 km) outside of Hilo town on the island of Hawaii. Since *D. pallivitta* oviposits its eggs either singly (Cock, et al. 1986) or in small clusters (up to 8-10 eggs) (Hara, et al., personal observations), all eggs were counted individually (Table 5.2). Four to six week old sorghum (*Sorghum bicolor*) seedlings in a six inch (15.24 cm) diameter pot were placed into a cage with mated female *D. pallivitta* adults. Females were allowed to oviposit on the seedlings overnight. The seedlings, with varying egg densities (Table 5.2), were removed the next morning and placed in the field at one of four locations in Panaewa where *D. pallivitta* infestations were known to occur – H. Eunice Nursery (HEN) (19° 38 min. 55 sec. N, 155° 3min. 19 sec. W / elev. 333 feet (101.5 m)), Hawaii Nurseries Incorporated (HNI) (19° 38 min. 8 sec. N, 155° 3 min. 27 sec. W / elev. 339 feet (103.3 m)), Waiakea Research Station (WRS) (19° 38 min. 38 sec. N, 155° 4 min. 47 sec. W / elev. 604 feet (157 m)) and at a private residence (Mr. Taka) in Ainalako (19° 39 min. 44 sec. N, 155° 5min. 33 sec. W / elev. 525 feet (160 m)). HEN is approximately 1.5 miles (2.4 km) from WRS and the private residence was approximately 1.75 miles (2.8 km) from WRS. During placement, the potted seedlings were put in a 12 in. (30.48 cm) diameter saucer which was half filled with water to provide the seedlings with water and serve as a deterrent against ants colonizing the potted seedlings and preying on the *D. pallivitta* eggs. Exclusion of ants was necessary as they were a potential mortality factor for *D. pallivitta* eggs in the field. Ants may attack eggs that have been parasitized making it impossible to determine if other mortality factors on *D. pallivitta* eggs currently exist in Hawaii. Sentinel egg placements were left in the field for one to four days before being brought back to the HDOA insectary for observation. Eggs from the field were kept under ambient conditions 72°-92°F (22.2°-33.3°C) with 75% RH at HDOA with ambient light supplemented by overhead fluorescent light from 0700h through 1600h each day. Any predators or parasitoids found attacking the eggs were recorded along with the number of eggs that were attacked.

Larval Field Collections

Between 1 – 35 larvae were field collected on a monthly basis from 13 locations from September 2004 until June 2005 (Table 5.1) in the Hilo to Keaau area where *D. pallivitta* is most prevalent. The distance between these two locations was approximately 13.5 kilometers (8.5 miles). Field-collected larvae were reared in individual disposable pint sized plastic containers on a general purpose artificial diet (Bio-Serv Inc. #F9772 Frenchtown, NJ) combined with crushed dried leaves of honohono grass (*Commelina diffusa*), a known host plant on which *D. pallivitta* larvae were commonly observed feeding on in the field. The possibility of insect pathogens from honohono grass contaminating the artificial diet was eliminated by boiling the water agar-based media. Once in the laboratory, larvae were kept under conditions of 71.8° ± 0.4°F (22.1° ± 0.4°C)

70% RH under 24h light conditions. Only 4th – 11th instar larvae were field collected since prior experimentation determined that artificial diets were not an optimal food source for limacodid larvae (M. Epstein, personal communication and C. Kishimoto, personal observation). Early instar *D. pallivitta* fed but did not develop on artificial diets; therefore middle through late instar larvae had the best chance to mature and emerge. Larvae were reared for two months and observed on a weekly basis to determine any mortality factors. Data taken on field-collected larvae were adult emergence, mortality at pupation, mortality due to failure of larvae to develop, and mortality due to parasitism or pathogen. Pupae were held for one month since eclosion normally takes place in less than one month (Nagamine and Epstein, unpublished data). Cocoons with no eclosion after one month were dissected to qualitatively determine death of the pupa. Mortality due to parasitism or pathogen infection was observed through the plastic container after which the container would be frozen and cause of larval mortality investigated. All larval mortality that showed no noticeable symptom of parasitism or pathogen infection, or larvae that had not pupated after two months post-collection were noted as "failing to develop "since after a two month period, any surviving larva did not exhibit any significant growth. This was attributed to inadequate nutrition for *D. pallivitta* larvae in the diet.

Koch's Postulates

Confirmation of CPV as the cause of mortality was achieved using Koch's Postulates. In collaboration with H. Kaya (UC Davis), field-collected diseased larvae from WRS were shipped to UC Davis where viral polyhedra were extracted by macerating larvae with a mortar and pestle. The larval tissue was then diluted with 60 ml purified water before filtering through cheese cloth and cotton. The titre of the resulting suspension was 3.5 x 10^7 polyhedral occlusion bodies / ml. The suspension was shipped back to Hilo, Hawaii where it was stored under refrigeration until use at 37.2° ± 3.9°F (2.9 ± 0.7°C).

All test larvae were obtained from the quarantine facility of HDOA on Oahu. Larvae were reared from eggs that were sterilized in a 0.5% sodium hypochlorate solution for 15 min. and rinsed three times with tap water to neutralize any potential CPV that could be transmitted transovum. The viral suspension was brushed onto 10.1 cm x 7.6 cm (4in. x 3in.) ti leaf (*Cordyline fruticosa*) cuttings and air dried them before placing them into a 32 oz. clear plastic container (# PK32T Fabri-Kal, Kalamazoo, MI) and introducing five 4th-6th instar *D. pallivitta* larvae to feed. Distilled water was used as a control and was applied to ti cuttings in the same manner as the viral suspension. Larvae were allowed to feed for a period of 10-14 days at 71.8° ± 0.4°F (22.1° ± 0.4°C) 70% RH under 24h light conditions. Dead and moribund larvae were collected and stored in 4 dram (13.79 ml) glass vials with screw on caps: One vial per treatment replicate and frozen at 2.7° ± 2.1° F (-16.3° ± 0.3°C) before being shipped to Dr. Kaya (UC Davis - http://ucdnema.ucdavis.edu/imagemap/nemmap/Kaya.htm) for confirmation of CPV infection. Four replications of Koch's Postulates were run with replicate consisting of five replicated controls and five CPV treated leaf cuttings giving a total of 100 treatment larvae and 100 control larvae.

5.4. Results

Sentinel Egg Surveys

Seven of the 70 sentinel *D. pallivitta* egg surveys (10%) conducted were parasitized (Table 5.2). The parasitoid was identified by HDOA Entomologist Patrick Conant and confirmed by HDOA Insect Taxonomist Bernarr Kumashiro as *Trichogramma papilionis* Nagarkatti (Hymenoptera: Trichogrammatidae). Sentinel egg surveys found *T. papilionis* in three locations –HNI, WRS, and HEN; all located in Panaewa. The two nurseries are approximately 500m apart and WRS is approximately 1.5 miles (2.4 km) from HEN. Six of the seven surveys that recovered *T. papilionis* were from HEN. The number of eggs per survey that were parasitized ranged from 1 egg to 162 eggs. Parasitism rates reached 100% (n = 67 eggs) in early November 2003 but parasitism of *D. pallivitta* eggs by *T. papilionis* continued for only a two month period (early September 2003 – early November 2003). A sentinel survey conducted in early February 2004 found one egg parasitized at HEN but following surveys discovered no further parasitism.

Parasitized eggs turned black within 2-3 days after being held at the HDOA's insectary in Hilo. Up to 6 adults would emerge from each egg within 10-12 days. The ratio of males to females emerging from each *D. pallivitta* egg averaged 3.31 : 1 but ranged from 2.78 : 1 to 5.27 : 1.

Larval Field Collections

During this experiment, a total of 213 larvae were collected, of which 160 larvae were middle instars and 53 larvae were late instar. Seventy-four larvae (34.74%) successfully pupated and emerged as adults, 31 larvae (14.55%) died during pupation, 62 larvae (29.11%) did not complete development (did not mature to pupation) and 46 larvae

(21.60%) died of a viral disease. The unknown viral disease was identified by Insect Pathologist Dr. Harry Kaya of the University of California Davis as a cytoplasmic polyhedrosis virus (CPV) in the family Reoviridae. All 46 field-collected larvae that became diseased were infected with CPV. Larvae that later became diseased were collected from nine of the thirteen locations (Fig. 5.1). CPV-infected larvae became sessile and stopped feeding a few days prior to death. Shortly after death, the cuticle of the larva turned dark brown to black in color (Fig. 5.3), becoming extremely brittle, and was easily ruptured, thus spreading the pathogen on the plant where it could infect other feeding larvae. Twenty-three of the 46 larvae that became infected with CPV were collected from the United States Department of Agriculture's Agricultural Research Service (USDA ARS) facility located at WRS. A total of 33 (71.74%) larvae that became infected with CPV were collected from various locations at WRS. CPV appeared to be spread easily to new sites. At one of the newest infestation sites which was reported during the summer of 2005 located in Hawaiian Paradise Park (approximately 15 miles (24.14 km) southeast of Hilo), a diseased larva has already been observed in the field.

Middle instar larvae became symptomatic before late instar larvae both in the field and in laboratory colonies indicating middle instar larvae may be more susceptible to the disease. In the laboratory, as the CPV outbreak progressed into the next generation of *D. pallivitta*, early instar larvae became infected as well.

With the exception of one unidentified tachinid species attacking a single *D. pallivitta* larva, no larval parasitism was observed during field collections.

Koch's Postulates

Koch's Postulates (Table 5.3) confirmed CPV was the cause of mortality for diseased larvae collected in the field. Of the controls 93 (93%) larvae tested negative for CPV infection while 2 larvae (2%) became infected with CPV and 5 larvae were unaccounted for. In the CPV treatments 84 larvae (84%) became infected with the virus, 3 larvae were not infected, and 7 larvae (7%) were unaccounted for. Positive infection of 6 CPV-infected larvae (6%) was undetermined since the virus could not be reisolated from them.

5.5. Discussion

Sentinel surveys revealed only one species, *T. papilionis* parasitizing the eggs of *D. pallivitta*. *Trichogramma papilionis* is originally from Asia (described from Japan) (Nagarkatti, 1974 and Oatman, et al. 1982). The finding of *T. papilionis* parasitizing *D. pallivitta* eggs is only the third record worldwide of *Trichogramma* spp. attacking limacodid eggs (Wei, 1985 and Hoong and Hoh, 1992) and represents the first identified species of *Trichogramma* to parasitize limacodid eggs. *Trichogramma* tend to be polyphagous (Hirose, et al, 1976 and Cock, et. al, 1987) and numerous species of *Trichogramma* exist in close proximity with many limacodid species. Cock, et al. (1987) state that *Trichogramma* have never been recorded from limacodid eggs. Although Cock, et al. (1987) theorize that the flattened shape of limacodid eggs might be a reason for lack of parasitism, observed rates of parasitism of up to 100% (Table 5.2) in this survey show that egg shape does not prevent oviposition by *T. papilionis*. However, our results suggest *D. pallivitta* does not appear to be a preferred host of *T. papilionis*. Possibly, an increase in population of another lepidopteran species which was a preferred host caused an increase of *T. papilionis* populations. This increase in *T. papilionis* populations allowed parasitization of *D. pallivitta*, normally not a host of *T. papilionis*. Prior to its discovery parasitizing *D. pallivitta* eggs, in the State of Hawaii, *T. papilionis* was only known to occur on the Island of Oahu (Nishida, 2002).

Although day to day observations could not be made after the discovery of the initial CPV outbreak at the USDA, ARS complex and the nearby WRS dumpsite location (in which 30 diseased larvae were observed during the initial discovery), a follow up survey two weeks later suggested that the virus had caused substantial mortality to the *D. pallivitta* populations at those locations with 40% (n = 15) of the larvae observed at the WRS dumpsite having succumbed to the virus. Though CPV was only positively identified during summer 2004, observations of larvae that had the same type of symptoms had been made in the field since larval surveys began in July 2003. Field-collected larvae that became CPV symptomatic while under observation were collected at various sites from Hilo to Keaau (Table 5.1). Although larvae collected from nine sites later became infected with CPV, this could also be the result of possible contamination during the transfer, observation, or feeding of the larvae. Larvae were sometimes held for almost two months before showing signs of infection making contamination while in the lab a high probability. However, many low level CPV infections may only become pathogenic to an infected larva if it has become stressed (H. Kaya, personal communication). Low concentrations of CPV may also require long incubation times to increase adequately for larvae to become symptomatic. The artificial diets used in this experiment may have been a stress factor that allowed CPV to increase to lethal levels.

Conventional artificial diets may lack one or several nutrients which could have affected field-collected larvae adversely, thereby allowing the CPV levels to increase in the larvae. If CPV is endemic (constantly present to a greater or lesser extent in a particular locality) in many areas, it is unclear as to how its presence there came about. Possibilities include infected larvae being spread via the movement of nursery plants, *D. pallivitta* larvae coming into contact with CPV-infected larvae of another species, infected moths moving into the area and disseminating the virus transovum (Sikorowski, et al. 1973 and Tanada and Kaya, 1993) though Fuxa, et al. 1999 state CPV is also transmitted transovarially

In addition to parasitism by *T. papilionis* and CPV infection, one unidentified tachinid larva was discovered parasitizing a larva of *D. pallivitta* by Dr. Harry Kaya while dissecting the *D. pallivitta* larva for CPV identification. There have been no further observations of larval parasitism by tachinids since then.

In conclusion, *D. pallivitta* currently has no natural enemies in Hawaii which effectively suppress current population levels. It is unlikely that *T. papilionis* has a preference for *D. pallivitta* eggs, although findings of high rates of parasitism may suggest that inundative releases of lab-reared *T. papilionis* could prove successful. CPV may have the most potential as a biocontrol agent for *D. pallivitta* in Hawaii because it is found in many areas. However, its susceptibility to UV from sunlight makes it harder to persist on the surface of vegetation for extended periods of time. Since the virulence of CPV may not be high, feeding damage to crops would continue, which remains a problem for Hawaii's foliage industry. However, applications of the virus onto areas with high populations of *D. pallivitta* should offer good control in the short term and the debilitating effects of the virus could help with control in the long term. Until a successful biological control agent is found, chemical controls or the biological insecticide, *Bacillus thuringiensis* (Chun, et al. 2005) may prove to be the most effective short term control for *D. pallivitta*.

5.6. Acknowledgements

The Hawaii State Department of Agriculture is greatly appreciated for their help and collaborative efforts. We also thank Bernarr Kumashiro for the *Trichogramma papilionis* identification, Dr. Harry Kaya for the CPV identification and Dr. Xingeng Wang for literature translations.

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Table. 5.1. Location of all larval collections with site, number of larvae collected, and observations.

Site Collected	# Larvae Collected	Adults Emerging	# Starved	# Died Pupating	# Diseased
Mahiai St.	13	3	3	6	1
Manuia Rd.	10	1	7	2	0
Manuia Rd. (Private Residence)	3	1	2	0	0
H. Eunice Nursery	15	6	1	4	4
Ainalako Rd.	1	0	0	0	1
Panaewa Zoo (Outside zoo)	2	0	0	1	1
Panaewa Zoo (Inside zoo)	27	19	2	3	2
Kealakai St. (Panaewa)	1	0	1	0	0
USDA Fruitfly Facility	86	29	30	5	23
Waiakea Res. Station Shadehouses	1	0	1	0	0
USDA Germplasm Building	13	2	6	3	2
Waiakea Res. Center Dumpsite	18	4	6	0	8
THP (Keaau)	23	9	3	7	4

Table 5.2. Sentinel egg surveys positive for parasitism by *T. papilionis* with number of eggs parasitized and percent parasitism.

Date	Location of Placement	# Eggs Used	# Eggs Parasitized	% Eggs Parasitized
9/4/2003	H. Eunice Nursery	75	30	40
11/4/2003	H. Eunice Nursery	135	95	70
11/5/2003	H. Eunice Nursery	67	67	100
11/6/2003	Hawaii Nurseries Inc.	54	4	7.4
11/7/2003	H. Eunice Nursery	181	162	89.5
11/10/2003	Waiakea Research Station	116	2	1.72
2/2/2004	H. Eunice Nursery	160	1	0.63

Table 5.3. Results of Koch's Postulates conducted with CPV isolated from diseased field-collected *D. pallivitta* larvae.

Date: Nov. 2004

Control 1		**Treatment 1**	
CPV-Positive	CPV-Negative	CPV-Infected	CPV-Negative
0	23	24	0

Date: Dec. 2004

Control 2		**Treatment 2**	
CPV-Positive	CPV-Negative	CPV-Infected	CPV-Negative
0	23	22	0

Date: Jan. 2005

Control 3		**Treatment 3**	
CPV-Positive	CPV-Negative	CPV-Infected	CPV-Negative
2	21	21	2

Date: Feb. 2005

Control 4		**Treatment 4**	
CPV-Positive	CPV-Negative	CPV-Infected	CPV-Negative
0	25	17	2

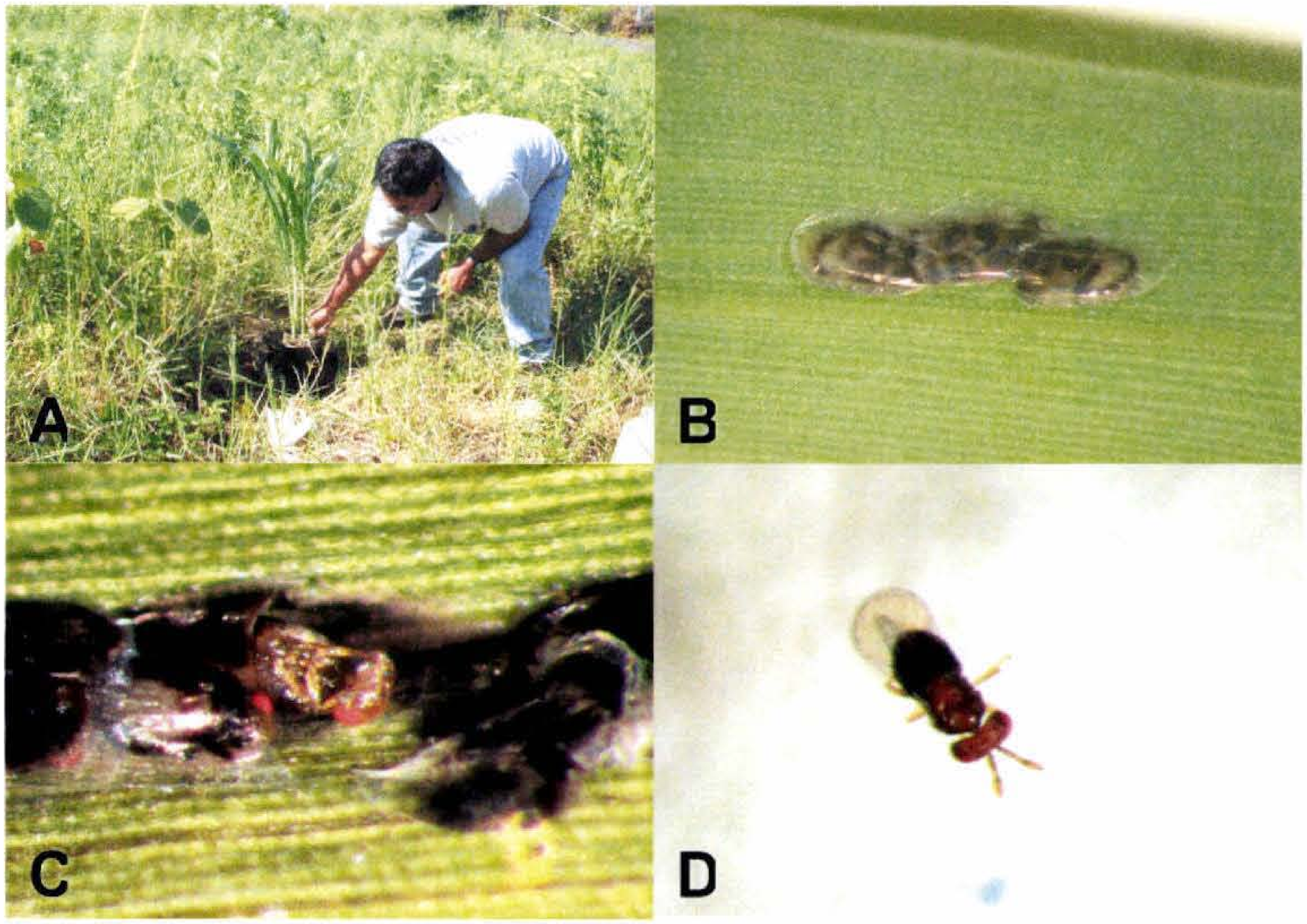

Fig. 5.2. Photos showing sentinel egg survey placement (A), parasitized *D. pallivitta* eggs (B), emergence of *T. papilionis* (C), and adult *T. papilionis* (D).

Fig. 5.3. Photograph showing a healthy *D. pallivitta* larva (left) and CPV-infected larvae (middle and right). Note the larva on the right that has ruptured dispersing the virus.