NON-TARGET IMPACTS OF INTRODUCED PARASITOIDS AND VALIDATION OF PROBABILITISC RISK ASSESSMENT FOR BIOLOGICAL CONTROL INTRODUCTIONS

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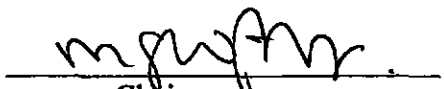

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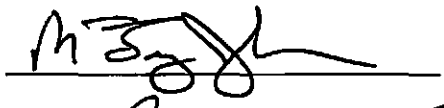

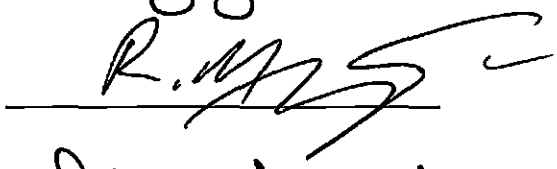

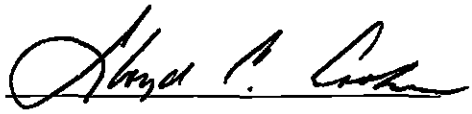

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ABSTRACT

This dissertation addressed non-target parasitism of the endemic Hawaiian moth *Udea stellata* (Butler) and validated a probabilistic risk assessment approach for biological control introductions. *Udea stellata* is distributed across a wide gradient of environmental conditions, which allowed an assessment of non-target effects under a range of ecological conditions. Seven parasitoid species were associated with *U. stellata* larvae. *Trathala flavoorbitalis*, *Casinaria infesta* and *Triclistus* nr. *aitkeni* are of adventive origin; *Cotesia marginiventris* and *Meteorus laphygmae* were purposely introduced to Hawaii; and *Diadegma blackburni* and *Pristomerus hawaiiensis* are of unknown origin.

Field surveys and partial life table studies were conducted to assess apparent mortality and marginal attack rate, respectively. Field surveys of larvae were conducted at eight different sites throughout the Hawaiian Islands, parasitism rates by individual parasitoid species varied significantly among study sites of varying ecological conditions. Adventive parasitoids rather than purposely introduced ones were responsible for the majority of *U. stellata* apparent mortality. Results from the life table studies showed that predation) was the major larval mortality factor at all study sites and that parasitism contributed minimally to total mortality. The two purposely introduced parasitoids were present at high altitude, in relatively undisturbed sites.

Multivariate analyses were used to detect patterns in species assemblage among sites. *Udea stellata* density, elevation, and level of habitat disturbance significantly explained variability in the parasitoid assemblage among sites. Most species increased in

abundance with higher densities of *U. stellata* and some were restricted to less disturbed sites.

By comparing the use of single point estimates versus probability distributions in quantitative risk assessment modelling, it was demonstrated that the use of point estimates can hide important variability and significantly impact the estimates of risk. It was also demonstrated that, at least in this study system, the use of apparent mortality significantly increased the estimate of risk compared to the use of marginal attack rate.

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GENERAL INTRODUCTION AND DISSERTATION STRUCTURE

Invasive species and control methods

The rate of non-indigenous species introductions, intentional and unintentional, around the globe has increased drastically with the increase of international trade (Keane and Crawley 2002). Some introduced species become invasive and cause direct and indirect effects on organisms living in the environment they invade and therefore threaten biodiversity, agriculture and human health (Stohlgren and Schnase 2006). Pimentel et al. (2000) estimated the annual accumulated costs attributable to invasive species at $122,639 million.

Suppression methods against invasive species include chemical, mechanical and biological control. Chemical control has traditionally been used for pest suppression, but their potentially negative health effects, environmental impacts, potential build-up of resistance and the necessity of repeated applications (Simberloff 1996) have prompted investigations for more sustainable control methods. Mechanical suppression methods have been used with success in combination with chemical control (Simberloff 1996); however, mechanical methods are often expensive and/or not feasible when target invasive species are widespread and/or located in remote inaccessible areas. Biological control is an attractive alternative to chemical and mechanical control, or in come cases the only alternative, (Simberloff 1996) because of its self dispersing, self-sustaining characteristics.

The philosophy of classical biological control is based on the enemy release hypothesis, which states that organisms become invasive in a new area because they have

escaped the natural enemies that suppress their populations in their area of origin, thus providing them an advantage over competitors in area of introduction, that are still suppressed by their indigenous natural enemies (Blumenthal, 2005). Therefore, this type of control works under the premise that reestablishment of top-down control by the introduction of natural enemies will reduce the populations of invasive species and therefore restore balance (Hoddle 2004).

History of biological control

The practice of biological control, as the transfer of beneficial organisms from one geographical region to another to control pests, was initiated more than 120 years ago. Since then, there have been more than 5000 introductions worldwide targeting 165 pests (Hill and Greathead 2000) with various rates of success (Greathead 1995).

The history of biological control provides remarkable examples of success of many programs (Caltagirone 1981). Prime examples include the introduction of the Australian lady beetle *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae) to control the cottony cushion scale, *Icerya purchasi* Maskell, an introduction that saved the California citrus industry, and the introduction of *Anagyrus lopezi* (DeSantis) (Hymenoptera: Encyrtidae) from South America to control the cassava mealybug *Phenacoccus manihoti* Matile-Ferrero, in Africa, credited with saving many people from starvation. Those are just two of many other remarkable examples. Besides the economic benefits of this practice, the use of biological control has also led to a reduction in the use and dependence on pesticides in numerous cropping systems.

Non-target effects of biological control

Many biological control practitioners have long considered this practice environmentally safe, benign, risk-free and a natural phenomenon (van den Bosch and Messenger 1973; DeBach 1974, Caltagirone and Huffaker 1980, Simmonds and Benett 1977). Even though awareness of potential negative effects was expressed over a century ago (Perkins 1897), it is only since the 1980's that classical biological control was first severely criticized (Howarth 1983, 1991). The center of this criticism was the issue of host specificity (Ehler 1999). Biological control agents were implicated in the reduction of populations of native and desirable species (Howarth 1983, 1991). Soon after, researches were calling for more rigorous screening methods in the USA (Ehler 1999) and revisiting means of predicting positive or negative impacts of biocontrol agents. Some authors went so far as to call for the cessation of biological control (Asquith and Miramontes 2001).

During the early 1900's, biological control introductions lacked careful planning prior to release of natural enemies into the new geographic area. Some of these introductions included generalist predators and parasitoids. In most of these cases, non-target effects on native species were not examined due to lack of concern for potential negative effects, or were not considered negative but a potential means of maintaining populations of biological control agents when the target pest was scarce. Some of these early releases account for the undesirable effects that some agents have had on native species and other desirable organisms.

Hawaii and non-target effects of biological control

The Hawaiian archipelago is the most isolated island chain on earth and is home to a greater proportion of endemic species than any other place of similar size on earth (Kaneshiro 1995). Hawaii also has a long history of biological control introductions, with more than 700 species released since 1894, (Funasaki et al. 1988), and has been the center of debate about environmental impacts of biological control introductions. Howarth (1983) claimed that population declines and even extinctions of some native Lepidoptera species in Hawaii were due to parasitism by purposely introduced biological control agents. This situation divided conservationists and biological control practitioners around the world and has contributed to a significant reduction of biocontrol introductions in Hawaii and also elsewhere due to rigid regulations (Messing 1999). No biological control species introduced into Hawaii since 1970 have been found to attack native fauna or exotic desirable species, suggesting that screening methods became more rigorous and were effective (Funasaki et al., 1988; Messing and Wright, 2006).

Retrospective studies and risk assessment

Natural enemies used in classical biological control are also non-indigenous species that have potential to become invasive themselves. Retrospective studies on biological control introductions provide an important tool in the evaluation of potential non-target effects of future biological control programs. They help build case histories that can provide patterns to aid identify key biological and ecological factors that need to be investigated to provide a robust estimate of the candidate's non-target potential (Louda et al. 2003). Retrospective studies do not only provide data on patterns on possible

mechanisms of host and habitat expansion, but can also contribute to developing more efficient risk assessment methodologies to predict outcomes of future introductions and in this way increase the safety of biological control.

No species introduction is absolutely "risk-free" and there are always potentially undesirable consequences of introducing species into new environments (Nechols et al.1992). Lonsdale et al. (2001), defined "risk assessment" as a set of analytical techniques for estimating how much damage or injury can be expected as a result of an event. Risk assessment in the field of biological control evaluates the likelihood that adverse ecological effects may occur as a result of a release of a purposely introduced biological control agent and exposure of indigenous species to these introduced natural enemies.

Since the center of criticism of biological control was based on issues related to host specificity, host specificity testing is now a key element in any risk assessment methodology, and is typically used to accept or reject potential introductions. Several protocols have been developed for selection of non-target species for screening and host range determination throughout the years and are available in the scientific literature (Barratt et al. 1997; Sands, 1998; Kulhmann and Mason 2003; Messing 2001; van Lenteren 2003, 2006; Kuhlmann et al. 2006). Some countries such as New Zealand, Australia and South Africa as well as countries within the European Union have developed their own regulations and risk assessment frameworks. For the most part they have similar criteria, but they involve different procedures and work under different guidelines.

In the current literature, two general risk assessment frameworks have been proposed. Van Lenteren et al. (2003) proposed a semi-quantitative environmental RA approach for inundative biological control agents, which was later improved and expanded to address classical biological control agents, in a stepwise procedure which identifies biological control agents with high potential risk early in the process, therefore avoiding unnecessary research and use of resources (Van Lenteren 2006a,b). Wright et al. (2005) proposed a probabilistic risk assessment approach for either classical or augmentative biocontrol agents, which is based on the development of 'precision trees', using conditional probabilities in a Bayesian approach to estimating risk. Chapter 4 of this dissertation presents an overview and critique of these two approaches.

Aims of this dissertation

The overall aims of this dissertation are to:

1) Assess current impacts some alien parasitoids on the endemic moth *Udea stellata*;

2) Refine and validate a probabilistic risk assessment approach proposed by Wright et al. (2005).

The study system

The study system used in this dissertation is the endemic Hawaiian moth *Udea stellata* (Butler), its associated larval parasitoids, on the endemic host plants *Pipturus* spp. This study system provides the opportunity to assess ecological impacts of three adventive parasitoids, two purposely introduced biocontrol agents and two parasitoids of unknown origin. *Udea stellata* is not a species of special concern in terms of conservation status,

but one distributed across a wide range of elevation and anthropogenic disturbance which offers the opportunity to examine the impacts of introduced and adventive parasitoids species in a range of circumstances.

Outline of the dissertation

This dissertation is set out in five chapters, each one with specific objectives, and providing complementary information for subsequent chapters.

Udea stellata, the non-target species subject of this study, was described by Butler in 1883. Since there have been no previous studies on the biology and ecology of this endemic moth, Chapter 1 describes basic aspects of the life cycle, larval phenology in the field and presents the parasitoid species associated with the larval stages. This chapter provides the basis for understanding how ecology of *U. stellata* may influence the levels of non-target use and impact by alien and purposely introduced parasitoid species.

Chapter 2 describes parasitism levels (apparent mortality) by alien wasps on populations of *U. stellata* at eight sites varying in elevation and levels of anthropogenic disturbance during a two year period. Chapter 2 also quantifies parasitism by larval stage collected, and specific stages utilized by the assemblage of parasitoid species that attack *U. stellata.*

Field parasitism (Chapter 2) provides background information on parasitoid assemblage composition, levels of parasitism of samples taken, and seasonal trends in different locations but does not often provide an effective measure of parasitoid impact at the host population level. Chapter 3 presents results of partial life-table studies conducted

at six sites. This study determined the relative contribution of the seven parasitoid species, to the population dynamics of *U. stellata*.

Field surveys (Chapter 2) and partial life-table studies (Chapter 3) were conducted at different sites varying in ecological conditions. Chapter 4 presents the results of a community analysis (multivariate analysis) that identifies key ecological factors that likely play a role in determining the composition of the parasitoid assemblage associated with *U. stellata*.

Chapter 5 provides an overview and criticism of proposed risk assessment approaches for wide adoption as well as a refinement and validation of a probabilistic risk assessment approach proposed by Wright et al. (2005).

CHAPTER 1

LIFE HISTORY, SEASONAL PHENOLOGY AND PARASITISM OF THE HAWAIIAN ENDEMIC MOTH *UDEA STELLATA* (LEPIDOPTERA: CRAMBIDAE)

Abstract

This study presents basic information on the life cycle, seasonal phenology and parasitism of the endemic Hawaiian moth *Udea stellata* (Butler), a species for which little biological information is available, even though it was described more than a century ago. By observation of ecdysis and measurements of corresponding head capsule widths under laboratory conditions it was determined that *U. stellata* undergoes six larval stages. All larval stages had distinct ranges in head capsule width, which corresponded to discrete groups. Duration of each larval stage as well as the egg and pupal stage are reported. Endemic host plants of *U. stellata*, *Pipturus* spp. (Urticacea) were sampled at eight field sites between July 2004 and July 2006. Temporal differences in density of larvae were most pronounced in medium and high elevation sites, possibly an effect of more marked seasonal temperature changes. The parasitoid assemblage associated with *U. stellata* comprised seven species: three adventive species, two purposely introduced species and two of unknown origin. Adventive parasitoids rather than purposely introduced ones were responsible for the greater part of the apparent mortality observed.

Key Words Life cycle, leaf samplings, *Pipturus* spp., adventive species, purposely introduced species

Introduction

The Hawaiian archipelago is the most isolated island chain on earth, which has resulted in a unique insect fauna (Kaneshiro 1995). Hawaiian insects are under-represented in many taxa, with only 50% of the world's insect orders and just 15% of the known families of insects native to Hawaii (Howarth and Mull 1992). Yet, Hawaii is home to a greater proportion of endemic insect species than any other place of similar size on earth (Kaneshiro, 1995), with approximately 98% of the native arthropod species endemic to the archipelago (Howarth and Ramsay 1991), which originated from species radiations.

Hawai'i has been severely impacted by invasive species, which have become environmental and agricultural pests. One response to invasive species has been biological control. The islands have an extensive record of biological control introductions, amounting to more than 700 species introduced since 1890 (Funasaki 1988). The first introduction in Hawaii was the vedalia beetle, *Rodolia cardinalis* (Coccinellidae), against the cottony cushion scale, *Icerya purchasi* (Hemiptera: Aleyrodidae), which provided a spectacular demonstration of the practice of classical biological control. Since then, biological control has been recognized as an effective tool for pest management and has played a significant role in Hawaiian agriculture and contributed to the suppression of 200 pest species (Funasaki et al. 1988).

Biological control introductions, many of which have tremendous potential to reduce host or prey populations, may also impact non-target insect populations, with possible irreversible effects (Howarth 1983, 1991; Gagne and Howarth 1985). Parasitism of native Hawaiian insects by introduced biological control agents has received

considerable attention recently (Follett et al. 2000; Henneman and Memmott 2001; Oboyski et al. 2004), with few studies showing the actual impacts they have cause on non-targets (Johnson et al. 2005).

The lack of basic information on the status, biology and ecology of native insects in Hawaii, as in most parts of the world, is a serious obstacle to their conservation (Howarth and Mull 1992). This lack of knowledge is typically attributable to the fact that there are large numbers of insect species, and many of these lack the 'charisma' to generate conservation priority (Samways 1993, 2005). Besides the lack of basic biological information on many described native insects in Hawaii (Howarth and Mull 1992), quantitative information of non-target impacts by alien species (including purposely introduced species) on these species is also sparse. Most studies on non-target use and non-target impact in the field of biological control in Hawaii and elsewhere have concentrated on species of economic importance or species observed to have serious population declines, and with clear evidence that these declines have been influenced by attacks of purposely introduced species (Boettner et al. 2000; Barron et al. 2003, 2004; Benson et al. 2003a, 2003b; Kellogg et al. 2003, Van Driesche et al. 2004; Johnson et al. 2005).

The indigenous Hawaiian Lepidoptera fauna includes at least 1149 described species of Lepidoptera, 957 of which are endemic to the islands (Zimmerman, 1958a, 1958b, 1978; Nishida 2002). Invasive Lepidoptera pest species have been the target of many biological control introductions, and biological control introductions have even in rare instances targeted native species considered pests in agricultural settings (Funasaki et al. 1988). Gagné and Howarth (1985) suggested that purposely introduced biological

control agents were the major factor in the putative extinction of 15 species of native moths in Hawaii. Some of these presumed extinct species have been recently re-discovered to be persisting in apparently healthy populations (Haines et al. 2004).

The genus *Udea* (Lepidoptera: Crambidae) is a relatively large group that occurs in the Americas, Eurasia and into the Pacific (Zimmerman 1958b). Hawaii has 44 endemic species in this genus (Nishida 2002). *Udea stellata*, the subject of this study, was first described by Butler in 1883. There have been no previous studies on the biology and ecology of this endemic moth. *Udea stellata* is a widespread species that occurs in a range of habitats with different levels of anthropogenic impacts. This species offers the opportunity to examine the impacts of introduced parasitoids in a range of circumstances, ranging from relatively undisturbed indigenous forest to highly modified systems with pressures from invasive species, habitat modification and with different densities of host plants. The objectives of this study were to investigate basic aspects of *U. stellata* life history traits and its seasonal abundance, larval phenology and parasitism, as the basis of an investigation of impacts of invasive and purposefully introduced parasitoids on this endemic Hawaiian moth.

Materials and Methods

Host plants

The study system consisted of the endemic host plant *Pipturus* spp. (Urticaceae, common name mamaki), the endemic moth *U. stellata* and the parasitoid assemblage associated with the larval stages of this moth. *Pipturus* is a genus of 30-40 species, ranging from the Mascarene Islands to Malaysia, Australia and many Pacific islands

(Wagner et al. 1999). The Hawaiian *Pipturus* are commonly known as "mamaki" or "mamake". This plant is a relatively common shrub of the mesic areas throughout the islands (Sohmer and Gustafson 1993). Mamaki flowers are inconspicuous, and the fruits are unusual white masses (Pratt 1998). *Pipturus albidus* was sampled on Oahu and Hawai'i. They are shrubs or small trees 2 - 6 m tall. *Pipturus kauaiensis*, shrubs (1.5 - 3m tall) were sampled on Kauai.

Sampling sites

To quantify the seasonal phenology of *U. stellata,* censuses of larvae were conducted at eight sites on three of the six major Hawaiian Islands (Table 1.1, Figure 1.1). Three sites were located along the Ditch Trail at Kokee State Park on the island of Kauai: Kokee P1, Kokee P2 and Kokee P3. Four sites were located on the island of Oahu: Kunia and Palikea are located in the Waianae Mountains and are managed by the Nature Conservancy of Hawaii and Pali and Tantalus, are located in the areas Nuuanu and Makiki respectively. The last site was located in the Kipuka Puaulu trail at the Hawaii Volcano National Park on the island of Hawai'i. These sites vary in ecological features, primarily in elevation and in level of disturbance, defined by prevalence of alien plants. Sites located at higher elevations, such as sites in Kauai and Volcano in Hawaii were the least disturbed; sites at lower elevations, such as Tantalus and Pali, were the most disturbed. Disturbance refers to the degree of presence of native plants. Disturbance level was categorized as low when more than 40% of the plants in the patch were native species, medium when 20-40% of the plants in patch were native species and high when less than 20% of plants in patch were native species (Table 1.1).

***Udea stellata* life history**

The number of larval stages that *U. stellata* undergoes was studied under laboratory conditions (22°C (± 2%) and ~62% (± 10%) RH). Before starting this study, laboratory colonies were established from field collected larvae. The day of collection, larvae were placed individually into labeled clear containers (28 ml) and provided with a fresh piece of leaf. Containers were cleaned and new plant material was added to the containers every day or every other day depending on the rate of feeding. Soon after eclosion, moths were placed into cages containing a potted host plant of about 1.5 feet tall and provided with a honey/water solution. Oviposition plants were changed every 2-3 days.

Newly hatched larvae were transferred individually using a fine paint brush from the oviposition plants into small Petri dishes (35x10 mm) with a fresh piece of plant material every other day. Petri dishes were sealed with Parafilm. At intervals of 1-2 days, larvae were observed microscopically. Molted head capsules were removed soon after edcysis and the head capsule diameter was measured using a stage micrometer. The duration of each instar (ecdysis to ecdysis) was recorded. Results are presented in box and whisker plots (box represents the 25th, 50th (median) and 75th percentiles, while the whiskers represent 10th and 90th percentiles).

Duration of the egg stage was determined by keeping record of the date potted plants were placed in the oviposition cage and the date at which larvae hatched from eggs deposited on those plant. In the same way, the duration of the pupal stage was determined by keeping record of the date the 6^{th} instar larvae molted into pupa and the date at which

adults emerged. Means ± standard error are reported for the duration of the different stages.

Udea stellata seasonal phenology

To assess the seasonality of *U. stellata* larvae, monthly censuses of larvae on mamaki plants were conducted at eight field sites between July 2004 and July 2006. Plants were selected haphazardly within mamaki patches. A total of 30 leaves per plant were inspected in the Oahu sites, whereas 60 leaves were inspected in the Kauai and Hawaii sites. The difference in number of leaves sampled per plant was because of differences in density of leaves per plant. The total number of plants sampled varied from 8 to 20 per site; some plants were sampled repeatedly each month. Plant sample sizes were dictated by the abundance and accessibility of the host plants in the various locations. The larvae found were categorized by larval instar based on head capsule measurements.

Statistical analysis: Number of *U. stellata* larvae per leaf per plant was subject to transformation (log +1) and the data were analyzed using a Generalized Linear Model (SAS Institute, 2003). Mean separations for the frequency of encounter of larvae (expressed as larvae per leaf) by site was performed using Ryan-Einot-Gabriel-Welsch Multiple Range Test. This test was used instead of Tukey's test in order to reduce Type I error. Data presented in graphs are the untransformed means, whereas the mean separations were conducted on transformed data.

Larval parasitism

Larvae found during the above surveys were placed in plastic containers with some plant material and stored in a cooler chilled with cold packs for transport to the University of Hawaii at Manoa. Once in the laboratory (22°C (± 2%) and ~62% (± 10%) RH) each larva was placed individually into a labeled plastic container (30 ml clear plastic container). Feces and old plant material were removed and new plant material was added to the containers every day or every other day depending on the rate of feeding. Host plants used for feeding the larvae were grown from seed in the greenhouse facility at the University of Hawaii. Field-collected larvae were reared to the adult stage, until parasitoids emerged, or until they died. Emerging parasitoids were pinned for identification. Specimens were identified using unpublished keys to the Hawaii Ichneumonidae (compiled by J.W. Beardsley) and also by comparing adult voucher specimens with specimens at the Hawaii Department of Agriculture insect collection, University of Hawaii Insect Museum and Bishop Museum. Identifications were confirmed by Dr. David Wahl at the American Entomological Institute (Gainesville, Florida). Vouchers specimens are kept at the American Entomological Institute. The identification at the species level of *Triclistus* nr. *aitkeni* (Cameron) was done by Dr. Gavin Broad at the National History Museum (London, United Kingdom). Voucher specimens of all species are to be deposited at the Bishop Museum and University of Hawaii at Manoa Insect Museum.

Statistical analysis

Percentage parasitism was calculated for larvae of known fate as: % Parasitism = Parasitized hosts/(parasitized + unparasitized hosts) x100.

Larvae that died during rearing were not dissected but data from dissected pupae (which died but from which no parasitoid emerged), were included. Parasitism rates were subject to transformation (log +1) in order to normalize the data. A mixed model Analysis of Variance was conducted using PROC MIXED (SAS Institute, 2003) to detect significant differences of mean percentage parasitism by parasitoid across all sites and all months. The mixed model approach was used owing to the repeated sampling of sites, disparities in sample sizes and resulting unbalanced design.

Results

Udea stellata larval stages

The measurements of head capsule widths and within-instar variability from laboratory reared larvae are shown in Figure 1.2. By tracking individual larval development (sample sizes are shown in parenthesis in Figure 1.2), it was possible to determine that *U. stellata* undergoes six larval instars. Head capsule widths ranged from 0.18 mm to 1.70 mm. All larval stages had distinct ranges in head capsule width, which corresponded to discrete groups (Figure 1.2).

Description of developmental biology

The *Pipturus* spp. host plants of *U. stellata* occurred under fairly variable conditions in terms of canopy cover, disturbance level, presence of invasive plant species

and elevation. During the search for collection sites it was observed that the host plants could occur from almost sea level up to 1,400 m. *Udea stellata* was never observed at elevations below 240 m (Kaufman, personal observation).

Eggs of *U. stellata* are flat and translucent. The time from oviposition to hatch ranged from 4 to 7 days and mean duration of 5.2 ± 0.2 days (n = 23) under laboratory conditions(22°C (± 2%) and ~62% (± 10%) RH). All larval stages fed mainly on the underside of the leaves, and were protected by a fine web that they spun. Table 1.2 shows the mean duration of each larval stage in days as well as sample sizes (for the same cohort used for head capsule measurements). The duration of each larval stage ranged from three to five days. The duration of pupal stage ranged from 11 to 15 days with a mean of 13.4 ± 0.25 days (n = 30).

The pupal stage was never found during the field surveys even though leaves, branches and soil around the plants were inspected on many occasions. On two occasions a ~1.50 m tall plant (planted in a 19 liter pot) was infested with 15 fifth instar larvae and caged to monitor where they pupate. The larvae left the plant to pupate on the screen of the cage rather than on branches or in leaf litter below the plant, which confirmed that the final instar larvae wander off the plant to pupate.

Udea stellata larval phenology and frequency of encounter

During the 25 months of sampling, a total of 2,400 trees and 105,000 leaves were sampled across all sites. Table 1.3 shows the total number of trees and leaves sampled by site as well as total number of larvae collected at each site. The site with the highest density of larvae per leaf was Palikea, whereas the sites with lowest number of larvae per

leaf were Pali, Kokee2 and Tantalus. There was no significant correlation between number of leaves sampled and number of larvae recorded per leaf (Pearson's correlation , $r = 0.456$, d.f. = 6, $P = 0.256$), suggesting that different sample sizes in different areas did not bias estimates of numbers of larvae.

There was no significant difference in the total number of larvae found per leaf between the two years for all study sites (Table 1.4, Figure 1.3). The analysis also showed significant variation in the counts of larvae by month and by site. An overall significant interaction was found between site and month as well as year and site (Table 1.4).

Figure 1.3 shows the phenology of *U. stellata* larvae at each of the eight collection sites for the two years of sampling. At the Kokee sites and Volcano site, slightly higher numbers of larvae were encountered from March to May each year. In the Palikea site, there was an increase in larval density in both years from February to July. *Udea stellata* numbers reached a peak in January 2005 in the Kunia and Palikea sites. There was no distinct seasonal pattern at the Pali and Tantalus sites.

Figure 1.4 shows the mean density (larvae/leaf) of *U. stellata* by study site across all months. There were significant differences in the mean number of larvae found per leaf by sites (Table 1.4). The Palikea site yielded significantly higher *U. stellata* encounter rates, and the Pali site had the lowest encounter rates (Figure 1.3).

During sampling, a mixture of larval stages was typically present at any sampling time, and in some cases all six larval stages were present at the same collection time, showing clearly that multiple overlapping generations occurred annually. Table 1.5

shows the occurrence of larval stages in monthly samplings for the Palikea site. This site is at a medium high elevation and was the one with the highest density of larvae.

Larval parasitism

Seven koinobiont solitary endoparasitoids were associated with the larval stages of *U. stellata*: *Diadegma blackburni* (Cameron, 1883), *Casinaria infesta* (Cresson, 1872), *Trathala flavoorbitalis* (Cameron, 1907), *Pristomerus hawaiiensis* (Perkins, 1910) and *Triclistus* nr. *aitkeni* (Cameron, 1897) (Ichneumonidae) and *Meteorus laphygmae* (Viereck, 1913) and *Cotesia marginiventris* (Cresson, 1865) (Braconidae). All these parasitoid species except *P. hawaiiensis* and *D. blackburni*, whose origins are unknown (either indigenous or adventive), are alien to Hawaii (Fullaway and Kraus., 1945; Stein., 1983; Oboyski et al., 2004). *Cotesia marginiventris* and *M. laphygmae* were purposely introduced to Hawaii in 1942 to control *Spodoptera exempta* (Walker) in sugarcane plantations (Funasaki et al. 1988).

There were significant differences ($F_{6;1211} = 489.65$, $P < 0.0001$) in mean percentage parasitism by species, across all months and all sites (Figure 1.5). Of the total larvae that survived laboratory rearing, 2.7% were parasitized by *D. blackburni*, 6.0% (136/2267) by *P. hawaiiensis*, 1.6% by *T.* nr. *aitkeni*, 28.8% (653/2267) by *T. flavoorbitalis*, 0.8% by *C. infesta*, 0.4% by *M. laphygmae* and 2.6% by *C. marginiventris*. The adventive parasitoid *T. flavoorbitalis* contributed the most to the mean parasitism compared to the other species ($t = 56.11$, $P < 0.0001$), and accounted for 67.1% (653/973) of all wasps reared.

Discussion

The only certain method to determine the number of instars in the development of an insect is by direct observation of insects reared individually throughout their entire larval development (Nealis 1987). Using this approach, it was possible to determine that *U. stellata* undergoes six larval stages. The lack of overlap in head capsule width measurements by instar suggests that it is possible to determine the larval stage of this species when collected in the field with high levels of confidence. Knowledge of the number of larval stages provided valuable information to determine stages susceptible to parasitism. This information was also crucial for the planning and evaluation of partial life-table studies (Chapter 3).

It is possible that development of larvae, and head capsule width, will be influenced by parasitism. Studies done on other organisms have shown that parasitism resulted in smaller head capsule size and retarded development, especially during later instars (Miller 1983; Nealis 1987). It is thus possible that field collected larvae categorized as emerged in the fifth instar might actually represent sixth instar.

The collection of multiple larval stages at the same sampling time(s) shows that *U. stellata* is a multivoltine species. Seasonal differences in frequency of encounter of larvae were most evident in medium and high elevation sites, which might be an effect of more marked seasonal temperature changes (Lee and Pemberton 2007). Low elevation sites appeared to be less suitable for *U. stellata*, given that *U. stellata* populations were not observed below 240m above sea level, and larval densities were lowest at low elevation sites (Pali and Tantalus). Besides elevation, sites varied in other ecological

features such as level of disturbance, presence of invasive species, and type of overstory and understory. This aspect will be addressed in a separate study (Chapter 4).

Natural habitats in Hawaii have been severely impacted by humans. Beginning with the arrival of the Polynesians in Hawaii and accelerating after the European contact, original native lowland landscapes were dramatically modified (Kirch 1982). Throughout the Hawaiian Islands, most land below 600m elevation is now dominated by non-native flora and fauna (Loope 1998). The study sites, Pali and Tantalus, are located below 460m and are dominated by alien plant species which probably harbor mainly non-indigenous arthropods. These sites had the lowest density of mamaki plants per area. The low density of host plants as well as the presence of non-native generalist predators, such as ants on the host plants (especially at Pali) may explain the low density of larvae per leaf found at these sites.

The Kunia (550m) and Palikea (781m) sites are also disturbed, but to a lesser degree, mainly by non-native overstory plants, and they have a higher incidence of native understory plants compared to Pali and Tantalus. Both sites are managed by the Nature Conservancy of Hawaii and are within fenced areas that exclude feral pigs and other exotic mammals. Out-plantings of native species and chemical control of non-native plant species is also done in the area. The increased mamaki density may contribute to the higher densities of larvae at these sites, especially in Palikea, which was the site yielding the highest number of larva per total number of leaves searched during this study.

Of the seven parasitoid species reared from larvae of *U. stellata* during the course of this study, *T. flavoorbitalis*, *D. blackburni*, *C. infesta* and *M. laphygmae* were already reported from this host by Zimmerman in 1958. I additionally reared *P. hawaiiensis*, *T.*

nr. *aitkeni* and *C. marginiventris*, which represent new records of parasitoids associated with this endemic moth. All parasitoids but *T.* nr. *aitkeni* are known to occur on all major islands (Nishida 2002). *T.* nr. *aitkeni* is a new adventive species to the Hawaiian Islands. The origin of *D. blackburni* and *P. hawaiiensis* has been questioned and remains largely unresolved. *Pristomerus hawaiiensis*, although listed as endemic (Nishida 2002) is possibly adventive to the islands (Fullaway and Kraus 1945; Stein 1983). On the other hand, *D. blackburni*, listed as adventive (Nishida, 2002) may be endemic to Hawaii (Oboyski et al. 2004).

Adventive parasitoids, specially *T. flavoorbitalis*, rather than purposely introduced species, inflicted the bulk of the parasitism in this study system. A separate study comprehensively reports field parasitism of *U. stellata* by sites and by parasitoid species (Chapter 2).

This study presents information on the life history traits, phenology and parasitism of the Hawaiiian endemic moth *U. stellata*, providing a basis for understanding how ecology of *U. stellata* may influence the levels of non-target use and impact by alien and purposely introduced parasitoid species. Most importantly, this study provides information about an endemic species for which little biological information was available, even though it was described more than a century ago.

The practice of biological control has long been recognized as an important tool for suppressing invasive species in agricultural settings. More recently, with the increase in numbers of invasive species threatening native species and native habitats, biological control is also becoming an important tool for biological conservation management (Hoddle 2004; Messing and Wright 2006). However, the conservation potential of

biological control is also dependent on the potential risk it poses to non-target species. Increasing our basic knowledge of native species will in turn facilitate non-target studies when biological control agents are to be introduced to suppress invasive species.

Table 1.1 Geographical data (in decimal degrees) for collection sites sampled for *Udea stellata*

Sites	Elevation (m)	Latitude	Longitude	Disturbance
Kauai				
Kokee P1	981	22.13159	-159.63171	Low
Kokee P2	1046	22.12790	-159.63472	Low
Kokee P3	1113	22.12135	-159.63582	Low
Oahu				
Pali	372	21.36579	-159.79398	High
Tantalus	460	21.32996	-157.82249	High
Kunia	550	21.46290	-158.09552	Medium
Palikea	781	21.41279	-158.09953	Medium
Hawaii				
Volcano	1229	19.43742	-155.30328	Low

Table 1.2. Mean number of days (± SEM) for each larval instar of *U. stellata*

Larval stage	N	Mean ± SEM
First	80	3.43 ± 0.07
Second	39	3.46 ± 0.10
Third	32	3.75 ± 0.10
Fourth	29	3.97 ± 0.14
Fifth	30	3.80 ± 0.15
Sixth	24	3.72 ± 0.15

Table 1.3. Total number of trees/leaves sampled and number of larvae collected per site

Sites	Trees	Leaves	Total Larvae
Kokee 1	200	12,000	232
Kokee 2	200	12,000	199
Kokee 3	200	12,000	434
Kunia	500	15,000	527
Pali	300	9,000	71
Palikea	250	7,500	1,033
Tantalus	250	7,500	134
Volcano	500	30,000	901
Total	2,400	105,000	3,531

Table 1.4. Summary statistics from a generalized linear model analysis of transformed (logx + 1) number of larvae per leaf

Source of Variance	d.f.	F - value	*P* – value
Year	1	0.15	0.6977
Month	11	14.58	< 0.0001
Site	7	219.48	< 0.0001
Site x Month	77	8.62	< 0.0001
Site x Year	7	4.33	< 0.0001

Table 1.5. Occurrence of different *Udea stellata* larval stages per sampling month at Palikea (Oahu)

Month	% Larvae collected at different larval stages						Sample size (n)
	1	2	3	4	5	6	
Jul'04	42.1	15.8	18.4	15.8	7.9	0.0	38
Aug'04	20.8	37.5	12.5	25.0	4.2	0.0	24
Sep'04	0.0	0.0	55.6	33.3	5.6	5.6	18
Oct'04	20.0	6.7	26.7	23.3	16.7	6.7	30
Nov'04	19.4	29.0	25.8	19.4	6.5	0.0	31
Dec'04	6.9	17.2	55.2	17.2	3.4	0.0	29
Jan'05	0.0	25.0	50.0	25.0	0.0	0.0	4
Feb'05	11.8	35.3	29.4	17.6	5.9	0.0	17
Mar'05	22.2	20.0	35.6	18.9	3.3	0.0	90
Apr'05	8.2	38.8	38.8	14.3	0.0	0.0	49
May'05	32.4	34.3	23.8	7.6	1.9	0.0	105
Jun'05	5.2	19.8	57.3	14.6	3.1	0.0	96
Jul'05	0.0	17.0	24.5	34.0	24.5	0.0	53
Aug'05	0.0	0.0	47.8	39.1	8.7	4.3	23
Sep'05	0.0	6.7	20.0	33.3	23.3	16.7	30
Oct'05	4.8	42.9	9.5	38.1	4.8	0.0	21
Nov'05	0.0	12.5	75.0	6.3	6.3	0.0	16
Dec'05	0.0	37.5	50.0	12.5	0.0	0.0	8
Jan'06	1.9	25.5	35.8	29.2	7.5	0.0	106
Feb'06	0.0	2.9	17.6	52.9	17.6	8.8	34
Mar'06	0.0	11.8	52.9	20.6	8.8	5.9	34
Apr'06	0.0	4.2	62.5	20.8	12.5	0.0	24
May'06	0.0	17.9	30.4	33.9	10.7	7.1	56
Jun'06	0.0	3.8	42.3	23.1	28.8	1.9	52
Jul'06	0.0	20.0	46.7	15.6	17.8	0.0	45

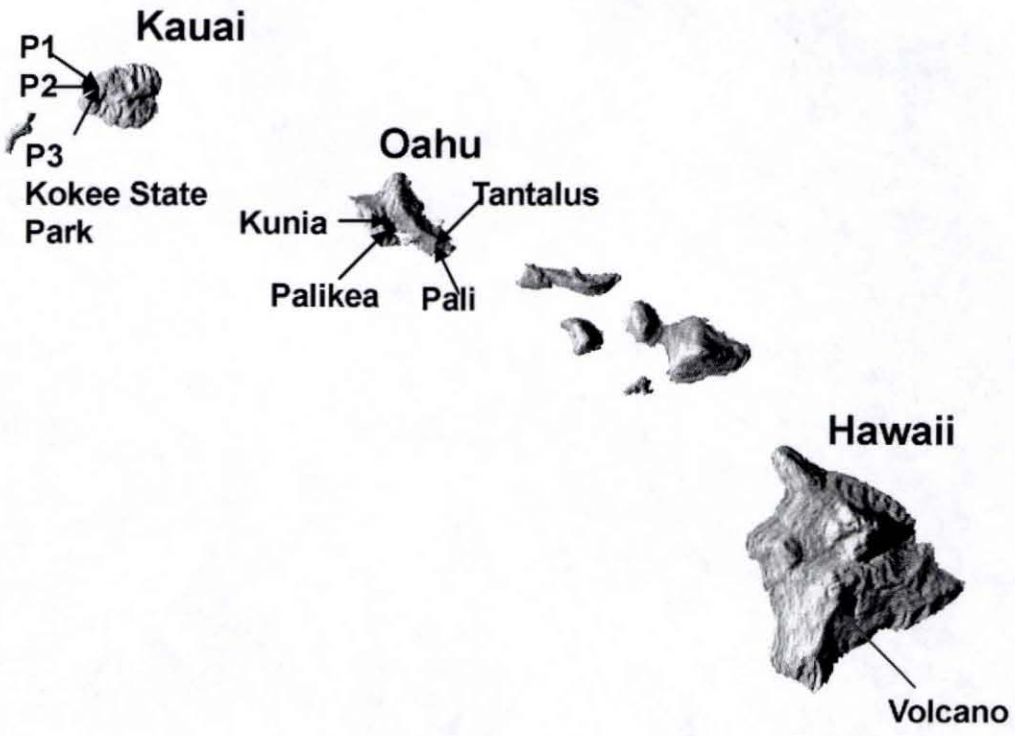

Figure 1.1 Map of the Hawaiian Islands indicating the eight sampling sites.

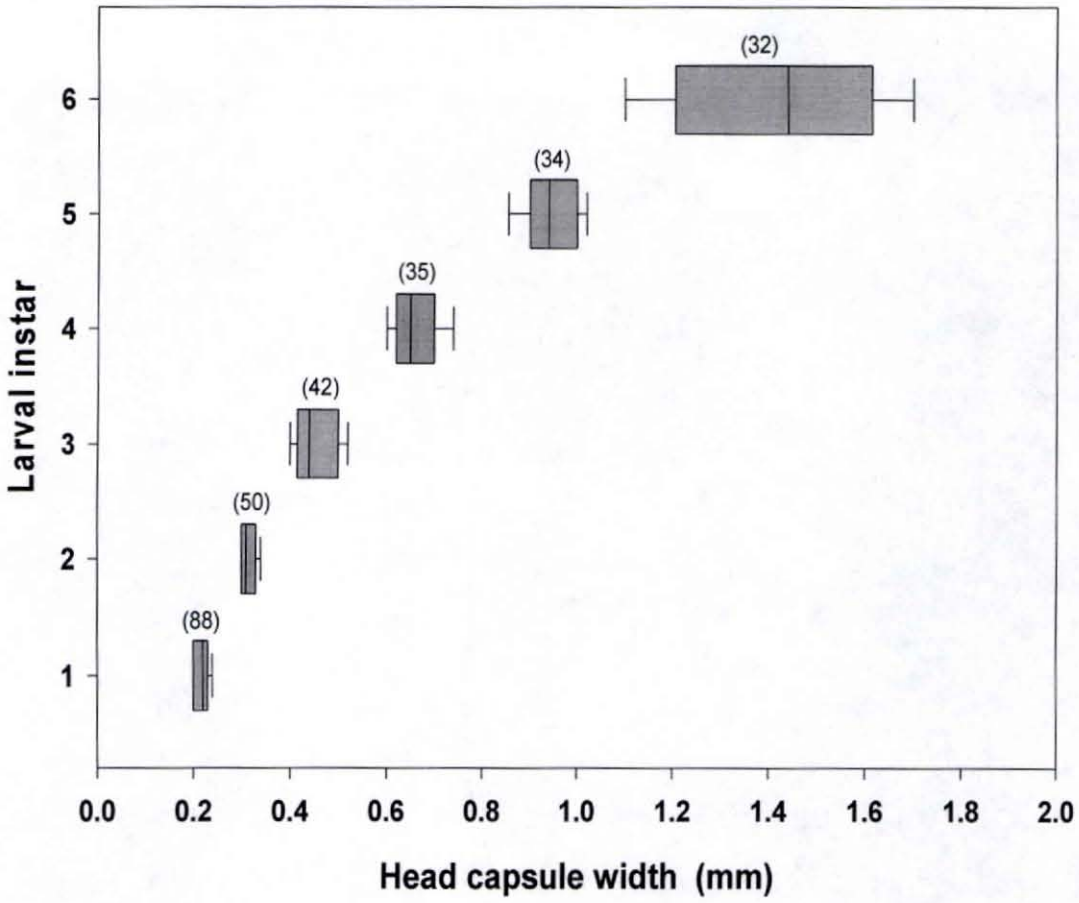

Figure 1.2. Box plots (showing the median with 25th and 75th percentiles, whiskers indicating 10th and 90th percentiles) describing the head capsule widths of the six larval instars of *Udea stellata*. Sample sizes for each instar are shown in parenthesis.

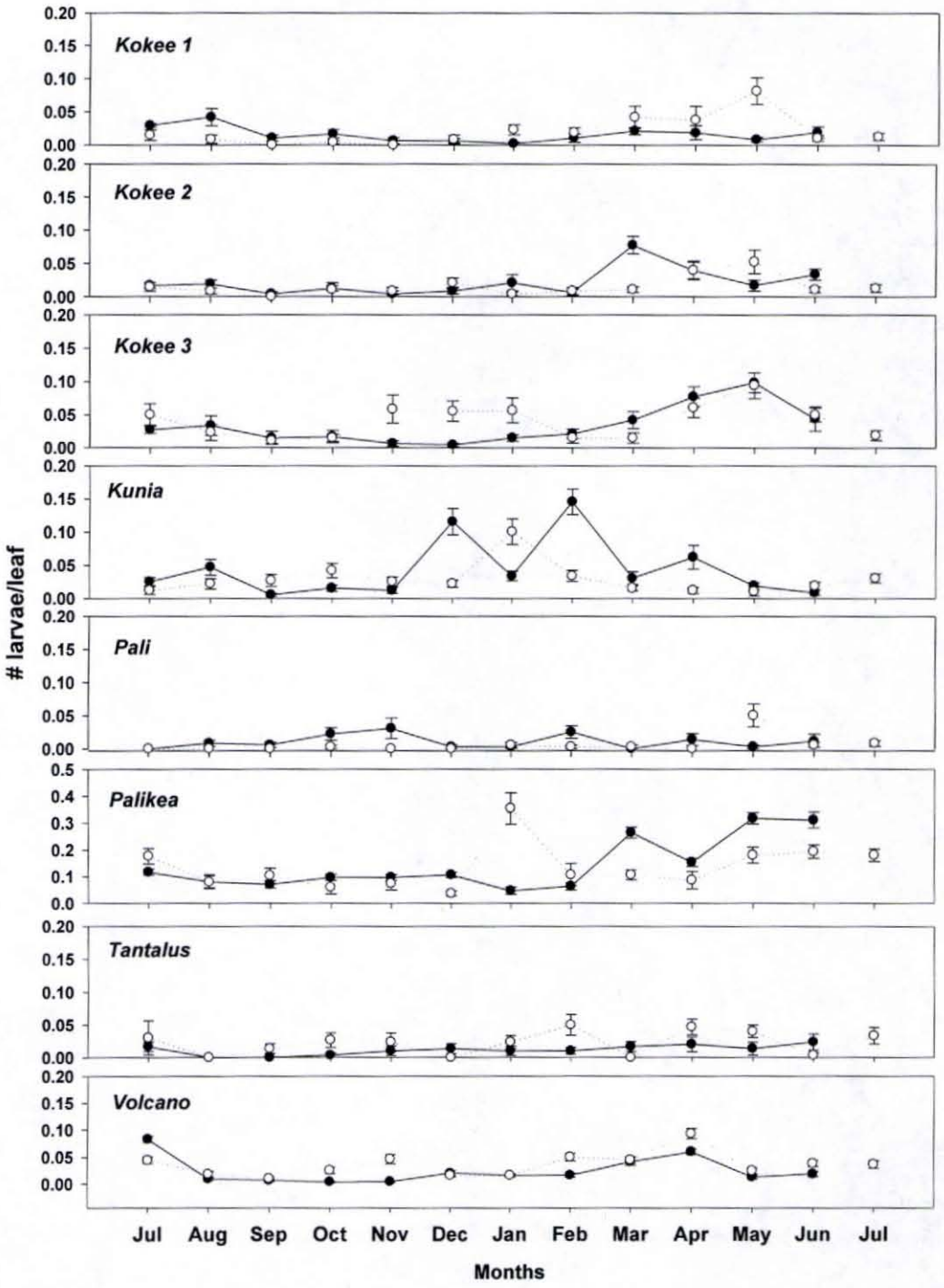

Figure 1.3. Phenology of *Udea stellata* at eight study sites in Hawaii (mean number of larvae per leaf ± standard error). Note difference in y-axis scale for Palikea. Solid lines with filled dots represent the year 2004/2005 and dotted lines with open dots represent the year 2005/2006.

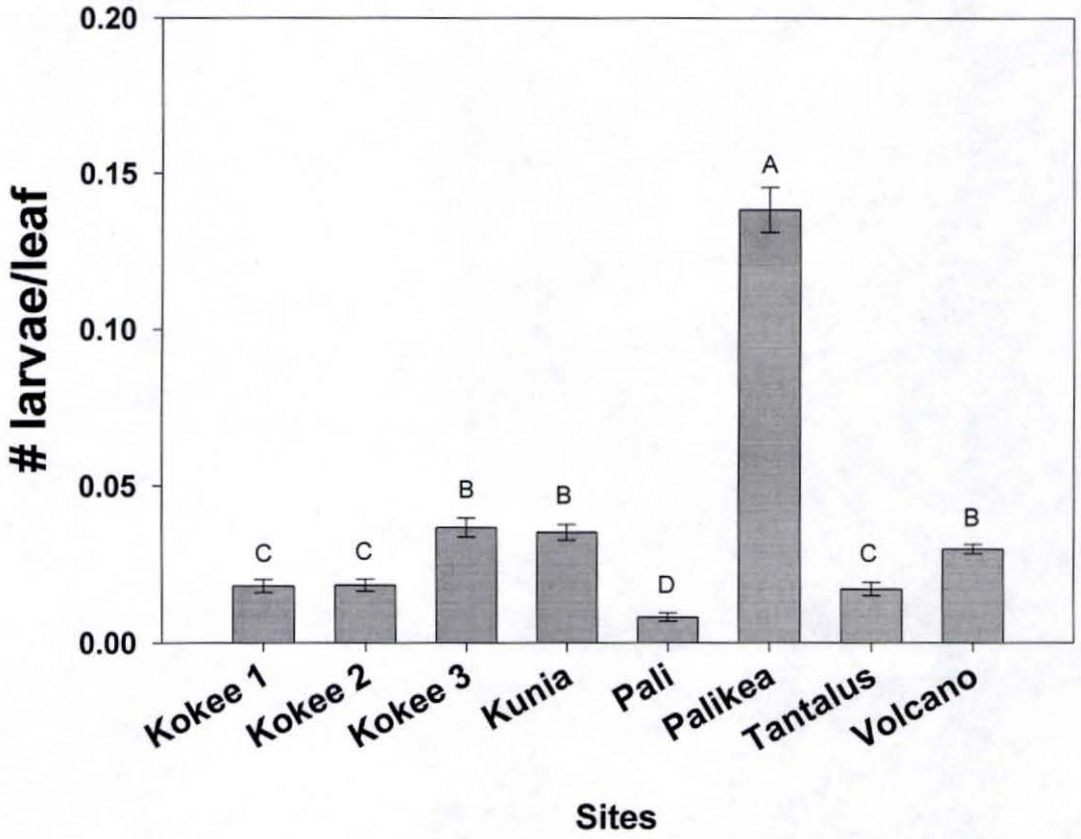

Figure 1.4. Mean number of *U. stellata* per leaf at eight sites. Different letters above bars indicate significant differences, Ryan-Einot-Gabriel-Welsch Multiple Range Test.

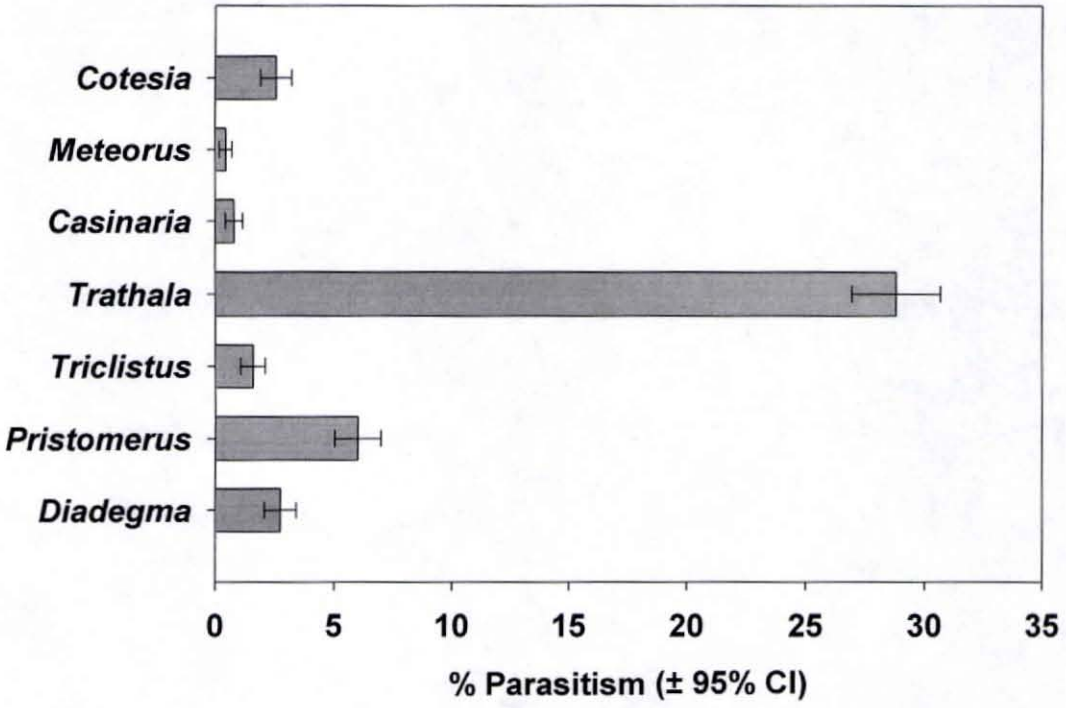

Figure 1.5. Mean (± 95% CI) percentage parasitism of *Udea stellata* by seven parasitoid species, across all months and all sites.

CHAPTER 2

PARASITISM OF THE HAWAIIAN ENDEMIC MOTH *UDEA STELLATA* (LEPIDOPTERA: CRAMBIDAE) BY INVASIVE AND PURPOSELY INTRODUCED HYMENOPTERA SPECIES

Abstract

The impact of invasive alien species on native organisms is a cause for serious concern. This concern is especially relevant in the Hawaiian archipelago due to its high level of endemicity, severe impacts of accidental introductions of invasive species, and long history of purposeful biological control introductions. Results from a previous study showed that the parasitoid assemblage associated with endemic moth *Udea stellata* (Butler) comprised seven species: three adventive species, two purposely introduced species and two of unknown origin. The objectives of this study were to assess the parasitism levels of alien wasps on populations of *U. stellata* at different sites and to determine the specific stages that were utilized by the spectrum of parasitoid species that attack *U. stellata*.

Standardized collections of wild larvae were conducted at eight sites, located on the islands of Kauai, Oahu and Hawaii. A total of 3,531 larvae were collected in a two year survey. Of these, 8.0% were collected as 1st instar, 23.0% 2nd instar, 39.0% 3rd instar, 21.0% 4th instar, 7.1% 5th instar and 1.8% 6th instar. Of the larvae that survived laboratory rearing, 43.0% were parasitized. Information collected in the surveys was complemented with data from life-table studies to determined stage specific parasitism. All larval stages

were susceptible to parasitism by at least one parasitoid species; second and third instars were susceptible to attack by all seven parasitoid species.

Adventive parasitoids rather than purposely introduced ones were responsible for the greater part of the apparent mortality observed. At low and low-medium elevations, the parasitoid assemblage was dominated by adventive species. The two purposely introduced parasitoids were present in remote relatively undisturbed sites on the islands Kauai and Hawaii. Addressing current ecological impacts of alien parasitoids on native species is of particular importance for developing more efficient means to quantify the risks of future biological control introductions.

Key Words: Non-target, biological control, parasitism rates, native organisms, Hawaiian archipelago.

Introduction

Biological control of insect pest species was initiated more than a century ago and has proved to be a valuable strategy to control pests in agricultural and natural systems, as an alternative to dependence on pesticide use (Pimentel, 1997). The practice of biological control was long considered an environmentally safe approach (van den Bosch and Messenger, 1973; De Bach, 1974, Simmonds and Bennett, 1977; Caltagirone and Huffaker, 1980), but it is now known that this strategy is not risk-free, and that it can potentially cause negative effects on native and desirable species (Howarth 1983, 1991, Gagne and Howarth 1985, Simberloff and Stiling 1996; Nechols et al. 1992, Lockwood 1993).

During the early 1900's, biological control introductions lacked careful planning prior to release of natural enemies into the new geographic area. Some of these introductions included generalist predators and parasitoids. In most of these cases, non-target effects on native species were not examined due to lack of concern for potential negative effects, or were not considered negative but a potential means of maintaining populations of biological control agents when the target pest was scarce. Some of these early releases account for the undesirable effects that some agents have had on native species and other desirable organisms.

Concerns about the effects of purposely introduced species on endemic fauna were expressed more than a century ago in Hawaii by Perkins (1897). Nevertheless, it was only since the nineteen-eighties that these concerns garnered international attention, with Hawaii being the center of debate. Howarth (1983) claimed that population declines and even extinctions of some native Lepidoptera species in Hawaii were due to parasitism by purposely introduced biological control agents. This situation divided conservationists and biological control practitioners around the world and has contributed to a significant reduction of biocontrol introductions in Hawaii and also elsewhere due to rigid regulations (Messing 1999).

After the publication of Howarth's (1983, 1991) which lacked any quantitative data, studies on the impacts of introduced natural enemies were initiated in Hawaii as well as in other parts of the world. Henemman and Memmott (2001) collected larvae of many native Lepidoptera species in a remote site on the island of Kauai spanning a two year period. They found that most of the parasitoid species associated with the immature stages of these native species were purposely introduced parasitoids and suggested that

the introduced species significantly altered food-web structure. Other more specific and comprehensive studies have been conducted, such as that of Duan and Messing (2000) on introduced parasitoids against Tephritid fruit flies. They found that impacts of purposely introduced species on native and desirable (weed biological control agents) Tephritidae were minimal compared to the ones caused by accidentally introduced parasitoid species. Similar results were reported by Johnson et al., (2005) who studied the effects of parasitoids introduced to control the southern green sting bug, *Nezara viridula* (Pentatomidae), on the endemic koa bug *Coleotichus blackburniae* (Scutelaridae). They also concluded that adventive predators had the greatest impact on *C. blackburniae* populations; whereas effects of intentionally introduced species were relatively minor.

It has been recognized that retrospective studies on past biological control introductions provide opportunities to improve the pre-release decision-making process for future biological control programs (Follett et al., 2000; Louda et al., 2003; Barratt et al., 2006). No biological control species introduced into Hawaii since 1970 have been found to attack native fauna or exotic desirable species, suggesting that screening methods became more rigorous and were effective (Funasaki et al., 1988; Messing and Wright, 2006).

Hawaii is a unique place to study non-target effects from biocontrol due to its high rate of endemism and its long history of biocontrol introductions (Funasaki et al., 1988). Hawaii is also remarkable for its invasive species richness, which pose significant problems not only in agricultural areas but also in remote natural areas, a situation that in many cases makes biological control the only feasible option for sustained suppression of invasive species and a tool for conservation (Hoddle, 2004). Each year an average of 17

arthropod species accidentally arrive in the Hawaiian Islands, many of which become permanently established and create severe environmental and economic problems (Messing and Wright, 2006). For example, one of the most recent invaders, the Erythrina gall wasp (*Quadrastichus erythrinae*, Eulophidae), has placed the endemic coral tree *Erythrina sandwichensis* under severe duress, with many conservation biologists and managers concerned the tree will be driven to extinction. Currently, biological control seems like the only feasible option for sustainable suppression of the gall wasps.

The Hawaiian entomofauna is diverse, with a high degree of endemism. The Hawaiian Lepidoptera is represented by 957 described native species in 17 families (Zimmermann 1958a, 1958b, 1958c, Howarth and Mull, 1992; Nishida, 2002). This Order has been the target of many biological control introductions against non-native Lepidoptera species, and even in rare instances against native species considered pests in agricultural settings (Funasaki et al., 1988).

The genus *Udea* (Lepidoptera: Crambidae) is a very large group that occurs in the Americas, Eurasia and into the Pacific (Zimmerman, 1958b). Hawaii has 44 endemic species in this genus (Nishida, 2002). *Udea stellata* was first described by Butler, in 1883 and is the non-target subject of this study. *Udea stellata* presents an appropriate organism to study the effects of introduced species, as it occurs across a broad range of elevations, and is present on all Hawaiian Islands in areas of varying ecological disturbance.

The parasitoid assemblage associated with *U. stellata* larvae includes seven koinobiont solitary endoparasitoids (Chapter 1). Two of them are listed species of adventive origin: *Casinaria infesta* (Cresson), *Trathala flavoorbitalis* (Cameron) (Nishida, 2000); *Triclistus* nr. *aitkeni* (Cameron) is a new adventive species to the

Hawaiian Islands; two species were purposely introduced for biological control purposes: *Meteorus laphygmae* (Viereck) and *Cotesia marginiventris* (Cresson) (Nishida, 2002); and two species are of uncertain origin. *Diadegma blackburni* (Cameron), listed as adventive (Nishida, 2002) is possibly endemic to Hawaii (Oboyski et al. 2004), and *Pristomerus hawaiiensis* (Perkins) listed as endemic (Nishida 2002) may be adventive to the islands (Fullaway and Kraus 1945; Stein 1983). All parasitoid species recorded are larval parasitoids expect for *T.*nr. *aitkeni.*, which is a larval-pupal parasitoid. All of them can exploit other species of Lepidoptera (Zimmerman a, b, c, 1958).

The present study had two specific objectives: 1) assess the parasitism levels of alien wasps on populations of *U. stellata* at different sites 2) determine the specific stages that were utilized by the spectrum of parasitoid species that attack *U. stellata.*

Materials and Methods

Study system

The study system consisted of the endemic host plant *Pipturus* spp. (mamaki), the endemic moth *U. stellata* and the parasitoid assemblage associated with the larval stages of this moth.

Pipturus spp. (Urticacea): A genus of some 30-40 species from the Mascarene Islands to Malaysia, Australia and many Pacific islands (Warner et al., 1999). The Hawaiian *Pipturus* are commonly known as "mamaki" or "mamake". This plant is a relatively common shrub of the mesic areas throughout the islands (Sohmer and Gustafson, 1993). Mamaki flowers are inconspicuous, and the fruits are unusual white masses (Pratt, 1988). *Pipturus albidus* was sampled on Oahu and Hawai'i. They are

shrubs or small trees 2-6 m tall. *Pipturus kauaiensis*, shrubs of 1.5 to 3m tall, were sampled on Kauai.

Udea stellata caterpillars are pale whitish-green, head testaceous without markings except the black eyes. They feed on the under-side of the leaves and are protected by a thin web that they spin (Zimmerman, 1958b). *Udea stellata* undergoes six larval instars (Chapter 1).

Study sites

An extensive field survey was carried out over a two year period from 2004 to 2006.

Udea stellata larvae were collected from eight sites on three islands of the Hawaiian archipelago (Table 1.1, Figure 1.1). Three sites were located along the Ditch Trail at Kokee State Park (Kokee P1, Kokee P2 and Kokee P3) on the island of Kauai. Four sites were located on the island of Oahu: Kunia and Palikea are located in the Waianae Mountains and are managed by the Nature Conservancy of Hawaii. The other two sites, Pali and Tantalus, are located in the areas Nuuanu and Makiki respectively. The last site was located in the Kipuka Puaulu (Bird Park) trail at the Hawaii Volcano National Park, on the island of Hawai'i. These sites vary in ecological features, primarily in elevation and in level of disturbance. Disturbance refers to the degree of presence of native plants. Disturbance level was categorized as low when more than 40% of the plants in the patch were native species, medium when 20-40% of the plants in patch were native species and high when less than 20% of plants in patch were native species (Table 1.1).

Sampling and rearing methods

Monthly sampling was conducted by haphazardly selecting plants within mamaki patches, and visually inspecting a predetermined number of leaves per tree. A total of 30 leaves per plant were inspected in the Oahu sites, whereas 60 leaves per plant were inspected in Kauai and Hawaii sites. The difference in the number of leaves sampled was because of differences in the density of leaves per plant; plants in the Oahu sites were taller and with fewer leaves, whereas plants on the Big Island and Kauai were more bushy and with more leaves. The total number of plants sampled varied from 8 to 20 per site. Plant sample sizes were dictated by the abundance and accessibility of the host plants in the various locations, hence the disparity in number of plants sampled. Larvae found during the above surveys were collected and returned to the laboratory to be categorized by larval instar based on head capsule measurements (Chapter 1). Each larva was placed individually into a labeled plastic container (30 ml clear plastic container). Feces and old plant material were removed and new plant material was added to the containers every day or every other day depending on the rate of feeding. Host plants used for feeding the larvae were grown from seed in the greenhouse facility at the University of Hawaii. Field-collected larvae were reared to the adult stage, until parasitoids emerged, or until they died. Emerging parasitoids were pinned for identification. Specimens were identified using unpublished keys to the Hawaii Ichneumonidae (compiled by J.W. Beardsley) and also by comparing adult voucher specimens with specimens at the Hawaii Department of Agriculture insect collection, University of Hawaii Insect Museum and Bishop Museum. Identifications were confirmed by Dr. David Wahl at the American Entomological Institute (Gainesville,

Florida). Vouchers specimens are kept at the American Entomological Institute. The identification at the species level of *T.* nr. *aitkeni* (Cameron) was done by Dr. Gavin Broad at the National History Museum (London, United Kingdom). Voucher specimens of all species are to be deposited at the Bishop Museum and University of Hawaii at Manoa Insect Museum.

Information on the larval stage at the time of collection and parasitoids reared from them (present study) was used in combination with data collected in a partial life-table study (Chapter 3) to determine the specific stages that were utilized by the spectrum of parasitoid species that attack *U. stellata*. Head capsule diameter at the time of emergence of the parasitoid was measured to determine the stage at which the host was killed. In the partial life-table studies sentinel larvae of all instars were deployed on sentinel host plants in the field, where they were exposed simultaneously to parasitoids and other sources of mortality for the duration of only one instar.

Data Analysis

Percentage parasitism was calculated for larvae of known fate as

% Parasitism = Parasitized hosts/(parasitized + unparasitized hosts) x 100.

Larvae were not dissected but data from dissected pupae (which died but from which no parasitoid emerged), were included. Parasitism rates were subject to transformation (log +1) in order to normalize the data. A mixed model Analysis of Variance was conducted using PROC MIXED (SAS Institute, 2003) to detect significant differences of mean percentage parasitism by parasitoid across all sites and all months, as well as mean percentage parasitism by sites across all parasitoids and all months. The mixed model

approach was used owing to the disparities in sample sizes and resulting unbalanced design, and the repeated sampling of the same plant patches. Analysis of Variance (ANOVA) using PROC MIXED (SAS Institute, 2003) was conducted to analyze percentage of dead larvae by instar. Due to disparity in numbers of larvae collected by site and by sampling time, means and 95% confidence intervals were plotted in graphs. Data presented in graphs are untransformed means.

Results

During the two years of the study, a total of 3,531 larvae were collected at all sites. Of these, 2,267 survived laboratory rearing whereas 1,264 died. Larvae that did not survive laboratory rearing died due to unknown bacterial and fungal infections or unknown causes. Of those that survived, 42.9% (973/2,267) were parasitized. Larvae that did not pupate were not taken into account for calculation of parasitism rates

Parasitism by species and by larval stage

Table 2.1 shows the total number of trees sampled and the total number of leaves inspected during the study. Total number of larvae found and corresponding fates are shown per site.

Of the 3,531 larvae collected, 8.0% were 1st instar, 23.5% 2nd instar, 39.1% 3rd instar, 20.6% 4th instar, 7.1% 5th instar and 1.8% 6th instar. There was a trend for older field-collected larvae to be parasitized more heavily than younger ones, except for the sixth instar (Figure 2.1). There was no significant correlation ($r = -0.486$; d.f. = 4; $P > 0.05$) between percentage of larvae collected by larval stage and percentage parasitism.

Figure 2.2 shows the percentage of larvae that died during rearing by instars the larvae had reached at the time of collection. The number of dead larvae decreased significantly as with increasing age of collection larval stages ($F = 29.2$, $P < 0.0001$). Figure 2.3 shows the mean percentage parasitism by the seven parasitoid species for each of the six larval stages collected. Only three of the seven parasitoid species (*D. blackburni*, *P. hawaiiensis* and *T. flavoorbitalis*) reared during the course of the study were found parasitizing the first instar, whereas all seven species were reared from larvae collected from second to sixth instar. For most parasitoid species recorded, there was a cumulative trend in parasitism rate by instar, except for *T. flavoorbitalis* and *P. hawaiiensis* which maintained a fairly consistent parasitism rate across all instars collected.

Figure 2.4 shows larval stages susceptible to parasitism as well as stages at which the host dies for each of the seven parasitoid species. Of the seven parasitoids reared, six were larval endoparasitoids, and *T.* nr. *aitkeni* was the only larval-pupal endoparasitoid. All larval stages were susceptible to parasitism by at least one parasitoid species (6^{th} instar was only parasitized by *T.* nr. *aitkeni*) The first five instars were susceptible to attack by multiple parasitoid species, from three (1^{st} instar) to all seven (2^{nd} and 3^{rd} instars). Of the six larval endoparasitoids, all killed their host in either the fifth or sixth larval stage.

Parasitism by sites

Pali and Tantalus were the sites with the smallest number of parasitoid species associated with *U. stellata* larvae. In the Oahu sites, the parasitoid assemblage was

mainly composed of adventive species, whereas when considering all Kauai sites together plus the site at Volcano, the complete assemblage of parasitoid species, including the two purposely introduced species was found (Table 2.2). The latter sites correspond to the higher elevations and were the most undisturbed sites (more than 40% of the plants in the patches were native species).

Figure 2.5 shows the mean percentage parasitism by study site, across all moths and all parasitoid species. There were significant differences in parasitism by sites ($F_{7,2259} = 20.2$, $P < 0.0001$). Total mean parasitism varied from 8.9% to 55.7% across sites. The lowest parasitism rates were found in the Kauai sites and the highest in Palikea and Kunia in the island of Oahu. The latter two sites had intermediate levels of disturbance relative to the other study sites (between 20 to 40% of the plants in the patch were native species).

Parasitism per species by site is presented in Figure 2.6. For most wasps, parasitism rates varied significantly across sites ($P < 0.050$), except for *M. laphygmae* ($P = 0.165$). Parasitism rates by *D. blackburni* and *T.* nr. *aitkeni* were significantly higher at the Kokee1 site in Kauai. The adventive species *T. flavoorbitalis* contributed the most to the mean parasitism in the Oahu sites, with parasitism rates that ranged from 37.6 % to 53.9 %. The other adventive species contributed less than 12% to the total parasitism at all sites. *Pristomerus hawaiiensis* was only present in sites above 550m. Parasitism by the purposely introduced species, *M. laphygmae* was recorded in Kauai, Palikea on Oahu and Volcano on Hawaii, with parasitism rates less than 3.1 %. *Cotesia marginiventris*, was only present in one site on Kauai and at the Volcano site on Hawaii, with parasitism rates 1.5 % and 9.8%, respectively.

Discussion

This paper presents information on the parasitoid assemblage attacking the larvae of the Hawaiian endemic moth *U. stellata* between July 2004 and July 2006. This research was conducted to address the non-target parasitism by purposely introduced species as well as adventive parasitoids at different sites on an endemic species in Hawai'i.

All larval stages were vulnerable to parasitism by at least one parasitoid species and in some instances vulnerable to attack by all seven parasitoid species. Instar at the time of collection is a good predictor of the time that the host has been exposed to parasitoids and other mortality factors under field conditions. Estimates of parasitism might be influenced by the distribution of larvae among instars, a factor that is often not recorded in general collections (Lill, 1999; Van Driesche, 1983; Van Driesche et al., 1991). Nevertheless, in this study, there was a trend for older field-collected larvae to be parasitized more heavily (except for the sixth instar), no significant correlation was found between larval stage at time of collection and percentage parasitism (Figure 2.1).

Parasitism of wild larvae collected in late stages does not necessarily mean that they were parasitized at the stage collected, but could be an accumulation of parasitoid attack of earlier stages. Therefore data from life-table studies, where larval age at exposure was known, were used to define boundaries of susceptible stages. The only parasitoid species that was not reared during the partial life-table studies was *D. blackburni*, perhaps because it occurs in very low densities at the sites where the studies were carried out. Nevertheless, parasitism of *U. stellata* by *D. blackburni* tended to

increase with larval stage at the time of collection in this study, which suggests that it parasitizes other larval stages besides the first.

Larvae collected as later instars suffered less mortality during laboratory rearing than those collected as early instars. Early instar larvae might be more susceptible to mortality due to unknown reasons. In the laboratory colony of *U. stellata*, mortality due to unknown reasons was also higher in early instars (Kaufman, personal observation). In addition, it is possible that field collected early instar larvae could be more susceptible to death due to parasitism or multiple parasitism during rearing. In this study dead larvae were not dissected, but in a partial life-table study (Chapter 3) no significant difference was found in death due to unknown causes between larvae that were exposed to parasitoids and larvae that were excluded from parasitoids under field conditions, suggesting that other causes such as natural mortality or stress during rearing, rather than parasitism, were the causal factors.

Trathala flavoorbitalis was the dominant parasitoid contributing the most to the total parasitism in each larval stage. No cumulative trend was observed on parasitism by instars for *T. flavoorbitalis*, which suggests that most of the field parasitism is inflicted in the early larval stages rather than in later stages, even though this species has been found parasitizing larvae from first to the fifth instar. This information was corroborated with data collected in the partial life-table studies (Chapter 3) where *T. flavoorbitalis* was found to have higher parasitism rates in larvae exposed as second and third instars.

By combining the information from the field surveys and data from the partial life-table studies it is known that *T.* nr. *aitkeni* parasitizes larvae from second to sixth instar, however only larvae exposed from fourth to sixth instar were parasitized during

the life-table studies which suggests that *T.* nr. *aitkeni* prefers parasitizing later larval instars, perhaps to avoid competition with other parasitoid species attacking earlier instars.

The information used to define susceptible stages in this study was based on field collected data. Laboratory studies exposing parasitoids to different larval stages may provide more definite information about stages physiologically acceptable for parasitism but not necessary stages exploited under field conditions.

The overlap in susceptible stages, especially for the second and third instar, creates the potential for a level of interspecific competition among all seven parasitoid species. However, differences observed in the level of parasitism by individual species by larval stage and the fact that each species parasitizes three to five different larval stages may mean that discrimination of larval stage for parasitism would be a way to avoid competition, and therefore shape the structure of the parasitoid assemblage in this study system.

Sites with the lowest density of host plants (such as Pali and Tantalus) also had the lowest density of larvae per leaf. Those sites had only two or three parasitoid species associated with them, whereas sites with the highest number of larvae per leaf (such as Palikea and Volcano) had six or seven species of parasitoids, suggesting that host abundance plays a role in reducing parasitoid competition and therefore composition of the parasitoid guild structure (Price, 1970; Sheehan, 1994).

At low-medium elevations, the parasitoid assemblage was dominated by adventive species. The two purposely introduced parasitoids were present in remote relatively undisturbed sites on the islands Kauai and Hawaii. Adventive parasitoids,

specially *T. flavoorbitalis*, rather than purposely introduced species, inflicted the bulk of the parasitism in this study system. This is consistent with previous studies of non-target impacts in insect biological control (Barron et al. 2003; Johnson et al. 2005) that similarly showed that adventive species had far more serious impacts than purposefully introduced species.

The fact that densities of larvae, parasitism rates and parasitoid guild varied among the three sites in the Ditch trail in Kokee State Park suggests that subtle ecological differences influence the occurrence of both the moth and their associated parasitoids significantly. At the Oahu sites (located between 372 and 781m), *T. flavoorbitalis* was the species that contributed the most to the mean parasitism, and ranged from 37.63% to 53.91%; whereas at the three sites at Kokee State Park (981, 1,046 and 1113m, respectively) parasitism rates by this species ranged from 0.00 to 2.01%. At the Volcano site (1,229m), parasitism by *T. flavoorbitalis* averaged 17.02%. Besides altitude, which correlates with temperature, other attributes of the habitat and ecology and biology of parasitoids seem to be important in determining the occurrence of certain parasitoid species as well as parasitism rates. A separate study focuses on the environmental factors that influence the parasitoid assemblage associated with *U. stellata* at different sites (Chapter 4).

All of the parasitoid species in this system, except for two species whose origin are unknown, were introduced to Hawaii through human intervention, either accidental or intended. As previously discussed, parasitism rates in this study system were sometimes apparently high (in some cases more than 50%), yet it is not know how these parasitism rates might be influencing *U. stellata* at the population level. If non-target mortality does

not result in changes in distribution and abundance of the non-target, then it does not cause impact at the population level (van Lenteren et al 2006). Caution should be taken when interpreting results presented from field parasitism, since these results do not typically provide an effective measure of impact at the population level, such as information on the density of the host and the measure of total losses to parasitism for a stage over a generation is rarely available (Chesson, 1982, Van Driesche, 1983, Van Driesche et al, 1991). To date, analysis of non-target effects have mostly concentrated on determining apparent mortality, and few studies attempt to determine the impact on host populations by conducting life-table studies or understand how the structure of parasitoid guild might mediate population impacts. The impact of parasitism by the seven species at the population level on *U. stellata*, using life-table analysis is the focus of a separate study.

Table 2.1. Total number of trees/leaves sampled per site, number of larvae collected and corresponding fates.

Sites	Trees	Leaves	Total larvae	Dead	*Udea* adult	*Diadegma*[1]	*Pristomerus*[1]	*T.* nr. *aitkeni*[2]	*Trathala*[2]	*Casinaria*[2]	*Meteorus*[3]	*Cotesia*[3]
Kokee 1	200	12000	232	83	115	17	3	9	3	1	1	0
Kokee 2	200	12000	199	65	122	4	5	3	0	0	0	0
Kokee 3	200	12000	434	134	250	9	25	3	4	0	4	5
Kunia	500	15000	527	182	155	1	1	2	186	0	0	0
Pali	300	9000	71	28	24	0	0	0	17	2	0	0
Palikea	250	7500	1033	394	283	11	5	13	312	14	1	0
Tantalus	250	7500	134	41	55	1	0	2	35	0	0	0
Volcano	500	30000	901	337	290	19	97	4	96	1	4	53
Total	**2400**	**105000**	**3531**	**1264**	**1294**	**62**	**136**	**36**	**653**	**18**	**10**	**58**

[1]species of unknown origin, [2]adventive species, [3]purposely introduced species.

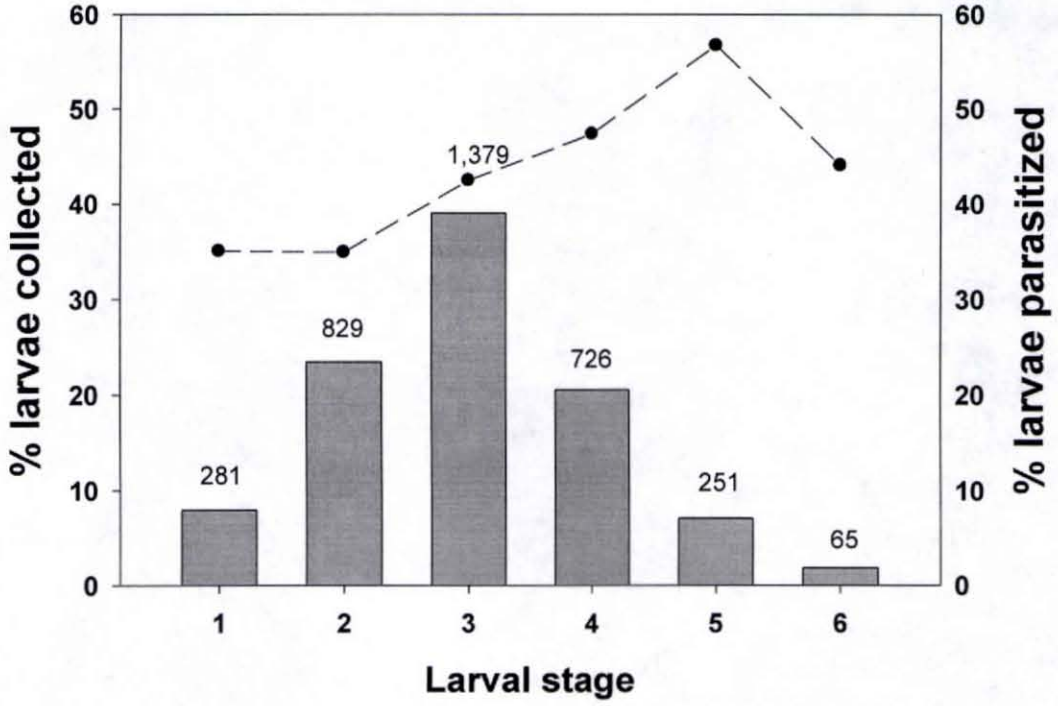

Figure 2.1. Frequency distribution of larval stages of *Udea stellata* collected pooled for all sites and all months (bars) and mean percentage of larvae parasitized by larval instar (filled dots). Numbers on top of the bars represent the actual number of larvae collected.

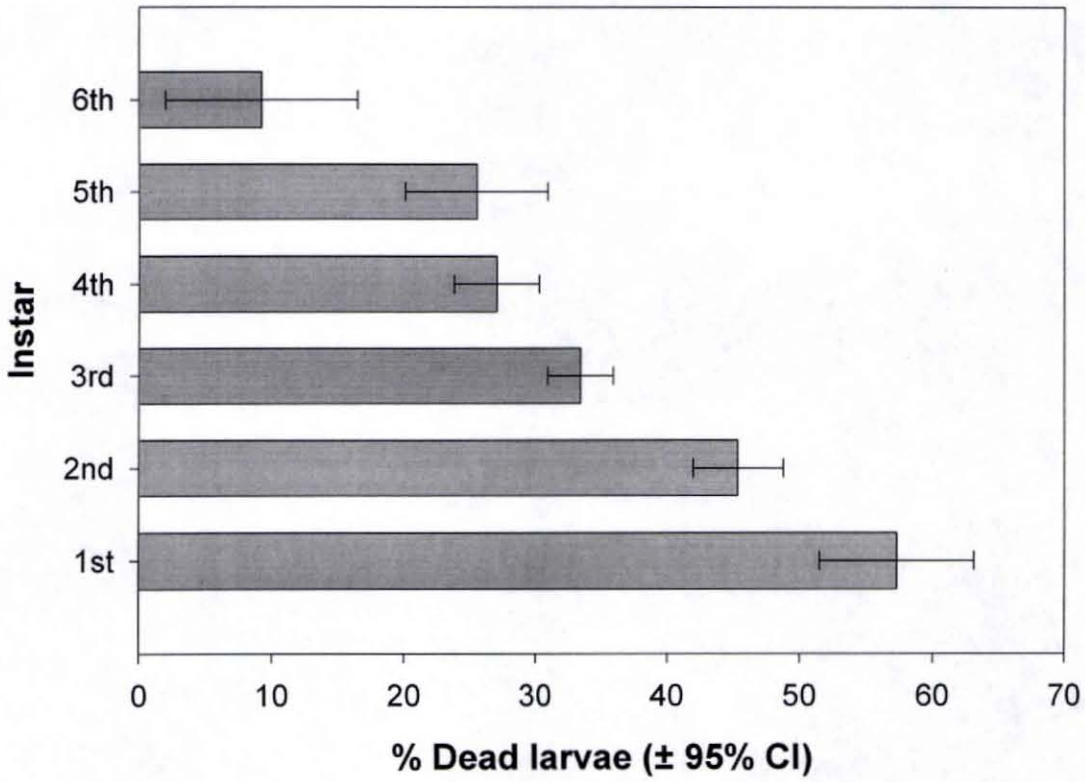

Figure 2.2. Mean (± 95% CI) percentage of dead *Udea stellata* larvae by instar at time of collection.

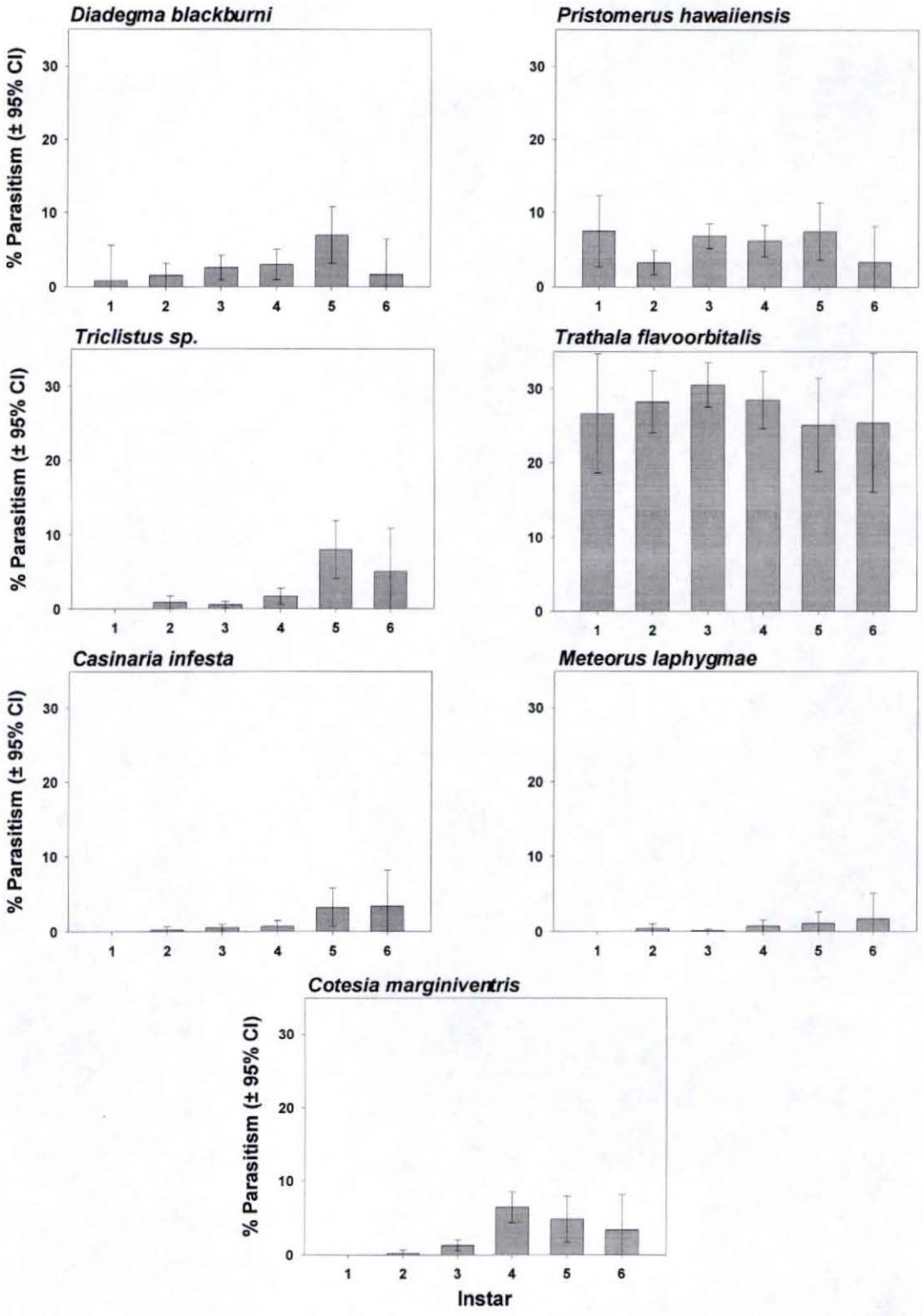

Figure 2.3. Mean (± SE) percentage parasitism of *Udea stellata* by seven parasitoid species for each larval stage.

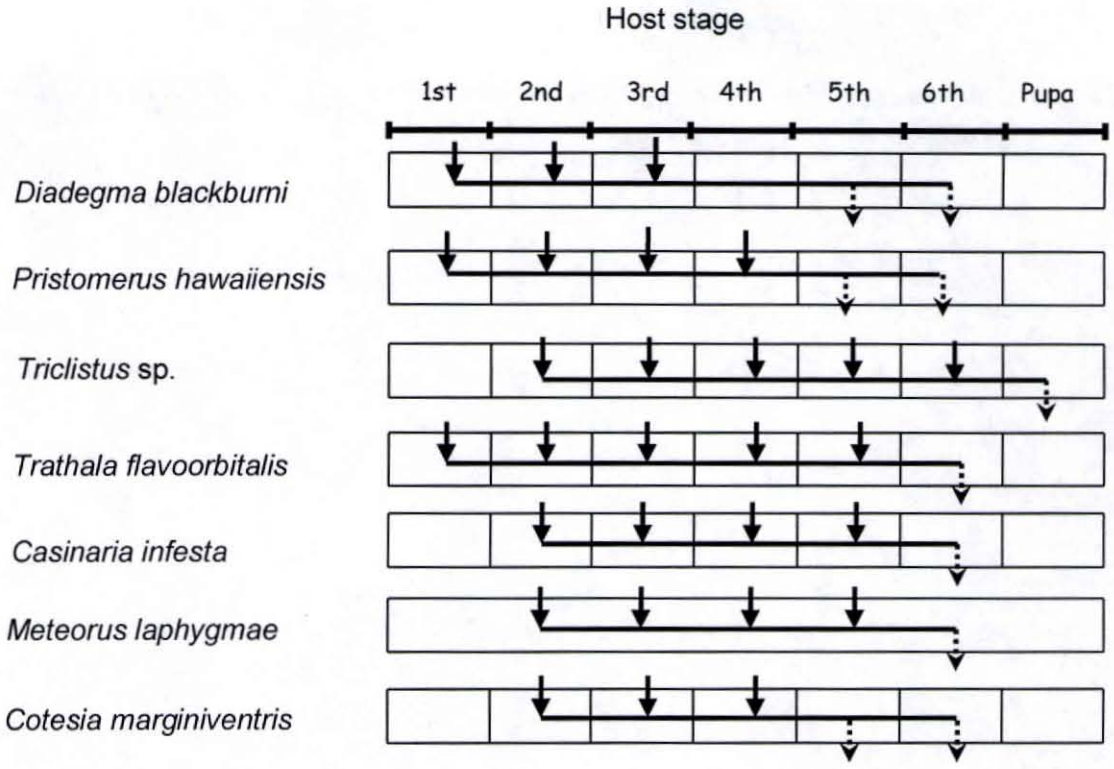

Figure 2.4. Larval stages susceptible to parasitoid oviposition (solid arrows) and stages at which host is killed and parasitoids emerge (dashed arrows). Horizontal lines represent host use by each of the seven parasitoid species divided into segments representing the six larval stadia of *U. stellata* plus the pupal stage.

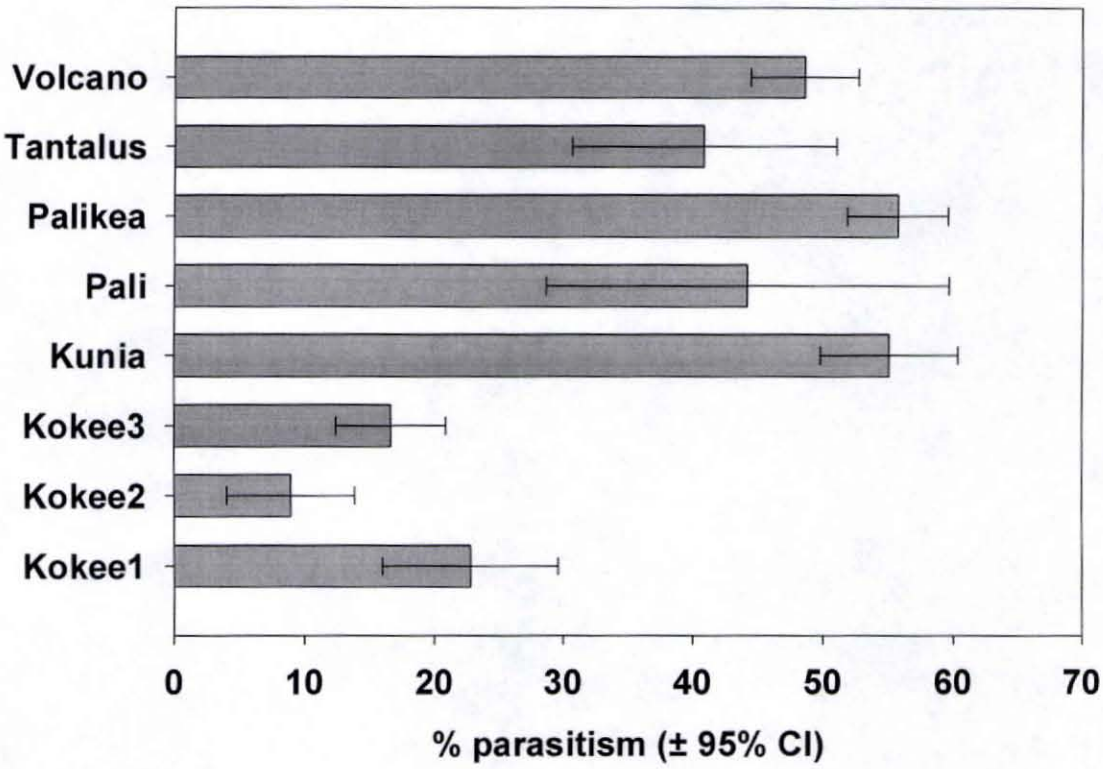

Figure 2.5. Mean (± 95% CI) percentage parasitism of *Udea stellata* by site, for all months and all seven parasitoid species.

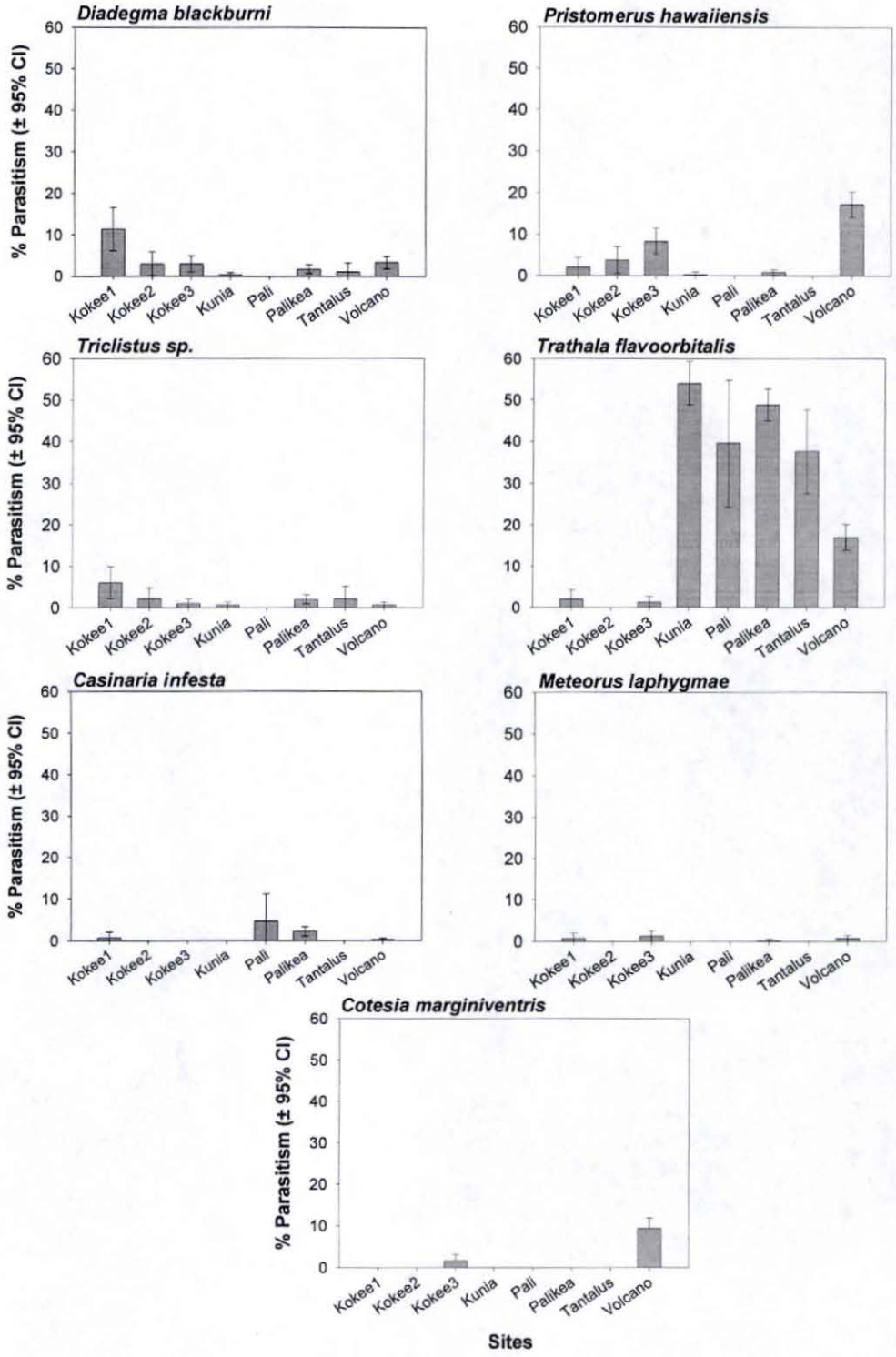

Figure 2.6. Mean (± 95% CI) percentage parasitism of *Udea stellata* by seven parasitoid species for each study site.

CHAPTER 3

QUANTIFICATION OF THE IMPACT OF EXOTIC PARASITOIDS ON POPULATIONS OF THE NATIVE HAWAIIAN MOTH *UDEA STELLATA* (LEPIDOPTERA: CRAMBIDAE) USING LIFE TABLE STUDIES

Abstract

The impact of alien species on native organisms is a cause for concern worldwide, with biological invasions commonplace today. Suppression efforts targeting many invasive species have included introductions of biological control agents. The numerous releases of biological control agents in the Hawaiian archipelago have resulted in considerable concern for non-target impacts, due to high levels of non-target parasitism observed to occur in some cases. This study investigated the impact of introduced Hymenoptera parasitoids on a Hawaiian moth. The endemic Hawaiian moth *Udea stellata* (Butler) has seven alien parasitoids associated with it, two purposely introduced, three adventive, and two of uncertain origin. The objective of this study was to determine the relative contribution of the seven parasitoid species, to the population dynamics of *U. stellata* by constructing partial life-tables. Marginal attack rates and associated *k*-values were calculated to allow comparison of mortality factors between experimental sites. Sentinel larvae were deployed on potted host plants and left in the field for three-day intervals in open and exclusion treatments. In the open treatment, larvae were exposed to natural enemies whereas in the exclusion treatment, natural enemies were excluded by placing a fine mesh over the plants. The factors that contributed to total mortality in the open treatment were: disappearance (42.1 %), death due to unknown reasons during

rearing (16.5 %) and parasitism (4.9 %). The open treatment incurred to significantly higher larval disappearance compared to the exclusion treatment (7.8 %), which suggest that in large part disappearance is the result of predation. Death due to unknown causes was not significantly different in the exclusion treatment (14.9 %) compared to the open treatment, which suggests that parasitism was not the cause of unknown mortality in the open treatment. Adventive parasitoids inflicted greater total larval mortality attributable to parasitism (97.0 %) than purposely introduced species (3.0 %).

Key words: Hawaiian archipelago, non-target impact, population dynamics, biological control.

Introduction

International and interregional commerce continues to break down biogeographical boundaries (Loope and Howarth 2003), and this is accelerating the rate of biological invasions to a degree without precedent. The practice of classical biological control for pest management has been commonly recognized as an effective suppression method for invasive species, and its use was encouraged reduce dependence on insecticides for the management of invasive insect pests. Biological control is an important component of any integrated pest management program as it is not only an option to control invasive species in agricultural settings but also a tool for conservation, when it targets invasive species that threaten native species and natural habitats (Hoddle 2004; Messing and Wright 2006).

Practitioners of classical biological control have traditionally regarded their method as environmentally safe (van den Bosch and Messenger 1973; De Bach 1974, Simmonds and Bennett 1977; Caltagirone and Huffaker 1980) and even though concerns about the potential negative effects of purposely introduced species on endemic fauna were expressed more than a century ago in Hawaii by Perkins (1897), it was only since the 1980's that there has been an increase in concerns about the environmental impact of introduced biocontrol agents on native species in the USA (Howarth 1983; 1991; Gagne and Howarth 1985; Simberloff 1992; Simberloff and Stiling 1996, Henemann and Memmott 2001).

Retrospective studies on biological control introductions provide an important tool in the evaluation of potential non-target effects of future biological control programs. They help build case histories that can provide patterns to aid identify key biological and ecological factors that need to be investigated to provide a robust estimate of the candidate's non-target potential (Louda et al. 2003). Adventive species (species that have been accidentally introduced to a new area) provide a further set of species for developing a greater understanding of non-target impacts, because they also offer opportunities to study impacts of new introductions, albeit accidental, upon indigenous species.

Hawaii provides an excellent set of circumstances to study non-target effects from biological control as well as accidental introductions. The Hawaii archipelago is home to a greater proportion of endemic species than any other place of similar size on earth (Kaneshiro 1995), has a steady rate of arrival of invasive species, and has a long history of biological control introductions; more than 700 species released in the past hundred

years (Funasaki et al. 1988). Hawaii has also been the center of controversy regarding non-target impacts on native and desirable species, and in some cases it has been suggested that extinctions have resulted from mortality caused by purposefully introduced species (Howarth 1983, 1991; Gagné and Howarth 1985). However, the actual impacts of introduced biological control agents on indigenous species have seldom been quantified.

Udea stellata Butler (Lepidoptera: Crambidae) is endemic to Hawaii and is widely distributed throughout the state. The larval stages of this moth feed on the endemic host plants *Pipturus* spp. (Urticaceae) (common name, mamaki) which occur in mesic forest, under fairly variable conditions in terms of canopy cover, disturbance level, presence of invasive plant species and elevation. A separate study (Chapter 2) assessed the parasitoid guild associated with *U. stellata* and quantified field parasitism rates at different sites. Results from these studies showed that the parasitoid guild associated with *U. stellata* larvae includes seven koinobiont solitary endoparasitoids. Two of the parasitoids are listed species of adventive origin: *Casinaria infesta* (Creson), *Trathala flavoorbitalis* (Cameron) (Nishida, 2002); *Triclistus* nr. *aitkeni* is a new adventive species to the Hawaiian Islands; two species were purposely introduced for biological control purposes: *Meteorus laphygmae* (Viereck) and *Cotesia marginiventris* (Cresson) (Nishida, 2002); and two are of uncertain origin. *Diadegma blackburni* (Cameron), listed as adventive (Nishida, 2002) is possibly endemic to Hawaii (Oboyski et al. 2004), and *Pristomerus hawaiiensis* (Perkins) listed as endemic (Nishida 2002) may be adventive to the islands (Fullaway and Kraus 1945; Stein 1983). In the two years of surveys of field parasitism (apparent mortality) reported above, 27.6 % of the total larvae collected (or

42.9 % of the larvae that survived laboratory rearing) yielded parasitoids, and parasitism rates varied significantly across sites (Chapter 2). Adventive parasitoids, notably *T. flavoorbitalis*, rather than purposely introduced species inflicted the majority of the field parasitism observed in samples. It was also noted that in low and low-medium elevations (below 900 m) the parasitoid assemblage was dominated by adventive species, whereas purposely introduced species where detected only from sites above 900 m.

Faunistic surveys provide good background information on parasitoid guild composition, levels of parasitism of samples taken, and seasonal trends in different locations. However, caution should be exercised when interpreting results from field parasitism since these data do not often provide an effective measure of parasitoid impact at the host population level, such as information on density of the host and the ecological role of the parasitoids in the population dynamics of the target or non-target hosts (Van Driesche et al. 1991; Duan and Messing 2000). Field surveys also do not provide information on the role of parasitism relative to other mortality factors influencing fluctuations in population densities. To date, there are indeed few detailed studies quantifying to what extent non-target attacks are impacting the populations of non-target species (Duan et al. 1998; Boettner et al. 1999; Duan and Messing 2000; Benson et al. 2003a; Benson et al. 2003b; Barron et al. 2003; Van Driesche et al. 2004; Johnson et al. 2005). This study aims to determine the relative contribution of parasitoid species with respect to other mortality factors, to the population dynamics of *U. stellata* by constructing life-tables, estimating marginal attack rates and their associated *k*-values, using artificial cohorts (Carey 2001) in the field. Life-tables studies provide important information on the contribution of different mortality factors for life stages over a

generation and among generations, and therefore for understanding the ecological impact of the mortality factors in insect populations. The calculation of marginal attack rates and associated *k*-values allow comparison of mortality factors between experimental sites. The marginal mortality estimates also provide data for a risk-assessment procedure validation in work based on this study and preceding studies.

Materials and Methods

Study sites

Field experiments were conducted at six locations (Table 3.1) on three of the Hawaiian Islands from August 2005 to October 2006. The site on the island of Kauai was located at the Ditch Trail at the Kokee State Park. Three sites were located on the island of Oahu: Kunia and Palikea are located in the Waianae Mountains and are managed by the Nature Conservancy of Hawaii. The last two sites were located on the island of Hawaii, Kipuka Ki at the Hawaii Volcanoes National Park, and Olaa which is located inside the University of Hawaii Volcano Experimental Station adjacent to the Olaa forest. Sites varied in many environmental features such as elevation, level of disturbance by alien species, type of overstory, percentage of overstory and type of understory. All study sites had naturally occurring mamaki plants. Effort was made to deploy sentinel plants in the vicinity of these plants.

Plant material

Potted *Pipturus* spp., locally known as mamaki, grown from seed were used as substrate for sentinel larvae exposed in the field. Plants for each experiment were grown

from seeds that were collected from the respective island in 2004 and 2005. Plants used at all sites were planted in 3.8 l pots and were approximately 50 cm tall when they were deployed at study sites.

Potted plants were chosen instead of wild plants in the field to avoid unobserved "recruitment" of wild larvae that could bias the data collected. Plants were grown at Kauai Research experimental station on Kauai, Gilmore Hall greenhouse facility at the University of Hawaii at Manoa on Oahu, and at the USDA Forest Service facility at Hawaii Volcanoes National Park on Hawaii.

Insect material

To ensure they were not parasitized before field exposure, larvae of *U. stellata* were reared in the laboratory (22°C (±2) and ~62% (±10%) RH) on mamaki plants, until exposed to create artificial 'cohorts' in the field. Since the experiments were conducted on three different islands, three colonies of *U. stellata* were maintained, each initiated from field collected larvae from the respective island; this was to ensure parasitism or other mortality factors measured in each study site were not influenced by the origin of the colonies. To produce larvae of specific instars, host plants were caged with moths that emerged from field-collected larvae. Oviposition plants were replaced every 2-3 days. Larvae emerging from eggs laid were used to initiate colonies. From quantification of larval morphometrics in the laboratory, it is known that *U. stellata* undergoes six larval stages (Chapter 1). All six larval instars of *U. stellata* were used for field experiments. Stages were differentiated by head capsule diameter (Chapter 1).

Field experiments

Sentinel larvae were deployed on potted host plants in the field, where they were exposed to parasitoids and other sources of mortality for a three-day interval. This interval was based on an estimate of the time that larvae take to molt to the next instar under laboratory conditions (Chapter 1). Potted plants with larvae were randomly placed into one of two treatments: exposed to natural enemies (open treatment) and caged to exclude natural enemies (exclusion treatment). Exclusion of predators and larval parasitoids was accomplished by placing a fine mesh bag over the plants. The exclusion treatment served to estimate predation levels and as a control for mortality due to transportation and stress during infestation of the plants. Nine deployments were conducted at six sites, and considered each of them a "generation".

To quantify mortality at each larval instar, groups of larvae of all instars were exposed at the same time, at a density of four larvae of similar instar per plant. Instars were kept separate on different plants. Plants were randomly placed within natural stands of mamaki at all sites. Since the fifth and sixth instar larvae are very mobile, plastic basins painted with fluon at the rim and provisioned with small drainage holes, were placed under each pot to facilitate recovery of larvae, in case they attempted to migrate to pupation sites. After three days sentinel plants were inspected in the field, and larvae found were retrieved and returned to the laboratory. Larvae that were not found during the retrieval were classed as "disappeared". Once returned to the laboratory (22°C and ~62% RH), each larva was placed individually into a labeled plastic container. Feces and old plant material were removed and new plant material was added to the containers every day or every other day depending on the rate of feeding. All larvae were reared to

the adult stage, or until they died or parasitoids emerged. Emerging parasitoids were pinned for identification. Specimens were identified by using unpublished keys to the Hawaiian Ichneumonidae (Beardsley, unpublished) and also by comparing adult voucher specimens with specimens at the Hawaii Department of Agriculture insect collection, University of Hawaii Insect Museum and Bishop Museum. Identifications were confirmed by Dr. David Wahl at the American Entomological Institute. Voucher specimens are deposited at the American Entomological Institute, Gainesville, Florida. Vouchers specimens are kept at the American Entomological Institute. The identification at the species level of *Triclistus* nr. *aitkeni* (Cameron) was done by Dr. Gavin Broad at the National History Museum, London, United Kingdom. Voucher specimens of all species are to be deposited at the Bishop Museum and University of Hawaii at Manoa Insect Museum.

Life-table construction

The number of larvae deployed at each stage and mortality data for each stage for both treatments were used to construct life-tables. Life-table were constructed using the method described by Morris & Miller (1954) and Morris (1963), where l_x denotes to the number of larvae that enter each stage (in this numbers deployed at each stage), dxF is the mortality factors acting during each stage, and dx the numbers dying during each stage. Proportion dying at each larval stage or q_x (also known as apparent mortality) was obtained by diving d_x by the corresponding l_x.

Calculation of marginal attack rates and associated k-values

Life-table data was used to calculate marginal attack rates, *k*-values.

Marginal attack rates

Since assessing the strength of individual mortality factors which act contemporaneously usually is not possible from simple analysis of numbers observed dying, marginal attack rates were calculated. Marginal attack rate is defined as the level of mortality that would have occurred if the agent had acted alone (Royama, 1981; Bellows et al. 1992; Elkinton et al. 1992). Marginal attack rates were calculated as:

$$m_i = 1 - (1 - q)^{q_i/q},$$

where m_i is marginal probability of attack from the *i*th cause, q_i is apparent mortality from the *i*th cause and q is mortality rate from all causes combined (Elkinton et al. 1992).

k-values

'Killing powers' or *k*-values were estimated as the negative logarithm of the estimated proportion surviving in each stage:

$$k_i = -\log 10(1 - m_i),$$

where k_i is the k-value for the ith cause. The sum of all successive mortality factors (k_1 - k_i) equals the total generational mortality (K). The major advantage of k-values as compared to percentages of organisms dying is that k-values are additive: the k-value of a combination of independent mortality processes is equal to the sum of k-values for individual processes.

Proportion of larvae 'disappearing' or dying from unknown causes was compared between exposed and exclusion treatments using two-way contingency table analysis, pooling data from all exposures. The null hypothesis that disappearance and mortality from unknown causes was not associated with exclusion or exposure was tested.

Results

The mortality factors that contributed most to the total generation mortality were: disappearance, unknown cause of mortality during rearing, and parasitism. From the 1975 larvae deployed across all sites in the open treatments, 1144 (57.9%) larvae were retrieve, while 831(42.1%) disappeared. Of the larvae retrieved, 28.5% (326/1144) died due to unknown causes during rearing, 8.5% (97/1144) were parasitized and 63.0% (721/1144) completed their life cycle and emerged as moths. Six parasitoid species were reared from the larvae recovered. Of the larvae parasitized, 1.0% (1/97) were *P. hawaiiensis*, 1.0% (1/97) *C. marginiventris*, 2.1% (2/97) *M. laphygmae*, 7.2% (7/97) *C. infesta*, 40.2% (39/97) *T. flavoorbitalis* and 48.5% (47/97) *T.* nr. *aitkeni.*

In the exclusion treatment a total of 295 larvae were deployed across all sites. Of these, 23 (7.8%) disappeared and 272 (92.2%) were recovered. Since this treatment excluded predators, and *Udea* larvae are not cannibalistic, it is reasonable to say that the

search efficiency exceeded 90%. Of those recovered, 16.2% (44/272) died for indeterminate reasons during rearing and 83.8% (228/272) emerged as moths. The open treatments incurred significantly higher 'disappearance' compared to the exclusion treatment ($\chi^2 = 127.06$; d.f. = 1; $P < 0.0001$). Death due to unknown causes was not significantly different in the exclusion treatment compared to the open treatment ($\chi^2 = 0.367$; d.f. = 1; $P = 0.545$). In the exclusion treatment, total mortality, as well as the impact of individual mortality factors decreased with increasing larval stage. The major mortality factor for all larval instars in exclusion cages was death by unknown causes during rearing.

Life-table data, marginal attack rates and *k*-values across all sites by larval stage for the open and exclusion treatments are presented in Table 3.2. In the open treatment, the major mortality factor across all sites in all larval stages was disappearance, which accounted for > 57% of the mortality. The highest observed *k*-values due to disappearance were in the first and sixth instar (0.366 and 0.317, respectively). The highest attack rates and *k*-values due to parasitism were incurred in the fourth and fifth larval instar (0.045 and 0.078, respectively) in which *T.* nr. *aitkeni* had the highest individual *k*-values (0.031 and 0.064, respectively). *T.* nr. *aitkeni* and *T. flavoorbitalis* were the species with the greatest *k*-values summed over all larval instars. Individual *k*-values for *T. flavoorbitalis* were the highest in second and third larval instar. The killing power due to parasitism by the purposely introduced species *M. laphygmae* and *C. marginiventris* summed across all six larval stages were 0.007 and 0.002 respectively, and together with *P. hawaiiensis* ($k = 0.002$), these were the species that contributed the least to the total mortality ($K = 2.651$).

Table 3.3 shows partial life-tables as well as marginal attack rates and *k*-values for *U. stellata* per larval instar, by site. Disappearance, which is an approximation of predation level, was the mortality factor with the greatest *k*-values at each site and in each larval stage, followed by death caused by unknown reasons. Not all mortality factors followed the same trend in different sites and not all parasitoid species were reared from all sites. In Kauai, mortality due to *T.* nr. *aitkeni* had the greatest *k*-value attributable to parasitism (0.171). Palikea had the highest total mortality summed over all larval stages (3.4014) and also the highest killing power due to parasitism when summed over all parasitoid species (0.373); Olaa had the lowest total mortality (1.835) and Tantalus the lowest killing power due to parasitism (0.031). The purposely introduced parasitoid species were reared from the sites with highest elevation. *Meteorus laphygmae* was reared from Kauai and Olaa and *C. marginiventris* from Kipuka Ki.

Life-tables were also constructed for each of the nine deployment times (nine artificial generations). The *k*-values by mortality factor for each generation are presented in Table 3.4. Mortality due to disappearance had the greatest *k*-value by generation and also the greatest average *k*-value (k_a = 1.8383) and therefore contributed the most to total generational mortality of larvae (K = 2.7086), followed by 'dead by unknown causes' (k_b = 0.6775). Although *T.* nr. *aitkeni* had the highest average *k*-value (k_c = 0.0938) among the various parasitoids, its contribution was only the highest for generations 3, 8 and 9 (Kauai'05, Kipuka Ki and Olaa, respectively), which did not correlate with the highest observed total mortality (K). *Trathala flavoorbitalis* had the second highest average *k*-value (k_c = 0.0716), and had the highest killing power in generations where total mortality (K) was also the highest (generations 1 and 2). *Casinaria infesta, P.*

hawaiiensis, *M. laphygmae* and *C. marginiventris* had the lowest contributions to total mortality in all generations.

Discussion

The occurrence and impact of *U. stellata* mortality factors varied across sites; however, comparison of marginal attack rates and *k*-values among sites and among larval instars in the open treatment showed that larval disappearance (most probably due to predation) was consistently the most important mortality factor. This is consistent with other studies on insects that report high rates of disappearance attributable to predation and or migration (Midega et al. 2005; Barron et al. 2003; Kellogg et al. 2003; Furlong 2004; Johnson et al. 2005). The significantly higher rate of disappearance in the open treatment when compared to the exclusion treatment, and the high recovery rate in the exclusion treatment (search efficiency was > 90%) suggests that predation was the main cause of disappearance in the open treatment.

It is generally difficult to quantify the effects of individual predators under natural conditions. Total mortality from predators can be roughly estimated by comparing rates of individuals recovered from caged and uncaged populations (Watanabe 1981). The significantly larger number of larvae recovered from the exclusion treatment may be attributed to reduced predation in part, but may be also confounded by reduced larval migration, especially in the last larval instars. The high rate of disappearance in the first larval instar was possibly mainly due to predation, whereas the high rate of disappearance in the last instar might have been also due to larval migration. This is supported by field observations; at the time of larval exposure leaves where larvae were placed were tagged

and mostly recovered the first three instars on the same leaf, whereas fourth, fifth and sixth instar larvae were more frequently recovered from leaves other than the tagged ones, and occasionally recovered these older larvae from the plastic basins below the plants. Active predation by Coccinellidae and spiders was observed.

This study attempted to evaluate the relative contribution of parasitism with respect to other sources of mortality as well as the impact of different parasitoids on *U. stellata* larvae. A previous study on parasitism rates of field collected larvae (Chapter 2) reported apparently high parasitism rates (27.6 % of all larvae collected or 42.9% of the larvae that reached adulthood). The present study, which employed rigorous demographic procedures, shows low impact of parasitoid species on *U. stellata* larvae. Even though larvae that died during rearing were not dissected, comparison of mortality due to unknown causes between open and exclusion treatments showed no significant differences, suggesting that other causes such as natural mortality or stress during rearing, rather than parasitism, were the causal factors .

Udea stellata is parasitized by up to seven parasitoid species in various locations throughout the Hawaiian Islands, but in this study only six species were reared from the larvae exposed in the field. *Diadegma blackburni* was not found parasitizing larvae of *U. stellata*, even though this species was occasionally reared from larvae collected from the wild at the same time the studies in some sites such as Kauai, Palikea and Kipuka Ki, albeit at a very low level.

In contrast with purposeful introductions of biological control agents, adventive parasitoids are introduced accidentally, obviously with no planning or concern for impacts upon indigenous species, and greatly outnumber those species that have been

introduced intentionally worldwide, often by a factor of 10 or more (van Lenteren et al. 2006). In this study, mortality resulting from parasitism by adventive species played a more important role as mortality factors than purposely introduced species in the study system. This is consistent with previous studies of non-target impacts in insect biological control (Duan and Messing 1996; Barron et al. 2003; Johnson et al. 2005) that similarly showed that adventive species had far more serious impacts than purposefully introduced species, and is consistent with observations of parasitism of wild larvae where adventive species were dominant (Chapter 2). As in the survey data, *M. laphygmae* and *C. marginiventris* species were only reared from the least disturbed high altitude sites (above 950 m), far from their original release areas and target habitat.

The role of parasitism by the six parasitoid species reared during this study differed among sites. The observed differences among study sites may be the result of contrasting ecological factors such as elevation, level of disturbance, presence of invasive species, and plants comprising the neighboring vegetation in the various sites sampled. A separate study focuses on the environmental factors that may influence the parasitoid assemblage associated with *U. stellata.*

Trisclistus nr. *aitkeni* was the species that in average contributed the most to mortality attributable to parasitism across all sites (48.5% of all parasitoids reared in the study), yet its contribution was restricted to sites at higher elevations (above 980 m). *Trathala flavoorbitalis* had the greatest contribution in low-medium elevation sites (below 800 m). These results are congruent with a previous study (Chapter 2) where *T. flavoorbitalis* was also found to contribute the highest parasitism rates in low-medium altitude sites and made a considerably lower contribution in the higher altitude sites.

However, the average contribution of *T. flavoorbitalis* in the faunistic study (Chapter 2) was by far the most important across all sites, whereas in the present study *T.* nr. *aitkeni* was the species responsible for the highest overall contribution to mortality attributable to parasitism. It may indeed be possible that *T.* nr. *aitkeni* plays a more important role than *T. flavoorbitalis* overall. In the field survey (Chapter 2), of the total number of larvae collected 8.0% were 1st instar, 23.5% 2nd instar, 39.0% 3rd instar, 20.6% 4th instar, 7.1% 5th instar and 1.8% 6th instar. In the present study the highest *k*-values due to parasitism incurred in the fifth larval instar (0.078), where *T.* nr. *aitkeni* played a major role (k_e = 0.064). From this and results from the study on field parasitism (Chapter 2), it is known that *T.* nr. *aitkeni* can parasitize larvae from second to sixth instar and the observed pattern in parasitism is consistent with accumulative parasitism over larval stages, however only larvae deployed from fourth to sixth instar were parasitized in the present study. Results from both studies suggest that *T.* nr. *aitkeni* prefers parasitizing later larval instars, perhaps to avoid competition with other parasitoid species that parasitize earlier instars (such as *T. flavoorbitalis*). Since the numbers of older larvae in the faunistic study were low compared to early-mid age larvae, the role of *T.* nr. *aitkeni* in the previous survey study could have been considerably underestimated. Early-mid larval instars are readably observable in the field, whereas fifth and sixth instar larvae are not regularly found; they are very mobile and drop easily from the leaves at the time of inspection. It is also possible that parasitized fifth and sixth instar larvae will hide and therefore be harder to sample in the field.

It is common to construct life-tables for economically important pests in agricultural and forest settings, providing an important component in the understanding

of the population dynamics of a species (Southwood 1978). The practical application of life-table studies in agroecosystems is to help identify key mortality factors that can be manipulated to reduce pest population densities. In the case of non-target studies in the field of insect biological control, life-table data have been used to assess the impact at the population level of species suspected to have experienced population declines due to attacks by purposely introduced species (Barron et al. 2003, Johnson et al. 2005). The application of such studies has been to the benefit of the practice of biological control itself, by providing quantitative data/evidence that non-target impacts are typically relatively small and build case histories that can help reduce negative impacts of current and future programs. Detailed life-table studies that do not only focus on the role of a specific biological control agent but take into account the full complement of mortality factors that act on a population could also be used in the field of insect conservation, since it can help identify key mortality factors, susceptible stages in the life cycle, and susceptible sites in order to develop efficient conservation strategies (e.g. if exotic predators are identified as important mortality factors then measures for control of those predators could be part of the agenda for conservation of that specific species of concern). Studies of this nature will likely be difficult to conduct, as it will often be difficult to acquire adequate sample sizes from the field to trace individuals through a generation or to start colonies to create artificial cohorts of rare species.

Asquith and Miramontes (2001) examined the composition of the braconid and ichneumonid fauna collected in malaise traps over a two year period in a mesic forest in Kokee State Park on the island of Kauai, in an area adjacent to one of the study sites used in the current study. The majority of wasps collected in their traps were exotic rather than

native species. The authors expressed concern since this site harbors a rich community of endemic species, and called for cessation of biological control introductions. The presence of purposefully introduced species in native areas is highly undesirable, but their presence alone is not evidence of attack on indigenous species, or measure of the severity of any such attack. Many nonnative Lepidoptera species are also present and readily attracted to light traps (Kaufman, personal observation). As in the study by Henneman and Memmott (2001) in a remote site in the island of Kauai spanning a two year period, they found that most of the parasitoid species associated with the immature stages of many native Lepidoptera species were purposely introduced parasitoids and suggested that the introduced species significantly altered food-web structure. From their data it is evident that these purposely introduced species are using native species as hosts, however it is difficult to translate on level impact on the non-target species involved.

Most studies on non-target use and non-target impact in the field of biological control have been done on species observed to have experienced serious population declines, and with clear evidence that these declines have been influenced by attacks of purposely introduced species. Attention has been concentrated on beneficial organisms, organisms of commercial, cultural, or aesthetic significance and some with conservation concern such as saturniid moths (Boettner et al. 1999; Kellogg et al. 2003). The field of biological control (and conservation) would benefit greatly if in addition to selecting a particular nontarget species with obvious high rates of field parasitism, researchers would select particular purposely introduced species such as *M. laphygmae,* known to attack many nontarget species in Hawaii (Funasaki et al. 1988; Henneman and Memmott 2001) with different rates of field parasitism by individual species (Henneman and Memmott

2001), and address a broader question such as: how has *M. laphygmae* impacted non-target native species in Hawai'i in a more general sense. To answer this, not only a particular species known have high rate of field parasitism should be included in the study but also other non-target species with not such evidence of high parasitism rates. This might give insights on what mediates the extent of impact on certain species and give a more realistic picture of the role that purposely introduced species have played on non-target species, and build stronger case histories. The lack of historical data makes it difficult to assess current status of non-target species (Stiling and Simberloff 2000; Follett et al. 2000; Kellogg et al. 2003; Barron et al. 2003, 2004). Current parasitism rates in the field may not reflect accurately the parasitoid's earlier parasitism rates when initially introduced, and original potential to destabilize nontarget populations (Follett et al. 2000), as it is also possible that some non-target species might be absent from part of their original range or have gone totally extinct (Henneman and Memmott 2001).

Udea stellata is not a species of special concern, but one distributed in sites with a wide range of ecological conditions. Results of this study have shown that *k*-values for the different parasitoid species vary among sites. For instance, *T.* nr. *aitkeni* had higher *k*-values at the least disturbed and highest altitude sites (Kokee, Kipuka Ki and Olaa), whereas *T. flavoorbitalis* had highest *k*-values at medium elevation and intermediate disturbance sites. This study system provides the opportunity to investigate environmental gradients, which could provide important information on causes and levels of impact of introduced parasitoids (Chapter 4). The marginal mortalities estimated in this study will contribute quantitative data for the validation of risk assessment procedures (Wright et al. 2005) and it is addressed in Chapter 5.

Table 3.1. Geographical data for *Pipturus* (mamaki) collection sites used for *Udea stellata* life table study (in decimal degrees).

Sites	Elevation (m)	Latitude	Longitude
Kauai			
Kokee	981	22.13159	-159.63171
Oahu			
Tantalus	460	21.32996	-157.82249
Kunia	550	21.46290	-158.09552
Palikea	781	21.41279	-158.09953
Hawaii			
Kipuka ki	1315	19.44339	-155.31633
Olaa	1245	19.47372	-155.26125

Table 3.2. Partial life-table for *U. stellata* combining all sites and all generations by larval instar for open (O) and exclusion (E) treatment

Larval Stage	l_x		Mortality factor (d_xF)	d_x		q_x		Marginal attack rate (m_x)		*k*-value	
	O	E		O	E	O	E	O	E	O	E
First	322	53	Disappeared	158	8	0.491	0.151	0.569	0.170	0.366	0.081
			Unknown death	64	11	0.199	0.208	0.289	0.227	0.148	0.112
			T. flavoorbitalis	3	0	0.009	0.000	0.016	0.000	0.007	0.000
			TOTAL	**225**	**19**	**0.699**	**0.358**	**0.874**	**0.397**	**0.521**	**0.193**
Second	425	48	Disappeared	180	5	0.424	0.104	0.492	0.114	0.294	0.053
			Unknown death	75	8	0.176	0.167	0.246	0.177	0.123	0.084
			T. flavoorbitalis	16	0	0.038	0.000	0.058	0.000	0.026	0.000
			C. infesta	1	0	0.002	0.000	0.004	0.000	0.002	0.000
			M. laphygmae	1	0	0.002	0.000	0.004	0.000	0.002	0.000
			TOTAL	**273**	**13**	**0.642**	**0.271**	**0.804**	**0.291**	**0.447**	**0.137**
Third	392	54	Disappeared	157	4	0.401	0.074	0.448	0.080	0.258	0.036
			Unknown death	50	8	0.128	0.148	0.172	0.154	0.082	0.073
			T. flavoorbitalis	15	0	0.038	0.000	0.055	0.000	0.025	0.000
			C. infesta	2	0	0.005	0.000	0.008	0.000	0.003	0.000
			TOTAL	**224**	**12**	**0.571**	**0.222**	**0.683**	**0.234**	**0.368**	**0.109**
Fourth	338	48	Disappeared	134	2	0.396	0.037	0.467	0.040	0.274	0.020
			Unknown death	59	6	0.175	0.111	0.242	0.151	0.120	0.059
			T. flavoorbitalis	4	0	0.012	0.000	0.019	0.000	0.008	0.000
			C. infesta	1	0	0.003	0.000	0.005	0.000	0.002	0.000
			T. nr. *aitkeni*	15	0	0.044	0.000	0.068	0.000	0.031	0.000
			P. hawaiiensis	1	0	0.003	0.000	0.005	0.000	0.002	0.000
			C. marginiventris	1	0	0.003	0.000	0.005	0.000	0.002	0.000
			TOTAL	**215**	**8**	**0.636**	**0.185**	**0.810**	**0.191**	**0.439**	**0.079**
Fifth	302	46	Disappeared	111	3	0.368	0.065	0.445	0.070	0.256	0.032
			Unknown death	50	6	0.166	0.130	0.233	0.135	0.115	0.063
			T. flavoorbitalis	1	0	0.003	0.000	0.005	0.000	0.002	0.000
			C. infesta	3	0	0.010	0.000	0.016	0.000	0.007	0.000
			T. nr. *aitkeni*	28	0	0.093	0.000	0.138	0.000	0.064	0.000
			M. laphygmae	1	0	0.003	0.000	0.005	0.000	0.005	0.000
			TOTAL	**194**	**9**	**0.642**	**0.196**	**0.842**	**0.205**	**0.447**	**0.095**
Sixth	196	46	Disappeared	91	1	0.464	0.022	0.518	0.023	0.317	0.010
			Unknown death	28	5	0.143	0.065	0.201	0.110	0.098	0.051
			T. nr. *aitkeni*	4	0	0.020	0.000	0.032	0.000	0.014	0.000
			TOTAL	**123**	**6**	**0.628**	**0.130**	**0.751**	**0.133**	**0.429**	**0.061**
									K =	**2.651**	**0.684**

Table 3.3. Partial life-table for *U. stellata* by site and by larval parasitoid

Site	Larval stage	l_x	Mortality factor (dxF)	d_x	q_x	m_x	k-value
Kokee, Kauai	First	80	Disappeared	40	0.500	0.592	0.394
			Unknown death	18	0.225	0.335	0.177
			T. flavoorbitalis	1	0.013	0.022	0.009
	Second	137	Disappeared	44	0.321	0.364	0.196
			Unknown death	28	0.204	0.250	0.125
	Third	140	Disappeared	62	0.443	0.477	0.281
			Unknown death	12	0.086	0.118	0.054
			T. flavoorbitalis	4	0.029	0.041	0.018
	Fourth	98	Disappeared	31	0.316	0.387	0.213
			Unknown death	24	0.245	0.315	0.165
			T. flavoorbitalis	2	0.020	0.031	0.013
			T. nr. *aitkeni*	3	0.031	0.046	0.020
	Fifth	82	Disappeared	28	0.341	0.459	0.266
			Unknown death	13	0.159	0.248	0.124
			T. nr. *aitkeni*	18	0.220	0.326	0.171
			M. laphygmae	1	0.012	0.022	0.010
	Sixth	36	Disappeared	19	0.528	0.594	0.391
			Unknown death	5	0.139	0.211	0.103
			T. nr. *aitkeni*	1	0.028	0.046	0.021
			TOTAL				**2.753**
Kunia, Oahu	First	32	Disappeared	18	0.563	0.629	0.431
			Unknown death	5	0.156	0.241	0.120
	Second	32	Disappeared	15	0.469	0.563	0.359
			Unknown death	5	0.156	0.241	0.120
			T. flavoorbitalis	3	0.094	0.152	0.072
	Third	36	Disappeared	7	0.194	0.253	0.128
			Unknown death	8	0.222	0.284	0.145
			T. flavoorbitalis	6	0.167	0.221	0.109
	Fourth	32	Disappeared	9	0.281	0.330	0.174
			Unknown death	3	0.094	0.125	0.058
			T. flavoorbitalis	1	0.031	0.044	0.019
			T. nr. *aitkeni*	4	0.125	0.163	0.077
	Fifth	32	Disappeared	12	0.375	0.424	0.239
			Unknown death	6	0.188	0.241	0.120
	Sixth	28	Disappeared	14	0.500	0.537	0.334
			Unknown death	3	0.107	0.152	0.072
			TOTAL				**2.576**
Palikea, Oahu	First	96	Disappeared	48	0.490	0.587	0.384
			Unknown death	22	0.224	0.333	0.176
			T. flavoorbitalis	2	0.020	0.037	0.016
	Second	120	Disappeared	62	0.517	0.646	0.451
			Unknown death	21	0.175	0.297	0.153
			T. flavoorbitalis	12	0.100	0.182	0.087
			C. infesta	1	0.008	0.017	0.007
	Third	52	Disappeared	21	0.404	0.489	0.291
			Unknown death	7	0.135	0.200	0.097
			T. flavoorbitalis	5	0.096	0.148	0.069
			C. infesta	2	0.038	0.062	0.028
	Fourth	48	Disappeared	28	0.583	0.715	0.545
			Unknown death	7	0.146	0.269	0.136
			C. infesta	1	0.021	0.044	0.020
			T. nr. *aitkeni*	4	0.083	0.164	0.078
	Fifth	42	Disappeared	17	0.405	0.487	0.290
			Unknown death	7	0.167	0.240	0.119
			T. flavoorbitalis	1	0.024	0.039	0.017
			C. infesta	3	0.071	0.111	0.051
	Sixth	34	Disappeared	14	0.412	0.463	0.270
			Unknown death	6	0.176	0.234	0.116
			TOTAL				**3.401**

Table 3.3. continued

Site	Larval stage	l_x	Mortality factor (dxF)	d_x	q_x	m_x	k-value
Tantalus, Oahu	First	52	Disappeared	25	0.481	0.559	0.356
			Unknown death	11	0.212	0.302	0.156
	Second	52	Disappeared	22	0.423	0.475	0.279
			Unknown death	8	0.154	0.209	0.102
			T. flavoorbitalis	1	0.019	0.029	0.013
	Third	76	Disappeared	35	0.461	0.498	0.299
			Unknown death	9	0.118	0.162	0.077
	Fourth	76	Disappeared	36	0.474	0.532	0.330
			Unknown death	11	0.145	0.207	0.101
			T. flavoorbitalis	1	0.013	0.021	0.009
			T. nr. *aitkeni*	1	0.013	0.021	0.009
	Fifth	76	Disappeared	40	0.526	0.608	0.406
			Unknown death	15	0.197	0.296	0.152
	Sixth	38	*Disappeared*	19	0.500	0.557	0.354
			Unknown death	6	0.158	0.227	0.112
			TOTAL				2.743
Kipuka Ki, Hawaii	First	26	Disappeared	14	0.538	0.567	0.363
			Unknown death	2	0.078	0.113	0.052
	Second	32	Disappeared	20	0.625	0.668	0.479
			Unknown death	3	0.094	0.153	0.072
	Third	36	Disappeared	15	0.417	0.456	0.264
			Unknown death	5	0.139	0.184	0.088
	Fourth	32	Disappeared	17	0.531	0.608	0.407
			Unknown death	3	0.094	0.153	0.072
			T. nr. *aitkeni*	1	0.031	0.054	0.024
			P. hawaiiensis	1	0.031	0.054	0.024
			C. marginiventris	1	0.031	0.054	0.024
	Fifth	26	Disappeared	8	0.308	0.357	0.192
			Unknown death	3	0.115	0.153	0.072
			T. nr. *aitkeni*	3	0.115	0.153	0.072
	Sixth	24	Disappeared	13	0.542	0.610	0.409
			Unknown death	2	0.083	0.135	0.063
			T. nr. *aitkeni*	2	0.083	0.135	0.063
			TOTAL				2.740
Olaa, Hawaii	First	36	Disappeared	13	0.361	0.402	0.223
			Unknown death	6	0.167	0.211	0.103
	Second	52	Disappeared	17	0.327	0.375	0.204
			Unknown death	3	0.192	0.241	0.120
			C. infesta	1	0.019	0.027	0.012
	Third	52	Disappeared	17	0.327	0.364	0.197
			Unknown death	9	0.173	0.213	0.104
	Fourth	52	Disappeared	13	0.250	0.293	0.151
			Unknown death	11	0.212	0.254	0.127
			T. nr. *aitkeni*	2	0.038	0.052	0.023
	Fifth	44	Disappeared	6	0.136	0.164	0.078
			Unknown death	6	0.136	0.164	0.078
			T. nr. *aitkeni*	7	0.159	0.188	0.090
	Sixth	36	Disappeared	12	0.167	0.377	0.206
			Unknown death	6	0.028	0.211	0.103
			T. nr. *aitkeni*	1	0.528	0.039	0.017
			TOTAL				1.835

Table 3.4. Estimated *k*-values by mortality factor per generation

	Mortality factors								
Generation	k_a	k_b	k_c	k_d	k_e	k_f	k_g	k_h	**K**
1	2.2342	0.9497	0.2190	0.1735	0.0968	0.0000	0.0000	0.0000	**3.6732**
2	2.3297	0.6525	0.0172	0.0000	0.0119	0.0000	0.0000	0.0000	**3.0113**
3	1.8791	0.7552	0.0420	0.0000	0.2788	0.0000	0.0126	0.0000	**2.9677**
4	2.2982	0.4458	0.0803	0.0000	0.0502	0.0000	0.0000	0.0000	**2.8745**
5	1.6649	0.6337	0.1998	0.0000	0.0774	0.0000	0.0000	0.0000	**2.5759**
6	1.3929	0.8351	0.0399	0.0000	0.0000	0.0000	0.0000	0.0000	**2.2678**
7	1.5738	0.7721	0.0459	0.0000	0.0399	0.0000	0.0000	0.0000	**2.4317**
8	2.1146	0.4186	0.0000	0.0000	0.1589	0.0240	0.0000	0.0240	**2.7400**
9	1.0575	0.6348	0.0000	0.0000	0.1308	0.0000	0.0120	0.0000	**1.8351**
Sum	16.5449	6.0975	0.6441	0.1735	0.8446	0.0240	0.0246	0.0240	**24.3771**
Average	1.8383	0.6775	0.0716	0.0193	0.0938	0.0027	0.0027	0.0027	**2.7086**

K= Total mortality = $k_a + k_b + k_c + k_d + k_e + k_f + k_g + k_h$

k_a =Disappeared, k_b =Unknown, k_c = *T. flavoorbitalis*, k_d = *C. infesta*, k_e = *T.* nr. *aitkeni.*, k_f = *P. hawaiiensis*, k_g = *M. laphygmae*, k_h= *C. marginiventris*

1= Palikea'05, 2 = Tantalus '05, 3 = Kauai'05, 4 = Palikea'06, 5 = Kunia'06, 6 = Tantalus'06, 7 = Kauai'06 8 = Kipuka Ki'06, 9 = Olaa'06.

CHAPTER 4

ECOLOGICAL CORRELATES OF NON-INDIGENOUS PARASITOID ASSEMBLAGES ASSOCIATED WITH THE HAWAIIAN ENDEMIC MOTH *UDEA STELLATA* (LEPIDOPTERA: CRAMBIDAE)

Abstract

Understanding what ecological factors might predispose indigenous habitats to invasion by invasive species is an important aspect of conservation management and invasive species management, particularly when biological control is considered for suppression of the invasive species. Hawaii presents many options for the study of invasive species and biological control, with high invasion rates and a long history of biological control. Biological control has been the focus of considerable opposition, based on the potential that introduced biological control agents have to impact indigenous non-target species. This study examines environmental factors correlated with infiltration of relatively undisturbed habitats in Hawaii by introduced Hymenoptera parasitoids. An endemic moth, *Udea stellata* (Butler), was used as the non-target organism. Previous surveys of *U. stellata* have shown that the parasitoid assemblage and parasitism rates vary by locality, and suggested that these differences were the result of contrasting ecological factors. The objective of this study was to identify ecological factors that might play a role in determining the structure (in terms of parasitoid species richness and abundance of individual parasitoid species) of the parasitoid assemblage associated with *U. stellata* larval stages in Hawaii. Multivariate analyses, specifically Principal Component Analysis (PCA) and partial Redundancy Analysis (RDA) were used to analyze parasitoid assemblage across a range of habitats varying in environmental

factors. A total of 14 environmental variables was measured at each of the 18 sites located on five of the six main Hawaiian Islands. Results of the RDA analysis showed that only three of the measured environmental variables (*U. stellata* density, elevation, and level of habitat disturbance) significantly explained variability in the parasitoid assemblage among sites. The application of this type of analysis in the field of biological control is discussed.

Keywords: Multivariate analysis, Principal component analysis (PCA), Redundancy analysis (RDA), ecological factors, parasitoid guild.

Introduction

The fact that some insect species purposely introduced as biological control agents in agricultural areas have established populations in relatively undisturbed habitats and maintain populations on native species of hosts or prey has been reported extensively (Gagné and Howarth, 1985; Howarth, 1991; Follett et al 2001, Hennemann and Memmott, 2001;Louda et al 2003; Banko et al., 2002; Oboyski et al 2004). To address this issue and to reduce unintended non-target effects on indigenous and desirable species, biological control practitioners have devoted great effort to develop protocols for host specificity testing in order to predict host ranges for new introductions and in this way reduce their nontarget risk (Neale et al., 1995; Briese et al., 2002; Babendreier et., 2005; Kuhlmann et al., 2006, van Lenteren, et al., 2006). While considerable effort has been made in terms of measuring host-specificity, less effort has been made to determine vulnerable nontarget habitats, based on key ecological variables which may determine the occurrence and parasitism rates of the prospective biological control agents.

Species are pre-adapted to occupy habitats based on their physiological limitations and ecological tolerances. Gradients in elevation, precipitation and other ecological variables create variation in habitat (Banko et al., 2002); this variation can influence different species in different ways. In the case of parasitoids used for biological control programs, knowledge of the ecological preferences of the prospective agent may help predict potential nontarget habitats (and potential success in target habitats) and in this way strengthen the ecological context in the prediction of realized host ranges.

The endemic Hawaiian moth *Udea stellata* (Butler), belongs to the family Crambidae. The larval stages of this moth feed on endemic host plants in the genus *Pipturus* (Urticaceae) (common name, mamaki) which occur under fairly variable conditions in terms of canopy cover, disturbance level, presence of invasive plant species and elevation. Previous studies elucidated that the parasitoid assemblage associated with *U. stellata* comprises seven koinobiont endoparasitoids: three adventive species, two purposely introduced species and two of unknown origin (Chapter 1).

Previous work showed that the composition of the parasitoid assemblage and field parasitism rates by individual parasitoid species varied significantly among study sites of varying ecological conditions (Chapter 2). The observed differences among study sites may be the result of gradients in ecological factors such as elevation, level of disturbance by alien plants species, host plant density, density of insect host, and percentage of canopy cover. Results presented in previous chapters have shown that adventive parasitoids rather than purposely introduced ones were responsible for the greater part of *U. stellata* apparent mortality (Chapter 2) and actual mortality (Chapter 3) observed. At low and low-medium elevations (between 240 and 900 m), the parasitoid assemblage was

dominated by adventive species. The two purposely introduced parasitoids that do attack *U. stellata* were present in relatively undisturbed sites on the islands Kauai and Hawaii, and never from lower elevation and ecologically disturbed sites.

The present study uses multivariate statistical analyses to analyze patterns in species composition, and to elucidate the influence of environment on parasitoids and parasitoid assemblages. Multispecies interactions are notoriously difficult to analyze in a clear and meaningful manner; multivariate methods can be used to unravel patterns in complex ecological data sets (Leps and Smilauer 2003). Unlike univariate analysis, multivariate analysis deals with multiple response variables (species composition data) and multiple explanatory variables (environmental data) simultaneously. Using these techniques it is possible to identify underlying trends or gradients in multivariate data that correlate with assemblage structure, which is often impossible with univariate techniques.

The primary objective of this study was to identify those ecological factors that likely play a role in influencing the parasitoid assemblage associated with *U. stellata* and parasitoid species densities.

Three key questions were addressed in this study:

1. Are there temporal patterns in the composition of the parasitoid assemblage associated with *U. stellata*?
2. Do any of the ecological factors quantified account for the variation in parasitoid assemblage and individual densities?
3. What ecological factors are correlated with *U. stellata* density?

Materials and Methods

Study sites and sampling procedure

In total, 18 sites were included in this study, located on five of the six main Hawaiian Islands (Table 4.1). At each site, standardized sampling of larvae was conducted four times: April 2006, July 2006, October 2006 and January 2007. At each sampling time 30 leaves per plant were searched for *U. stellata* larvae in the Oahu sites, whereas 60 leaves per plant were searched in sites located in other islands. The discrepancy in number of leaves searched was due to differences in density of leaves per plant and size of plants. The data were standardized as number of larvae per leaf. The number of plants sampled per site varied from 10 to 15; this number was dictated by the abundance and accessibility of plants at each site. The number of larvae found during sampling was recorded, and larvae were collected for rearing in the laboratory. Larvae were reared in the lab (22°C (± 2%) and ~62% (± 10%) RH) until parasitoids emerged, or until they died or metamorphosed to adults. Emerging parasitoids were identified and their density per leaf was calculated. Specimens were identified using unpublished keys to the Hawaii Ichneumonidae (compiled by J.W. Beardsley) and also by comparing adult voucher specimens with specimens at the Hawaii Department of Agriculture insect collection, University of Hawaii Insect Museum and Bishop Museum. Identifications were confirmed by Dr. David Wahl at the American Entomological Institute (Gainesville, Florida). Vouchers specimens are kept at the American Entomological Institute. The identification at the species level of *Triclistus* nr. *aitkeni* (Cameron) was done by Dr. Gavin Broad at the National History Museum (London, United Kingdom). Voucher

specimens of all species are to be deposited at the Bishop Museum and University of Hawaii at Manoa Insect Museum.

Throughout this paper, "species data" refers to species richness and density (frequency of encounter – an index of abundance) of parasitoids. The data comprises a species X sample site data matrix (Table 4.2), where parasitism data for each sample site is given as a measure of density (number of larvae that yielded a respective parasitoid by leaf). The parasitoid species reared from *U. stellata* larvae and the codes used for them in the figures are presented in Table 4.3.

Environmental variables

At each sampling site, putative explanatory variables (or environmental variables) (Table 4.4) were quantified to characterize the site and to attempt to identify those factors structuring the parasitoid assemblage and influencing *U. stellata* parasitism rates under the range of environmental conditions sampled. Minimum temperature, rainfall, percentage overstory, leaf nitrogen content, leaf wood content (percentage of leaf dried weight) and elevation were analyzed as quantitative variables; host plant abundance, site disturbance, island, date of sampling and land use/land cover (LULC) were categorical variables; plant canopy type and distance to agricultural land were entered as indicator variables (also called dummy or binary variables). Table 4.5 shows the data for each of the measured explanatory variables by site. Table 4.6 lists the plant species in field plots.

Shapefiles (Geographical Information System databases) with data on maximum and minimum temperature and precipitation were obtained from the PRISM Group, Oregon State University, http://www.prismclimate.org (2007). Shapefiles containing

information on land cover and land use (LULC), canopy type and distance to agricultural land were obtained electronically from the office of planning, state of Hawaii (http://hawaii.gov/dbedt/gis/). ArcGIS (ArcGIS 9, ESRI 2006) was used to map and retrieve data from shapefiles.

Data analysis

Ordination techniques were used to explore relationships between parasitoid assemblages and environmental factors. Ordination provides a graphical summary of the data, and aims at representing sample and species composition relationships as faithfully as possible in a low-dimensional space (Gauch 1982). More recent developments in ordination techniques have added many quantitative analyses to the basic graphic summaries, allowing in depth investigation of species-species and species-environment associations (Ter Braak and Smilauer 2002). When using ordination methods, an important first step is the decision whether to apply linear or unimodal models in the analyses. Model selection is usually made on the basis of the gradient length estimated in a preliminary Detrended Correspondence Analysis (DCA), which estimates the heterogeneity in community composition (Leps and Smilaur 2003, Ter Braak 1995, Legendre and Legendre 1998). A unimodal model (DCA gradient length ≥ 4) assumes that the species have an optimum in the environmental gradients measured, whereas a linear response (DCA gradient length < 4) assumes that the species have a monotonic response (Leps and Smilauer 2003). Results from a preliminary DCA of the parasitoid assemblage associated with *U. stellata* showed short gradient lengths of the ordination axes (< 1.22), supporting the use of linear ordination methods for further analysis of the

data. Therefore, Principal Component Analysis (PCA) was chosen for indirect gradient analysis and Redundancy Analysis (RDA) for direct gradient analysis. Indirect gradient analyses are ordination methods that utilize only the species data. If environmental data are included, the indirect analysis uses them indirectly as an interpretative tool with respect to the species ordinations. PCA involves a rigid rotation of a multidimensional cloud of points (the original data matrix), and by doing so it creates new autocorrelated (hypothetical) variables that are linear combinations of the original dataset and projects them into a reduced numbers of dimensions (axes), which maximally account for the structure (their relative positions) of the original cloud of points in multidimensional space (Gauch, 1982). The first axis, or principal component, accounts for the majority of the variance in the ordination, subsequent axes account for the remaining variance.

Direct gradient analysis integrates techniques of ordination and multiple regression. This method utilizes species data (response variables) and environmental/ecological data (explanatory variables). The ordination process is directly influenced (or constrained) by the set of explanatory variables and seeks to determine the axes through the data points that are best explained by a linear combination of explanatory variables (canonical axes). There are as many canonical axes as there are explanatory variables in an analysis (Legendre and Legendre 1998). By using both indirect and direct gradient analysis, it is possible to detect if important explanatory variables were not taken into consideration for the direct gradient analysis. If the results of both analyses are incongruent, then important variables were omitted.

The analyses were conducted using CANOCO 4.5 and CanoDraw software (Ter Braak and Smilauer 2002). Since the original density values included many zeros and

were small (between 0.0 and 0.1), species data were log (100x + 1) transformed to stabilize variance. Only the first two axes in the PCA and RDA were retained for interpretation since they explained most of the variance. PCA was performed to examine patterns of community structure based solely on species abundance among sites. RDA was performed to identify which of the measured environmental variables were responsible for the variation in the species data.

The variables contributing most to explaining variation in the data for analysis with RDA were identified with the CANOCO forward selection procedure. This procedure ranks variables according to the amount of variance in the response variables they explain. The statistical significance of each selected variable was tested for deviation from randomness by Monte-Carlo permutation tests (499 permutations; $P < 0.05$). Autocorrelated variables were identified by examining the variable inflation factors and excluded from the analysis. The variance inflation factor is a diagnostic tool in CANOCO which measures how much the variance of the canonical coefficients is inflated by the correlations among explanatory variables. Inflation factors greater than 20 suggest that a variable is highly correlated with another variable and does not contribute unique information (Ter Braak, 1988). Intra-set correlations were examined to evaluate the significance of the correlations between environmental variables and the canonical axes. Ordination diagrams were used to visualize the results of the analyses.

The scaling used for all the analyses were based on inter-species correlation. To find out which environmental variables were correlated with *U. stellata* density (larvae/leaf), explanatory data (or environmental data) were entered as primary data in

the PCA, since the cosine of the angles between two vectors provide an accurate estimation of the correlation.

Results

No new larval parasitoid species were recorded during these studies besides the ones already reported in Chapters I and II and III, even though sampling was conducted over a larger area than the previous studies. This suggests that the sampling effort was adequate to include all species of parasitoids attacking *U. stellata.*

A first PCA and RDA analyses conducted on the entire data set showed that date of sampling made no significant contribution to explaining the variation in species data (species richness and density of individual species by sites) (Table 4.7), therefore subsequent preliminary analyses were done by pooling the species data across sampling dates, by sites. For the preliminary partial RDA, climatic data used were averaged for all seasons. The final partial RDA was done only with significant variables (Table 4.7).

PCA on species data

The amount of variance accounted for by each of the principal components is given by its eigenvalue. The eigenvalue of each of the principal components (axis) measures the importance of the ordination axis (principal component), expressed as the amount of variability accounted for in the primary data (response variables, the species data). The first two PCA axes accounted for 87% of the variability in the species data (represented in the ordination biplot, Figure 4.1), and cumulatively, PCA axes 1 - 4 accounted for 89.9% of the variability in the species data. Since PCA is an indirect gradient analysis, the axes are constructed without reference to ecological factors. When

ecological data are available, the axes are first constructed and thereafter related to environmental variation. The sum of all canonical eigenvalues indicated that a total of 87% of the variability in species data could be explained if all measured explanatory variables (ecological variables) are used. The percentage variance of the species-environment relationship values (Table 4.8) represents percentages of the sum of all canonical eigenvalues (87 %). PCA axes 1-2 explained 89.9% of the variation of the species-environment relationship whereas PCA 1 - 4 accounted for 98.0% in the species-environment relationship (Table 4.8).

In the PCA ordination biplot (Figure 4.1) species are represented by arrows (vectors) and sample sites are represented by symbols. Species vectors point in the direction in which the species abundance (e.g. density) increases, and the vector's length in this case does not reflect its relative density with respect to the other species. Angles between species vectors reflect their correlation, which can be estimated as the cosine of the angle between any two vectors; arrows (species vectors) pointing in the same direction have a large positive correlation, those pointing in opposite directions are negatively correlated, and arrows at right angles means that the occurrence of those species are not correlated (or are independent from each other). Species plotted close to sample sites on the diagram are associated with those sites more strongly than with sites further from their plotted position.

Occurrence of *D. blackburni* (Sp1), *P. hawaiiensis* (Sp2) and *C. marginiventris* (Sp7) were highly correlated, suggesting that they probably have similar ecological requirements or tolerances. They were primarily associated with the sample sites Kokee 3 on Kauai and Kipuka Puaulu, Kipuka Ki 1, 2, 3 on Hawaii, all relatively undisturbed

sites. The association of *D. blackburni* and *P. hawaiiensis* with these less disturbed sites resulted in these sites being clustered separately on PCA2, from the other sampling sites in the ordination. Occurrence of *T. flavoorbitalis* (Sp4), *C. infesta* (Sp5) and *M. laphygmae* (Sp6) were also highly correlated. The sites Tantalus on Oahu, Olaa on Hawaii and Kula on Maui were mainly associated with *T. flavoorbitalis* (Sp4). The Palikea site was dominated by *Triclistus sp.* (Sp3) and *C. infesta* (Sp5) and *T. flavoobitalis* (Sp4). Axis 1 of the PCA accounted for 75.6% of the variation in parasitoid assemblage structure (in terms of species richness and density of individual species), and was primarily accounted for by level of disturbance ($r = 0.521$, $P < 0.05$) and Axis 2 with *U. stellata* density, ($r = 0.629$, $P < 0.05$). The PCA ordination (Fig. 1) showed a clear gradient in parasitoid assemblage structure in sampling sites ranging from undisturbed to disturbed (left to right on Axis 1), with spp. 3 – 5 associated most consistently with sites of higher disturbance.

PCA on environmental data

PCA results for the correlation between *U. stellata* density and other explanatory variables is presented in Figure 4.2. The first two principal components explained 99.9% of the variability in environmental variables (treated as response variables) among the 18 sites. The environmental variables were analyzed separately from the species data here, so as to seek correlations among them and *Udea* occurrence. The variables are represented by arrows in the biplot, and their length indicates the amount of variance accounted for by each variable in each component. The cosine of the angle between *U. stellata* vector and other environmental factors approximates their correlation (Table 4.9).

Udea stellata density was strongly positively correlated ($P < 0.001$) with host plant abundance, elevation, nitrogen content of the leaves and distance to agricultural land. Minimum temperature and level of disturbance were strongly negatively correlated with *U. stellata* density ($P < 0.001$, Table 4.9), which means that higher *U. stellata* densities tend to be associated with lower minimum temperature (at higher elevation) and with sites with a low level of disturbance.

Partial RDA

In RDA analysis (the canonical form of PCA), the axes are constrained to be linear combinations of the environmental variables (Rao 1964), and therefore represent only the variation that can be explained by the measured environmental variables. The same rationale explained above for PCA on species data was followed to interpret the results of the partial RDA analysis presented in Table 4.8 and read the ordination diagrams (Figure 4.3 and 4.4), with the exception that these diagrams included environmental factor vectors. In the partial RDA ordination triplot (sites, parasitoids and environmental variables), environmental variables are also represented by arrows (dashed). The longer the environment factor vector, the greater the contribution is has in explaining an environmental gradient, the smaller the angle between an environmental arrow and an axis, the more closely correlated that variable is with the gradient of points plotted. The cosine of the angles between species and explanatory arrows, and between species arrows or explanatory arrows themselves, is equivalent to their correlation (Legendre and Legendre, 1998; Leps and Smilauer 2003).

Considering *U. stellata* density as a main explanatory variable in the analysis caused all the parasitoid species to be clustered together, as they are intimately associated with *U. stellata*, this precludes clear interpretation of the other selected explanatory variables. The effect of *U. stellata* density was therefore factored-out by considering it a covariable in the analysis (partial RDA).

A preliminary partial RDA was conducted with all measured environmental variables (Table 4.8 and Figure 4.3). Preliminary partial RDA axes 1-2 accounted for 77.9% of the variation in the species data (represented in Figure 4.3), and axes 1-4 explained 84.8 % of variability in the species data. The sum of all canonical eigenvalues indicated that all measured environmental factors could explain 76% of the variability in species data. Axes 1-2 explained 90.1% of the species-environment relationship (Table 4.8). The ordination triplot (Figure 4.3), shows high levels of collinearity for some environmental variables. The positions of the arrows for environmental factors suggest that host plant abundance and elevation are highly positively correlated. Level of disturbance of the site, minimum temperature and canopy type are positively correlated which mean that higher levels of disturbance by alien plants tend to be associated with higher minimum temperature (at lower elevation) and with alien canopy type. A closer inspection of the CANOCO Log View confirmed that these variables were indeed correlated.

Of all explanatory variables considered for inclusion in the gradient analysis, disturbance level, elevation and *U. stellata* density were the only variables that contributed significantly to explaining the variation in the species data among sites based on the results of the forward selection diagnostic tool (Table 4.7). These variables also

showed low inflation factors (low collinearity) among the selected variables, and therefore were retained for further analyses.

Results of the partial RDA analysis conducted on significant variables only are presented in Figure 4.4 and Table 4.8 Axes 1 and 2 explained 60.5 % of the total variation in the species data among sites (Figure 4.4). The environmental variables included explained 54% of the total variability in the species data. Axes 1-2 accounted for 100% of the species-environment relation. The ordination triplot shows the negative correlation between elevation and level of disturbance in the study sites (Figure 4.4).

As in the PCA ordination diagram (Figure 4.1), the horizontal axis (Axis 1) of the partial RDA ordination diagram (Figure 4.4) represents a disturbance gradient (less to more disturbed: left to right). Level of disturbance contributed significantly in defining Axis 1 ($r = 0.75$, $P < 0.05$). The vertical axis (Axis 2) of the partial RDA represents an elevation gradient, but it did not contribute significantly in defining that axis (since this axis only explained 1.3 % of the variation in species data). Visual examination of the angles between species arrows and environmental variable arrows in the partial RDA ordination triplot (Fig. 4) show that *C. infesta* (Sp5) and *Triclistus* sp. abundance was strongly negatively correlated with elevation, *Triclistus* sp. occurrence was strongly positively correlated with level of disturbance, whereas *P. hawaiiensis* (Sp2) and *C. marginiventris* (Sp7) occurrence was strongly negatively correlated with level of disturbance (they are associated with *U. stellata* at low level of disturbance).

Discussion

The fact that no new parasitoid species were reared during the samplings even though field sites were increased suggests that these seven parasitoids are probably the only ones regularly associated with the larval stages of *U. stellata* in Hawaii. This provides confidence that the sampling in this study provided representative samples of the parasitoids.

Of the seven parasitoid species associated with *U. stellata* larvae, *T. flavoorbitalis* was the one with the highest frequency of occurrence (Table 4.2). *Trathala flavoorbitalis* was principally associated with disturbed sites and its occurrence was strongly positively correlated with *Triclistus* nr *aitkeni* and to a lesser degree with *M. laphygmae* and *C. infesta* occurrence. Sampling was conducted over a period of one year (four times at three-month intervals). A preliminary analysis of the data did not show significant temporal changes in the structure of the parasitoid assemblage, and thus showed no seasonal trends. This corroborates the data reported in Chapter 2 (covering a period of two years), where it was shown that *U. stellata* density also did not have a marked seasonal trend at most sites (except for the higher altitude sites) which might explain the monotonic response of the associated parasitoid species found in this study which covers a period of one year. This further justifies the use of linear models (monotonic response) in the current analysis of the data, rather than unimodal models, as suggested by the results of DCA (short gradient). This study was conducted to identify underlying ecological factors that play a role in determining the structure of the parasitoid community associated with *U. stellata* throughout the Hawaiian Islands. Of the 14 environmental variables measured at each of the 18 sites, *U. stellata* density, level of

ecological disturbance in study sites and elevation significantly explained the variation in parasitoid community composition. The purposely introduced species, *C. marginiventris* and *M. laphygmae*, attacking the nontarget host *U. stellata* predominated in high elevation sites with low level of disturbance. These polyphagous species, which can occur across a long gradient of elevation, were released to control the sugarcane pest *Spodoptera exempta* in low-elevation agricultural lands and were effective in controlling the intended target host (Lai, 1988). *Cotesia marginiventris* and *M. laphygmae* have also been implicated in attacks on many nontarget species in remote native habitats in Hawaii (Funasaki et al., 1988, Henemman and Memmott, 2001). Even though these latter species are present at low elevation sites in Hawaii (Peck et al. 2008), it is possible that presence and abundance of alternative, possibly preferred hosts, may have precluded the association of these wasps with *U. stellata* at low elevation sampling sites. The association of these species with *U. stellata* at high elevation sampling sites may not necessarily infer that these species have a preference for these sites, but perhaps lack of invasive alternative hosts and abundance of indigenous hosts.

Level of ecological disturbance contributed significantly to defining the environmental gradients identified in the analyses. Level of disturbance refers to the degree of infestation by alien plants in sampling sites. Most of the parasitoid species were associated with less disturbed sites, which are typically located at higher elevations, with some exceptions. In this study, some of the high altitude sites were coded as intermediately disturbed due to the predominance of alien plant species (e.g. Makawao, Kula, Kamakau 1). It is possible that disturbed sites may also be exploited by a greater number of exotic predators such as ants, which may have an impact on the level of

abundance of the insect host itself, and in this way indirectly affect parasitoid occurrence. Level of disturbance also seems to reflect level of host plant abundance. As seen in the PCA on the environmental data, level of disturbance was strongly negatively correlated with host plant abundance. Sites with medium and high disturbance levels were associated with low host plant abundance, which as suggested above, may have also had an influence on the abundance of *U. stellata* and thus their associated parasitoid species.

In a previous chapter (Chapter 2) it was reported that sites with low abundance of insect hosts also had fewer parasitoid species associated with *U. stellata* in those sites. This is confirmed by the results of the present study, and supports the idea that relative abundance of the host is of primary importance in determining parasitoid species richness (Price 1970; Sheenan, 1994; Hawkins et al., 1990; Mills, 1992). In Chapter 2, it was also suggested that host plant abundance had a direct influence on *U. stellata* density. Results of the PCA using environmental variables as primary data showed that host plant abundance was indeed positively correlated with *U. stellata* abundance, and even though it did not have a significant direct effect in defining the structure of parasitoid guild it had an indirect effect by affecting *U. stellata* abundance.

The degree of congruence between the indirect (PCA) and direct (partial RDA) gradient analysis provides a means to check whether important variables were omitted from the analyses. The partial RDA analysis explained substantial variance (~ 70%) in the species data used in the PCA analysis (Table 4.8), confirming that the explanatory variables used were good predictors of the ecological gradients structuring the parasitoid guild composition locally. The unexplained variance could be ascribed to the fact that all parasitoid species involved in this system do not have restricted diets (Zimmerman, 1958

a,b,c ; Funasaki et al., 1988; Henemann and Memmott, 2001) and it is possible that relative density of alternative hosts and the level of preference over alternative hosts influenced the structure of the parasitoid assemblage in different localities. Considering that most of the parasitoids in this study were adventives species, this aspect will probably be less important in the case of prospective biological control agents intended for new introductions, since most of them should be host specific parasitoid species, determined during quarantine screening.

Multivariate techniques have been used in the field of entomology to elucidate how ecological factors structure community composition and this information has been used for management and conservation planning and decision-making (Spitzer et al., 1997, Dennis et al., 1997, Antvogel and Bonn 2001, Progar and Schowalter 2002, Summerville et al., 2005, Sadler et al 2006, Crist et al., 2006, Small, 2006). Sujii et al. (1996) proposed the use of multivariate techniques (indirect gradient analysis) as a tool to help selection of weed biological control agents when conducting field surveys for potential natural enemies of the target weeds. They proposed seeking potential candidate species on the basis of appropriate environmental characteristics (identified using multivariate techniques), which should ensure pre-adaptation to the intended area of introduction. In their study, Sujii et al. (1996) underscored the relevance of this type of community analysis to identify weed biological control agents that could be considered for multiple introductions, based on species co-occurrence, frequency of encounter and feeding guild, which may identify species that are unlikely to compete with each other.

With univariate techniques it would be possible to identify variables that influence individual species; with multivariate techniques it is possible to identify

meaningful variables responsible for community structure. The identification of underlying ecological factors that determine natural enemy community structure can help biological control practitioners reconstruct synthetic communities through classical biological control, determine the introduction strategies (single vs. multiple introductions) as well as select appropriate 'strain' of natural enemies, based on their ecological compatibility with the new area of introduction.

Environmental gradients identified as important in structuring natural enemy communities (the pool of potential biological control agent candidates) in the area of origin of the target host could help characterize areas that are likely to be susceptible for invasion in the intended area of introduction (non-target habitats) by a specific candidate species (or group of species in case of simultaneous introductions from the same locality). These data can be used to assess the risk that the potential species for new introductions will overlap spatially. Nevertheless, the area of origin of prospective biological control candidates may not include all potential suitable habitats and conditions that the parasitoids being considered might be pre-adapted to occupy, and which may be present in the place of intended introduction. Information on spatial distribution and habitat preference of species within and outside their native range can provide more meaningful information in the identification of potentially susceptible non-target habitats. Data from places with introductions of the species of interest can provide greater understanding and predictive power for other prospective places of introduction. To make this type of information useable, biological control workers and other biologists need to capture ecological information when reporting records of parasitism in the area of origin and novel areas of introduction.

Current methods used to create preliminary lists of nontarget species for host range testing often rely on published records of parasitoids reared from various hosts. These records are usually detached from any ecological information that might be relevant to the herbivore parasitoid interactions (Godfray, 1994). With the overwhelming task that biological control practitioners currently face, to incorporate ecological information in the prediction of host ranges, information on the key ecological factors that may determine parasitoid occurrence and associated parasitism rates should be of great value in order to detect possible habitats that may harbor nontarget species, and should contribute significantly in assessing the risk posed by an introduction.

Table 4.1. Geographical data (in decimal degrees) for collection sites sampled for *Udea stellata* larvae and associated parasitoids. Sites codes match with Figure 4.1, 4.3 and 4.4.

Island	Site	Code	Elevation (m)	Latitude	Longitude
Kauai	Kokee 1	K1	981	22.13159	-159.63171
	Kokee 2	K2	1046	22.12790	-159.63472
	Kokee 3	K3	1113	22.12135	-159.63582
Oahu	Kunia	Kn	550	21.46290	-158.09953
	Pali	Pa	372	21.36579	-157.79398
	Palikea	Pk	781	21.41279	-158.09953
	Hawaii Kai	Hk	276	21.31462	-157.72592
	Tantalus	Ta	460	21.32996	-157.82249
Molokai	Kamakau 1	Km1	1107	21.13027	-156.92167
	Kamakau 2	Km2	1150	21.11158	-156.9069
Maui	Iao Valley	Iv	241	20.88224	-156.53708
	Kula	Ku	942	20.79732	-156.30168
	Makawao	Mk	1152	20.81267	-156.27004
Hawaii	Kipuka Puaulu	Kp	1229	19.43742	-155.30328
	Kipuka Ki 1	Kk1	1272	19.44083	-155.30845
	Kipuka Ki 2	Kk2	1315	19.44339	-155.31633
	Kipuka Ki 3	Kk3	1368	19.44243	-155.32698
	Olaa	Olaa	1245	19.47372	-155.26125

Table 4.2. Species X sample site data matrix (species data). Sample site scores are given in number of larvae that yielded a respective parasitoid per leaf

Sample site codes	Species codes						
	Sp1	Sp2	Sp3	Sp4	Sp5	Sp6	Sp7
K1	0.0011	0.0000	0.0006	0.0000	0.0000	0.0000	0.0000
K2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
K3	0.0000	0.0033	0.0000	0.0000	0.0000	0.0000	0.0017
Kn	0.0000	0.0004	0.0000	0.0042	0.0000	0.0000	0.0000
Pa	0.0000	0.0000	0.0000	0.0029	0.0007	0.0000	0.0000
Pk	0.0000	0.0017	0.0033	0.0097	0.0025	0.0000	0.0000
Ta	0.0008	0.0005	0.0000	0.0132	0.0000	0.0000	0.0000
Kp	0.0004	0.0010	0.0002	0.0023	0.0000	0.0002	0.0031
Kk1	0.0013	0.0004	0.0004	0.0033	0.0000	0.0004	0.0033
Kk2	0.0000	0.0021	0.0000	0.0004	0.0000	0.0000	0.0021
Kk3	0.0000	0.0004	0.0000	0.0021	0.0000	0.0000	0.0000
Ol	0.0000	0.0008	0.0008	0.0054	0.0000	0.0000	0.0000
Hk	0.0000	0.0000	0.0000	0.0017	0.0000	0.0000	0.0000
Km1	0.0000	0.0000	0.0000	0.0057	0.0000	0.0000	0.0000
Km2	0.0000	0.0000	0.0000	0.0075	0.0004	0.0004	0.0000
Iv	0.0000	0.0000	0.0000	0.0021	0.0000	0.0004	0.0000
Mk	0.0000	0.0000	0.0005	0.0067	0.0000	0.0016	0.0000
Ku	0.0000	0.0000	0.0000	0.0133	0.0000	0.0000	0.0000

Table 4.3. Parasitoid species codes and origin status (adventives = accidental introduction; Introduced = introduced purposefully as a biological control agent)

Family	Species	Code	Origin status
Ichneumonidae	*Diadegma blackburni* (Cameron)	Sp1	Unknown
	Pristomerus hawaiiensis (Perkins)	Sp2	Unknown
	Triclistus nr. *aitkeni* (Cameron)	Sp3	Adventive
	Trathala flavoorbitalis (Cameron)	Sp4	Adventive
	Casinaria infesta (Cresson)	Sp5	Adventive
Braconidae	*Meteorus laphygmae* (Viereck)	Sp6	Introduced
	Cotesia marginiventris (Cresson)	Sp7	Introduced

Table 4.4. Explanatory variables measured at each of the 18 collection sites

Explanatory variables	Description
Minimum temperature	Monthly mean (°C)
Rainfall	Monthly mean (mm)
Udea density	Expressed as larvae per leaf (frequency of encounter – an index of abundance)
Host plant abundance	Coded as: 1 = low (< 4 plants per 10 x 10 m plot); 2 = medium (between 4 and 8 plants per 10 x 10 m plot); 3 = high (> 8 plants per 10 x 10 m plot)
Site disturbance (presence of alien plants)	Coded as: 1 = low (< 60% of the plants in each plot were alien species), 2 = medium (60-80% of the plants in each plot were alien species), 3 = high (> 80% of plants in each plot were alien species). Table 4.4 list plant species in plots.
Plant canopy type	Coded as 1 = endemic, 2 = alien
Overstory	Percentage of canopy cover, visually estimated as percentage of canopy directly above each tree sampled
Leaf nitrogen content	Variable assumed to influence larvae density, determined using the Kjeldahl method
Leaf wood content	Expressed as percentage of dry weight.
Island	Coded as: 1 = Kauai, 2 = Oahu, 3 = Molokai, 4 = Maui, 5 = Hawaii
Sampling date	Coded as: 1 = April'06, 2 = August'06, 3 = October'06, 4 = January'07
Elevation	Meters above sea level
Distance to nearest agricultural land	Coded as: 0 = distant, 1 = proximal. Proximal if site was within 2000 m of agricultural areas, and not distant if distance of site to any agricultural land exceed 2000m.
Land use/ land cover (LULC)	Variable used as habitat descriptor and coded as: 1 = cropland or pasture, 2 = shrub and brush rangeland , 3 = evergreen forest land

Table 4.5. Scores of each of the measured explanatory variables by site

Site codes	Environmental variables												
	Tmim	Rainfall	Elev.	LULC	Distur.	Abund.	Wood	Nitrog.	Overst.	Island	Distan.	Can. type	*Udea**
K1	10.64	166.54	981	2	1	2	27.99	2.48	17.75	1	0	1	0.46
K2	11.10	165.01	1046	3	1	3	20.32	2.92	17.00	1	0	1	0.56
K3	10.45	157.02	1113	3	1	3	21.33	3.33	12.00	1	0	1	0.77
Kn	16.07	111.50	550	3	2	3	20.80	2.90	27.50	2	1	2	0.53
Pa	17.89	224.96	372	2	3	1	19.01	2.57	5.25	2	0	2	0.20
Pk	13.41	91.09	781	3	2	2	13.96	3.30	23.75	2	1	2	1.04
Ta	18.17	239.12	460	3	3	1	20.15	2.58	9.75	2	0	2	0.64
Kp	11.51	158.57	1229	2	1	3	21.13	2.42	33.75	5	0	1	0.73
Kk1	11.12	171.79	1315	3	1	3	22.57	3.40	19.50	5	1	1	0.86
Kk2	11.24	162.50	1272	3	1	3	22.27	3.17	10.75	5	1	1	0.34
Kk3	10.87	180.67	1368	3	1	2	20.87	3.24	21.75	5	1	1	0.48
Olaa	11.27	201.56	1245	3	2	2	21.94	2.64	15.75	5	1	1	0.41
Hk	19.15	133.83	276	3	3	1	17.33	2.01	33.75	2	1	2	0.24
Km1	12.68	176.25	1107	3	2	2	20.56	2.06	15.00	3	0	2	0.51
Km2	12.18	213.88	1150	3	1	3	21.95	2.30	21.75	3	0	1	0.78
Iv	18.53	206.78	241	3	3	2	18.64	2.62	43.50	4	1	2	0.34
Mk	12.37	215.37	1152	3	2	3	26.98	3.19	10.00	4	1	1	0.56
Ku	13.34	149.34	942	1	3	3	26.19	1.44	22.50	4	1	2	0.49

* The original density were log (100x +1) transformed (since the parasitoid species data was subject to transformation for the analyses)

Table 4.6. Plant species in field plots at 18 sites. Site codes match with Table 4.1.

Site code		Alien species	Native species
K1	Kauai	*Psidium cattleianum*	*Pipturus kauaiensis*
		Passiflora molissima	*Metrosideros polymorpha*
		Hedychium gardnerianum	*Coprosma sp.*
		Rubus rosifolius	*Dodonea viscosa*
			Hedyotis terminalis
			Cheirodendron platyphyllum
			Scaveola glabra
K2	Kauai	*Psidium cattleianum*	*Pipturus kauaiensis*
		Passiflora molissima	*Metrosideros polimorpha*
		Hedychium gardnerianum	*Coprosma sp.*
			Dodonea viscosa
			Acacia koa
			Pisonia brunoniana
			Hedyotis terminalis
			Scaveola glabra
K3	Kauai	*Lantana camara*	*Pipturus kauaiensis*
		Rubus rosifolius	*Dianella sandwicensis*
		Hedychium gardnerianum	*Coprosma sp.*
			Dodonea viscosa
			Acacia koa
Ku	Oahu	*Cordyline fructicosa*	*Pipturus albidus*
		Psidium cattleianum	*Hedyotis terminalis*
		Aleurites moluccana	*Charpentiera* spp.
		Eucalyptus globulus	*Pisonia brunoniana*
		Clidemia hirta	
Pa	Oahu	*Lantana camara*	*Pipturus albidus*
		Clidemia hirta	
		Hedychium gardnerianum	
		Schefflera actinophilla	
		Ficus spp.	
		Moluccan albizia	
		Ricinus comunis	
Pk	Oahu	Araucaria sp.	*Pipturus albidus*
		Rubus rosifolius	*Pisonia brunoniana*
		Psidium cattleianum	*Hedyotis terminalis*
		Clidemia hirta	*Coprosma sp.*
			Scaveola glabra
			Charpentiera spp.
			Cyanea spp.

Table 4.6. Continue

		Alien species	Native species
Hk	Oahu	*Psidium cattleianum*	*Pipturus albidus*
		Clidemia hirta	*Acacia koa*
		Moluccan albizia	
		Ricinus comunis	
		Acacia sp.	
		Hedychium gardnerianum	
		Eucalyptus sp.	
Ta	Oahu	*Schefflera actinophilla*	*Pipturus albidus*
		Ficus spp.	*Acacia koa*
		Coffea arabica	*Metrosideros polymorpha*
		Eucalyptus globulus	*Peperomia* spp.
		Psidium guajava	
		Psidium cattleianum	
		Hedychium gardnerianum	
		Cordyline fructicosa	
		Aleurites moluccana	
Km1	Molokai	*Eucalyptus spp.*	*Pipturus albidus*
		Rubus rosifolius	*Metrosideros polymorpha*
		Pinus spp.	*Acacia koa*
		Hedychium gardnerianum	
Km2			*Pipturus albidus*
			Metrosideros polymorpha
			Acacia koa
			Hedyotis terminalis
			Coprosma sp.
Iv	Maui	*Psidium cattleianum*	*Pipturus albidus*
		Coffea arabica	*Pisonia sp.*
		Schefflera actinophilla	
		Syzygium malaccense	
		Syzygium jambos	
		Ricinus comunis	
Ku	Maui	*Syzygium jambos*	*Pipturus albidus*
		Persea americana	
Mk	Maui	*Pinus sp.*	*Pipturus albidus*
		Hedychium gardnerianum	*Acacia koa*
		Rubus rosifolius	
		Eucalytus globulus	

Table 4.6. Continue

		Alien species	Native species
Kp	Hawaii	*Sapindus saponaria*	*Pipturus albidus*
		Rubus argutus	*Charpentiera sp.*
			Acacia koa
			Metrosideros polymorpha
			Peperomia spp.
			Ipomea indica
Kk1		*Sapindus saponaria*	*Pipturus albidus*
			Dodonea viscosa
			Metrosideros polymorpha
Kk2			*Pipturus albidus*
			Acacia koa
			Metrosideros polymorpha
Kk3		*Solanum pseudocapsicum*	*Pipturus albidus*
			Acacia koa
			Dodonea viscosa
			Sophora chrysophylla
Olaa	Hawaii		*Pipturus albidus*
			Dodonea viscosa
			Acacia koa

Table 4.7. Results of the forward selection procedure with entire data set. Test statistics for the environmental variables used in the RDA, based on Monte Carlo permutation tests. Lambda-A gives the additional variance explained by each variable as it is included in the analysis.

Variable	Lambda - A	*P*	F - ratio
Udea	0.18	0.002	15.42
Disturbance	0.18	0.002	19.41
Elevation	0.04	0.018	4.07
Wood content	0.01	0.198	1.54
Temp. mim.	0.01	0.244	1.35
Rainfall	0.01	0.280	1.24
Nitrogen	0.01	0.374	1.00
Island	0.01	0.480	0.81
Distance to ag. land	0.00	0.724	0.47
Overstory	0.01	0.478	0.77
Host plant abundance	0.00	0.764	0.45
Canopy type	0.01	0.966	0.17
Date of sampling	0.00	0.946	0.17
Land cover/land use (LULC)	0.00	0.938	0.19

Table 4.8. Summary results of PCA, and partial RDA analyses of the parasitoid assemblage associated with *U. stellata* in Hawaii

Analysis conducted	Ordination Axes				Total variance
	Axis 1	Axis 2	Axis 3	Axis 4	
PCA on species data					
Eigenvalues	0.76	0.12	0.07	0.03	
Species-environment correlation	0.95	0.92	0.91	0.66	
Cumulative percentage variance					
of species data	75.60	87.00	94.30	97.50	
of species – environment relation	78.10	89.90	96.30	98.00	
Sum of all eigenvalues					1.00
Sum of all canonical eigenvalues					0.87
Preliminary partial RDA					
Eigenvalues	0.63	0.06	0.05	0.01	
Species-environment correlation	0.95	0.88	0.94	0.73	
Cumulative percentage variance					
of species data	70.90	77.90	83.40	84.80	
of species – environment relation	82.10	90.10	96.50	98.20	
Sum of all eigenvalues					0.88
Sum of all canonical eigenvalues					0.76
Partial RDA w/significant variables					
Eigenvalues	0.52	0.01	0.188	0.07	
Species-environment correlation	0.87	0.40	0.00	0.00	
Cumulative percentage variance					
of species data	59.20	60.50	81.70	89.30	
of species – environment relation	97.90	100.00	0.00	0.00	
Sum of all eigenvalues					0.88
Sum of all canonical eigenvalues					0.54

Table 4.9. Angles between the *Udea stellata* vector and other environmental factors plotted by principal component analysis in Figure 4.2. Cosine of the angle approximates the correlation coefficient.

Vectors	Angle	Cosine
Udea and min. temp.	149.5°	-0.862**
Udea and rainfall	129.0°	-0.629*
Udea and host plant abundance	16.0°	0.962**
Udea and disturbance	153.0°	-0.891**
Udea and canopy type	129.0°	-0.636*
Udea and % overstory	90.5°	-0.009
Udea. and nitrogen	25.0°	0.906**
Udea and wood content	71.0°	0.009
Udea. and island	60.5°	0.492
Udea and elevation	38.5°	0.782**
Udea and distance to ag land	57.0°	0.544*
Udea and land cover/land use (LULC)	92.0°	0.034

* $P < 0.05$, ** $P < 0.01$

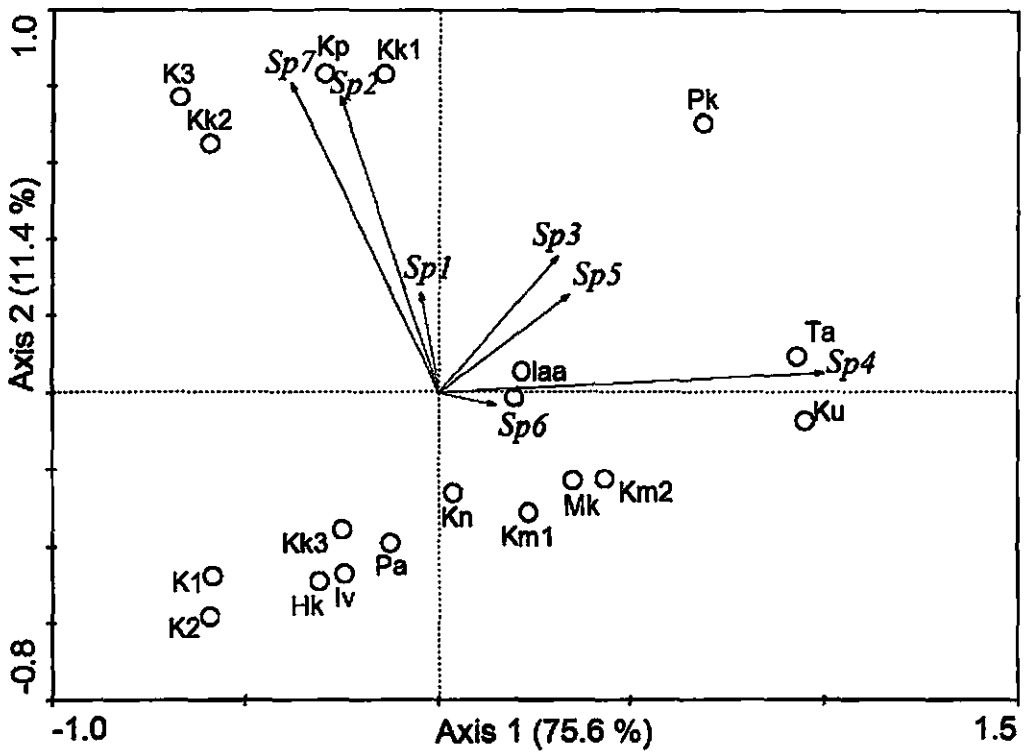

Figure 4.1. PCA ordination of the 18 study sites (site codes are detailed in Table 4.1) based on parasitoid densities (species codes are detailed in Table 4.2).

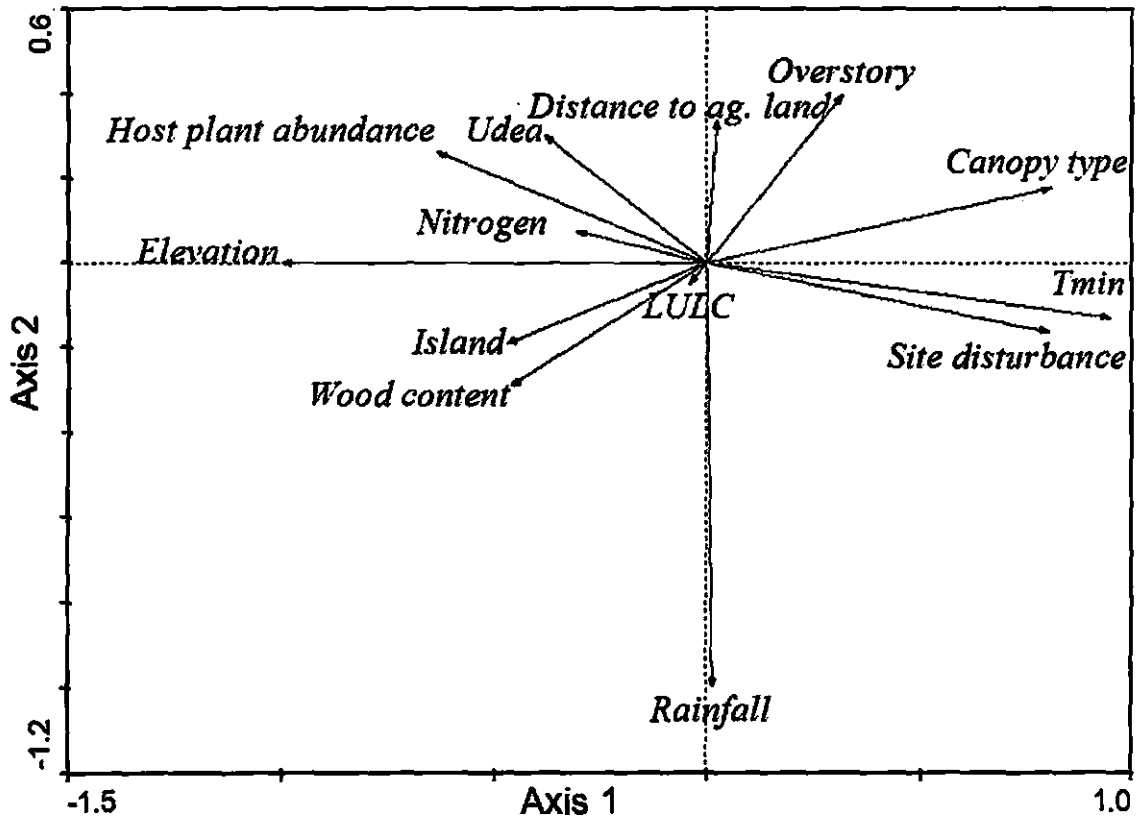

Figure 4.2. Principal component biplot showing the correlation among *Udea* density and environmental variables measured in 18 mamaki sites.

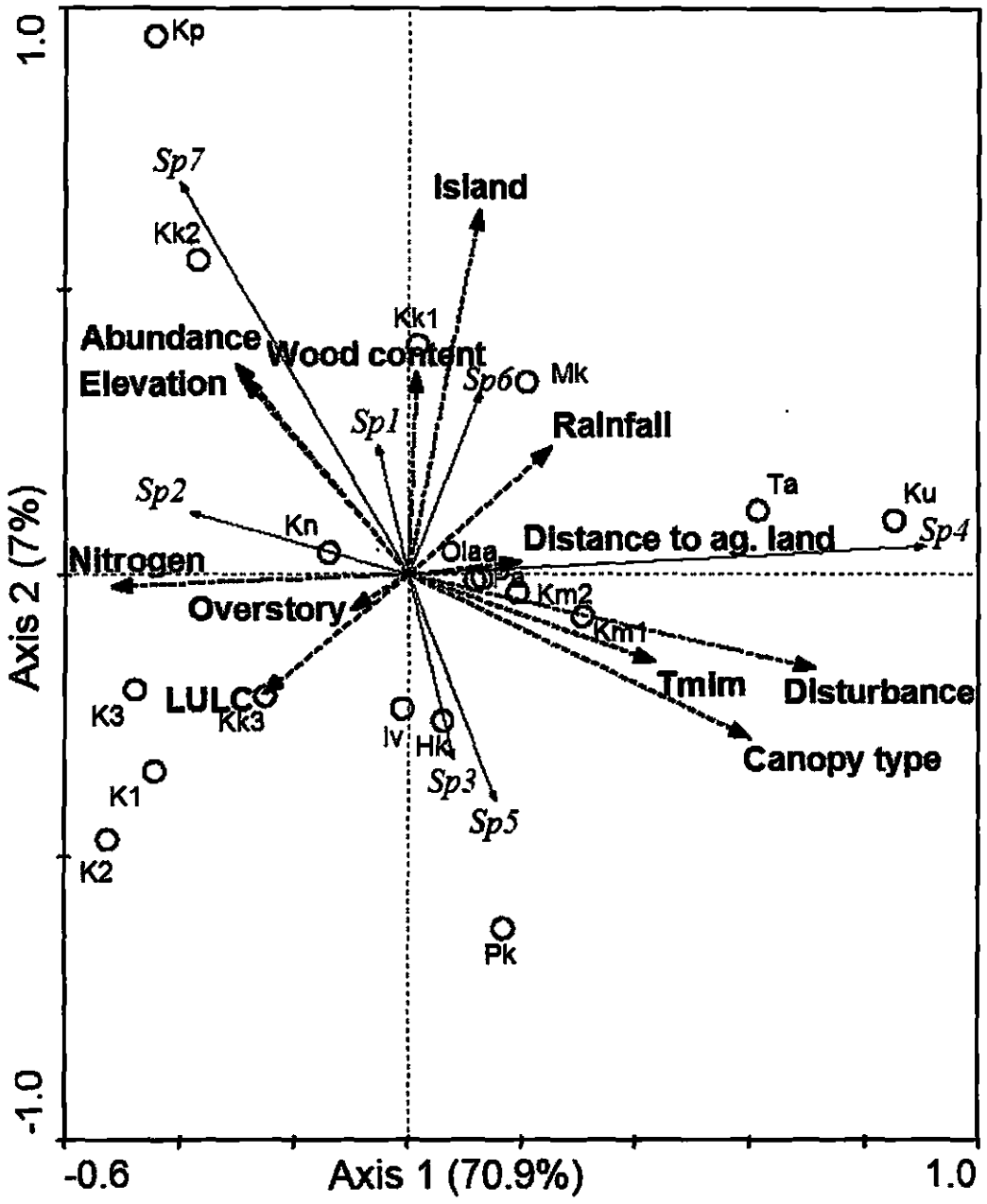

Figure 4.3. Preliminary partial RDA triplot of parasitoid species associated with *U. stellata*, sample sites and environmental variables. Circles represent sites (site codes are detailed in Table 4.1), solid arrows represent species (species codes are detailed in Table 4.2), dashed arrows represent environmental variables.

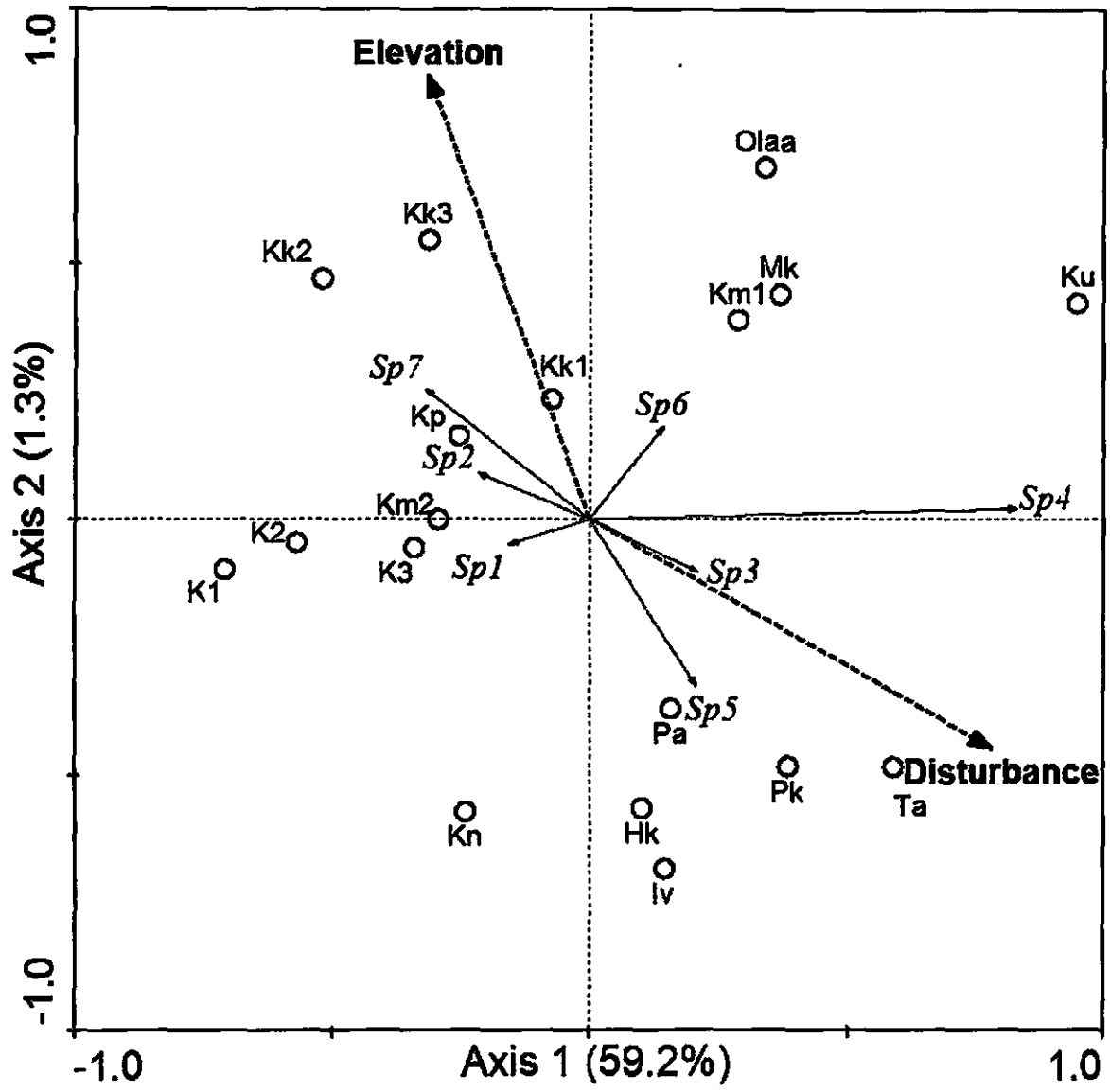

Figure 4.4. Partial RDA triplot of parasitoid species associated with *U. stellata*, sample sites and significant environmental variables. Circles represent sites (site codes are detailed in Table 4.1), solid arrows represent species (species codes are detailed in Table 4.2), dashed arrows represent environmental variables.

ASSESSING APPROACHES FOR RISK ASSESSMENT FOR INSECT BIOLOGICAL CONTROL INTRODUCTIONS

Introduction

The rate of biological invasions has increased dramatically in the past 500 years, due to an increase in human activities such as transportation, migration and commerce (Mack et al. 2000). Invasive species cause direct and indirect effects on organisms living in the environment they invade, and therefore threaten biodiversity, agriculture and human health. Besides the environmental impacts, invasive species cause major economic loses in different sectors of the US economy (Pimentel 2005). The practice of classical biological control (CBC), as the intentional transfer of natural enemies from one place to another, has traditionally been used as a tool to fight invasive species in agricultural settings and is now also being used to control invasive species in natural areas (Hoddle 2004; Messing and Wright 2006).

The basic philosophy of CBC is based on the enemy release hypothesis. This hypothesis states that organisms become invasive in a new area because they have escaped the natural enemies that suppress their populations in their area of origin, thus providing them advantage over competitors in area of introduction, that are still suppressed by their indigenous natural enemies (Blumenthal, 2005). Therefore, this type of control works under the premise that reestablishment of top-down control by introduction of natural enemies will reduce the populations of invasive species and therefore restore balance (Hoddle 2004).

The history of biological control provides remarkable examples of success of many programs (Caltagirone 1981). Prime examples include the introduction of the Australian lady beetle *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae) to control the cottony cushion scale, *Icerya purchasi* Maskell, an introduction that saved the California citrus industry, and the introduction of *Anagyrus lopezi* (DeSantis) (Hymenoptera: Encyrtidae) from South America to control the cassava mealybug *Phenacoccus manihoti* Matile-Ferrero, in Africa, credited with saving many people from starvation. Those are just two of many other remarkable examples. Besides the economic benefits of this practice, the use of biological control has also led to a reduction in the use and dependence on pesticides.

The potential and realized positive effects of biological control have been recognized for over a century. Many practitioners have long considered this practice environmentally safe, benign, risk-free and a natural phenomenon (van den Bosch and Messenger 1973; DeBach 1974, Caltagirone and Huffaker 1980, Simmonds and Benett 1977). Even though awareness of potential negative effects was also expressed over a century ago (Perkins 1897), it was only in the 1980's that this practice was first severely criticized (Howarth 1983, 1991). The center of this criticism was the issue of host specificity (Ehler 1999). Biological control agents were implicated in the reduction of populations of native and desirable species, and were blamed in some cases to be agents of extinction (Howarth 1983, 1991). Soon after, researchers were calling for more rigorous screening methods in the USA (Ehler 1999) and revisiting means of predicting positive or negative impacts of biocontrol agents. Some authors went so far as to call for the cessation of biological control (Asquith and Miramontes 2001).

Throughout the history of biological control, the most adverse effects in terms of non-target attacks have been due to the release of highly polyphagous species (Boettner et al. 2000, Evans 1996). Generalist biological control agents were at one point considered superior not only because they could potentially control several pests but they could also persist on native insects at the time the target pest was rare (Williams 1931). These releases were made before concerns for environmental impacts were highlighted. In the specific case of Hawaii, after concerns were raised the State experienced an overall reduction of biological control introductions due to rigid regulations (Messing, 1999).

At the time the potential for environmental impacts of introduced biological control agents was recognized in the US, the need for regulations and guidelines for introductions as well as for comprehensive risk assessment (RA) frameworks became apparent. Since the center of criticism was based on lack of host specificity, host specificity testing is now a key element in any RA methodology, and in many cases is used to accept or reject proposed introductions. Several protocols have been developed for selection of non-target species for screening and host range determination throughout the years and are available in the scientific literature (Barratt et al. 1997; Sands, 1998; Kulhmann and Mason 2003; Messing 2001; van Lenteren 2003, 2006; Kuhlmann et al. 2006). Some countries such as New Zealand, Australia and South Africa as well as countries within the European Union have developed their own regulations and RA frameworks. For the most part they have similar criteria, but they involve different procedures and work under different guidelines.

The United States has no comprehensive RA methodology adopted for insect biological control introductions, and this situation is further complicated by the different

regulations at the state level. Hawaii represents a unique case, with a long history of biological control introductions, and has been the center of controversy regarding non-target effects. This has resulted in overly-restrictive regulations being implemented at the state level, leading to (Messing 1999). The state of Hawaii and the US in general will greatly benefit from an adoption of a general RA framework that could quantitatively identify the risk of proposed biological control introductions.

Risk assessment in the field of biological control evaluates the likelihood that adverse ecological effects may occur as a result of a release of a purposely introduced biological control agent. Two general RA frameworks have been proposed with great potential to be widely adopted. van Lenteren et al. (2003) proposed a semi-quantitative environmental RA approach for inundative biological control agents, which was later improved and expanded to address classical biological control agents, in a stepwise procedure which identifies biological control agents with high potential risk early in the process, therefore avoiding unnecessary research and use of resources (Van Lenteren 2006a,b). Wright et al. (2005) proposed a probabilistic RA approach for either classical or inundative biocontrol agents, which is based on the development of 'precision trees', using conditional probabilities in a Bayesian approach to estimating risk. Even though other approaches have been used for specific biological control introductions, the two approaches mentioned above have been proposed for wide adoption.

It has already been shown that the risk posed by biological control agents can vary spatially and temporally within the area of introduction (Follett et al. 2000b, Le Corff et al. 2000, Stiling and Simberloff 2000, Johnson et al. 2005, Barratt et al. 2007). Nevertheless, current RA procedures lack comprehensive incorporation of spatial and

temporal components to characterize the risk. In order to predict how the biological control agent will behave in the area of introduction it is important to elucidate its behavior in the area of origin and other areas of distribution, to understand the nature of the relationship with target and potential non-target species.

The specific aims of this chapter are to:

- Provide an overview and critique of proposed approaches of RA for wide adoption
- Validate the probabilistic risk assessment methodology.

  Specific questions to be answered in the validation process:

  1) Would it have been possible to predict that *Cotesia marginiventris*, *Meteorus laphygmae* and *Trathala flavoorbitalis* would attack *U. stellata* based on literature data, and would it have been possible to predict their level of impact on this non-target species using PRA?

  2)Does using single point estimates as opposed to probability distributions to estimate exposure significantly impact the final risk assessment?

  3) How useful are estimates of marginal vs. apparent mortality (and measures of uncertainly in both) in conducting PRA? Is either preferable?

  4) Are there any key ecological variables that would be worth considering in the hypothetical case that any of the species will be considered for introduction in another place or are worth considering in any risk assessment?

Overview and critique of proposed methodologies for risk assessment for insect biological control

Environmental risk assessment

Van Lenteren et al. (2003) proposed an environmental risk assessment protocol for biological control agents used for inundative release programs. This first general framework was based on a semi-quantitative system that integrates information on potential of establishment, dispersal, host range, direct effects and indirect effects. Van Lenteren et al. (2006 a, b) later proposed a refined framework for both augmentative and classical biological control programs that distinguish between native and exotic agents in a stepwise system. Van Lenteren et al's approach includes seven steps. In the first two steps the risk assessor distinguishes between native and exotic natural enemies as well as the type of biological control program intended. The other steps refer to each the risk components in the following order: establishment, host range, dispersal and direct and indirect effects.

The van Lenteren approach uses predetermined qualitative and quantitative scales to describe the likelihood and magnitude for each of the five risk components identified to calculate risk indexes. In the first proposed approach (van Lenteren et al. 2003) a numerical value (1 to 5) was added to the each descriptor of likelihood and magnitude, thereafter the calculation of the overall risk was done by first multiplying the respective numerical value for likelihood and magnitude for each of the five risk components and subsequently summing those scores. Once the overall risk index is calculated, it is ranked based on a threshold scale that classifies the risk into low, intermediate or high risk categories. The authors identified some shortcomings of this approach: information about

likelihood and *magnitude* for each of the risk components had to be available before the evaluation, even for obviously risky agents, causing unnecessary research. Also, summing scores of risk components that are not completely independent from each other (most components are conditional events) was considered inadequate, therefore in the refined approach (van Lenteren 2006a, 2006b) weighting factors are provided for the calculation of the environmental risk index (ERI) for the following risk components: establishment, dispersal and direct and indirect effects. If the ERI falls above a given threshold for each of the risk components, the RA is recommended to be discontinued at that step, or it may continue upon request.

The main purpose of the stepwise approach is early identification of prospective species that pose a high risk of negative direct and indirect effects; these species are eliminated early in the process, avoiding unnecessary further research. The van Lenteren stepwise framework guides the risk assessor throughout the risk analysis process and clearly distinguishes between augmentative and classical biological control agents, as well as native and exotic agents. The strength of this approach is that it is possible to use qualitative and quantitative information. In many situations, quantitative data are difficult to obtain, making a framework that incorporates qualitative information more flexible. When assessing the risk of augmentative species already in use, the quick scan method (which differs from the full stepwise assessment in that information does not need to be generated but is already available)promotes the continuation of successful programs and the discontinuation of programs that are too risky for certain ecoregions. A full risk assessment is only necessary for new species.

One of the main risk components of this approach is establishment in a non-target habitat, which is only taken into consideration for augmentative biological control (ABC) agents and not for classical biological control (CBC) agents. Even though the main difference of both BC programs is establishment, when this happens in a non-target habitat then it is not desirable for both augmentative and classical BC.

One weakness of this approach is that risk components identified by the authors are indeed important, but they are not used in an integrated way. In this system the steps are not in a logical sequence of events, which is evident when assessing the risk of an inundative biological control agent. The host range issue is considered before dispersal to suitable habitats. In a situation where there is no chance of establishment, dispersal to potential suitable habitats (where potential non-targets may be present) will play a significant role in determining the ecological host range. Following this framework, agents that do not have restricted diets may be eliminated early in the process (or it may continue upon request of the applicant) based on their fundamental host range without consideration of filters such the ability to disperse to habitats where non-targets may be exposed to the agent. Therefore, it may be possible to fall into the bias of eliminating agents that do not pose a real risk if exposure is not clearly evaluated. This approach is therefore centered on the premise that resources can be saved by eliminating risky species early in the process based primarily on their fundamental host range. Nevertheless even when host range indicates that valued species can be used as non-target hosts, the agent may still cause no or low risk of impact at the population level (Barron et al. 2003, Benson et al. 2003, Haye et al. 2005, Johnson et al. 2005). Further research on promising agents can therefore be justified if the benefits outweigh the risks. Also, this approach is

used to infer the risk posed by an introduction to all non-target species, which loses resolution on the level at which each non-target species is affected.

Finally, this approach lacks incorporation of uncertainty. Suter (1993) highlighted three drawbacks of the use of single point deterministic approaches:(1) it is inconsistent (because it can hide inherent variability); (2) conservative assumptions *tend* to hide uncertainties and error from the decision maker by burying it in estimates of exposure and effects; and (3) conservatism assumes that there are no societal environmental costs of regulating false positives.

Probabilistic risk assessment (PRA) for biological control introductions

This approach was proposed by Wright et al. (2005) and is based on Bayesian reasoning to predict risk posed by prospective biological control agents to non-target species. Probabilistic risk assessment (PRA) uses probability distributions to characterize variability or uncertainty in risk estimates. Note that the ability to perform a PRA often is limited by the availability of probability distributions that adequately describe one or more of the input parameters. The quantitative analysis of uncertainty or variability can provide a more comprehensive characterization of risk than conservative approaches which rely on single point estimates. Results of a PRA provide a range of all possible outcomes (e.g. levels of parasitism) and their likelihood of occurrence, therefore allowing one to ask "what if" questions.

Event trees (also known as precision trees or fault-trees) are appropriate for displaying the order of events that result in the 'risk event' being realized, and also dependency between them (conditional probability) (Bier 1997). By modeling the

possible events that can occur after the release of a biological control agent, the risk assessor may identify potential routes of risk that need to be quantified. Event trees consist of a network of nodes and connecting branches, and outcomes of occurrences. Nodes indicate decision points and chance events. Branches correspond to the impact of each decision alternative or event outcome emerging from a node.

The use of the PRA approach in biological control requires quantification of key biological and ecological factors playing a role in non-target impacts, estimation of probability distribution to express variation for relevant factors measured, and articulation/construction of the precision tree in the appropriate sequence of events. The overall probability of impact from a series of events is estimated by multiplying the *P* values along each branch.

Wright et al. (2005) used the augmentative egg parasitoid *Trichogramma ostrinae* Pang and Cheng (Hymenoptera: Trichrogrammatidae) as a case study. This parasitoid is unable to survive winters in the northeastern United States. For the construction of the precision trees they considered aspects such as physiological host range of the parasitoid, dispersal capacity within and out of cornfields (the target release habitat), searching and host-location behavior in corn and crops of different architectures, and searching efficiency in indigenous deciduous forests. The authors underscored the importance of including key ecological traits into the RA to adequately estimate the probability of an adverse effect, rather than relying only on laboratory screening which only provides information on potential physiological host range, not potential ecological/realized host range. In their case study they presented a simplified application of probabilistic analysis,

presenting only two possible outcomes (the average and the worst case scenario, based on probability distributions) instead of a range of all possible outcomes.

Uncertainty is a fundamental characteristic of RA. Uncertainty may arise from unknown data or incomplete knowledge as well as from natural variability in a dataset. In decision tree or Bayesian statistical analysis there are different sequences of events that could contribute to the overall risk, therefore there is a need to assess how the estimate of risk is affected by uncertain components. An accurate prediction of the risk is therefore a daunting task. Uncertainty analysis provides a range of risk values that could be taken into consideration to evaluate risk under different scenarios.

Among the most important strengths of this approach is the incorporation of uncertainty in the analysis. Modeling different scenarios allows the risk assessor to answer "what if" questions. This aspect could be valuable for the risk benefit analysis phase. An additional strength of this approach is the incorporation of ecological data into the analysis that optimizes the estimate of risk posed by the introduction. Contrary to the environmental risk assessment approach (van Lenteren 2003, 2006a, b) this approach can not be used to infer the risk to all non-target species but only to selected species selected for scrutiny. This approach can provide high resolution regarding the extent to which effects may occur for a specific species of concern.

Some weaknesses of this approach:

The primary disadvantages of PRA are that it generally requires more time, resources, and expertise on the part of the assessor and reviewer, than a point estimate approach, and therefore should be conducted only in cases where the benefits are thought to outweigh the risks. Probability distributions of the different events are often difficult to

obtain which can therefore limit the use of this approach. The proposed framework lacks incorporation of spatial and temporal components to characterize the risk. A clear incorporation of spatial and temporal components can provide insights about areas that are at low or high risk of non-target effects, information that can be used in the risk management phase. The Wright et al. (2005) approach was proposed using an augmentative biological control agent already in use as a case study, therefore the generation of required ecological data such as dispersal outside the release area and host searching in different habitats was readily collected. The use of this approach as a predictive tool for new species will be limited to information that is available in the current literature from other places and also in information generated from rigorous field studies in the country of origin. The proposed approach had no general defined structure as far as phases in the problem formulation, analysis phase and risk characterization, situation that makes this approach difficult for widespread adoption.

Refined probabilistic risk assessment

PRA should be conducted in situations where the proposed candidate has demonstrated great potential for controlling the target pest and also when potential non-target hosts have been identified to be species of concern based on results of host specificity testing in the laboratory. PRA is then directed to estimate the probability that effects on selected non-target species will materialize and the magnitude of those effects. Phases to follow in PRA are problem formulation, analysis phase and risk characterization.

Problem formulation

This is a fundamental component of any risk assessment (USEPA 1992, 1998). The problem formulation should include the following information:

- Description of the biological control agent (stressor).Description of the basic biology, life cycle, known host range and other relevant information. The determination of the fundamental host range can be determined from literature records and/or during field surveys in the agent's country of origin.
- Non-target hosts (assessment endpoints) considered as of concern are selected for PRA. These are those entities that represent economic, ecological or cultural value for society and that are to be protected, and are selected based on their susceptibility to the biological control agent (stressor) (USEPA 1992, 1998). Species are selected based on results from host specificity studies and high likelihood of being used as non-target hosts under field conditions.
- Identification of habitats where the agent occurs in the area of origin or areas of distribution. These are habitats within which effects may potentially occur in the area of introduction (USEPA 1992). Data from published literature, data collected during field surveys in the area of origin and/or data from museum records can be used to determine the type of habitats that the stressor utilizes in the native range or in other areas where they may have been introduced previously. Ecological factors correlated with the relative abundance of the prospective agent can be useful for RA (such as altitude, vegetation type and other relevant environmental factors).

- Identification of vulnerable habitats in the proposed area of introduction. Once habitats where the agent does occur have been identified, it may be possible to match those habitats with the similar types of habitats present in the area of introduction.
- Identification of habitats that support non-target species. Once non-target species are identified the distribution of these species within the risk region need to be obtained as well as information on temporal occurrence.

Analysis phase

This phase involves relating exposure and effects to each other (USEPA 1998). During this phase of the RA, the exposure of non-target species to the stressor and the magnitude of the effects are characterized.

The assumptions (or risk hypothesis) considered in the analysis phase are the following:

- The sensitivity of non-target species to an introduced BCA varies among habitats (Wiegers et al. 1998, Colnar and Landis 2007).
- For a non-target species to be at potential risk of non-target effects it will have to be exposed to the agent spatially and temporally.

Exposure assessment

Parasitoids will only put native species at risk if they search the habitats of those species and locate and parasitize them in those habitats (Sands and Van Driesche 2004). Here the potential for spatial overlap of a BC agent with non-target species is described. To be able to overlap spatially the BC agent must successfully disperse to non-target habitats, establish in that habitat and overlap spatially and temporally with the agent.

In the problem formulation phase, potential non-target habitats in the area of introduction are identified based on habitats where the biological control agent is known to occur in other areas, either its place of origin or other areas where the species has been introduced previously. This information provides a baseline prediction of the potential distribution of the BCA in the proposed area of introduction.

Dispersal capacity of the BC agent is an important determinant of the likelihood of exposure of non-target species. Several techniques have been proposed to assess dispersal capacity for augmentative BC agents and could be incorporated in the PRA framework (Wright et al. 2001; van Lenteren et al. 2003, 2006; Mills et al. 2006). With the use of GIS, suitable habitats within the dispersal potential of the BC agent could be identified. The probability that agents will leave the release site and find non-target habitats where susceptible non-target species are present can be performed by using techniques such as mark-release-recapture techniques. Results of the dispersal experiments can be used to develop probability distributions for potential spatial overlap. This assessment can be for already in use ABC agents in the area of introduction or also on prospective ABC agents in their respective area or origin or distribution, preferably with similar type of ecosystems.

If ABC agents are likely to establish in either the target habitat or non-target habitats within their dispersal range, RA should be done as if it was an introduction for CBC (considering other non-target habitats outside its immediate dispersal potential should be considered).

For agents used for CBC the dispersal potential to non-target habitats will be more difficult to determine. Procedures used for ABC are inadequate since these

dispersal experiments are usually carried out over a relatively short period time, which will not represent the actual opportunity for long term dispersal. After establishment, CBC agents (those with both active and passive dispersal) could disperse over time to all potential non-target habitats vulnerable to invasion, unless there are insurmountable geographical barriers that preclude dispersal. In the case of CBC, the distribution of agents in the area of origin and in other areas (e.g. other places where the agent has been released for biological control) can provide useful information on their dispersal potential.

Once potential spatial overlap of non-target species and agent has been analyzed, temporal overlap needs to be assessed. Temporal overlap is based on the time scale of establishment and degree of synchronization with susceptible stages of the non-target hosts (Stiling 1993). Records of museum collections, published literature as well as survey data can help determine the likelihood of temporal overlap between the BC agent and potential non-target species. It is expected that probability distributions for temporal overlap may be difficult to construct; in such cases, the following scale can be used to account for synchronization with susceptible stages of the non-target host:

0.00 No synchronization with susceptible stages of the non-target host

1.00 Synchronization with susceptible stages of the non-target host.

Effects assessment

Given exposure, effects to non-target species can occur via direct or indirect mechanisms. Direct effects such as predation or parasitism are readily observed and measured in field and/or laboratory studies, and therefore relatively readily incorporated

into risk assessments. Indirect effects are effects of one species on another mediated by at least one intermediate species such as the case of apparent competition and enrichment. It has long been recognized that indirect effects could have substantial effects on non-target populations, nevertheless methods to measure those effects are only recently appearing in the scientific literature (Messing et al. 2006).Effects assessment centers on direct effects, since most non-target species selected for PRA in the problem formulation phase are expected to be based on potential direct effects. If the selection is based on potential indirect effects, proposed methods should be followed to quantify these effects (Messing et al. 2006).

Many studies have shown that data collected during laboratory studies can identify species that are in the agent's physiological host range but not in its ecological host range, and that the former is often greater than the latter (Onstad and McManus 1996, Cameron and Walker 1997, Benson et al. 2003). Also, laboratory studies often can not predict the actual impact on non-target host populations, which can only be quantified during field studies. Non-target species selected for PRA are considered species with high probability of being used as non-target hosts based on results from laboratory studies. During the effects assessment phase, the magnitude of these non-target effects may be assessed by conducting ecological studies in the agent's area of origin or other areas of distribution. The advantage of conducting this type of study is that the agent and the target host are present, which cannot be done on any realistic scale in the intended place of introduction. Open field experiments in the area of origin of the candidate can provide a more realistic estimation of the magnitude of the non-target use through providing a measure of host specificity under field conditions (Clement and Cristofaro

1995, Briese et al. 2002, Briese 2005) and provide more realistic information and ecological context than information gathered only from quarantine studies. These experiments have the constraint that non-target species that are native to the proposed area of introduction are not present in the agent's area of origin. Surrogate species in the area of origin should then be selected based on phylogenetic and ecological similarities (Briese 2005). Results from these studies may provide data that can be used for calculating the percentage of target hosts parasitized in the respective habitat in the area of origin, and to construct probability distributions (describing the probability that a certain percentage predation or parasitism will take place). Experiments of this nature can also provide valuable insights regarding indirect effects (Messing et al. 2006). When field experiments in the area of origin are not feasible, literature records on parasitism rates in different habitats could potentially be used if reliable information is available.

When working with oligophagous or polyphagous species, non-target effects can be influenced by the presence of the preferred or other alternative hosts, which may considerably alter estimates of risk (Briese et al. 2002, Briese 2005). Open field experiments with surrogate species closely related to the target host may also provide insights on how effects may vary in the presence and absence of the preferred or other alternative hosts in different habitats (Briese et al. 2002). Field results can be compared with laboratory tests. Life table studies on surrogate species closely related to the non-target hosts may also provide useful information on the relative impact of the agents on population of non-target species. Life table studies on non-target hosts in the country of proposed introduction can also provide useful information regarding the extent to which current mortality factors (e.g. existing natural enemies) affect the population of the non-

target species, and estimate the level of effect that can be posed if the agent is released (Johnson et al. 2005).

Collaboration with universities and research centers in the area of origin of the proposed candidate can provide a means to conduct such studies.

Risk characterization

This phase of the RA involves fitting data into the precision tree. The estimation of risk requires the integration of exposure and effects data and the evaluation of any associated uncertainties (USEPA, 1998).

In this phase, probabilistic risk assessment is implemented by constructing precision trees. Figure 5.1 shows a conceptual decision tree that incorporates aspects such as potential to disperse to suitable habitats in the area of release, potential to overlap temporally with non-target species, and potential to cause direct or indirect effects on non-target species. The conceptual decision tree can incorporate some other variables that may be important in determining the risk in specific cases, whereas other components can be excluded if not important for the specific case. In this conceptual tree, the probability to disperse to suitable habitats is a fundamentally important factor determining the risk of exposure to non-target species, also spatial and temporal overlap with non-target species are of major interest in characterizing the risk. The 'overlap with non-target species' node can be further divided into multiple branches for as many non-target species that are be included in the assessment.

The probability that the agent will cause an effect on non-target populations (and therefore be part of its ecological host range) will be conditional on a habitat overlap of non-target species with the biological control agent that in turn, is conditional on the successful dispersal of the agent to non-target habitats. Data collected in the analysis phase are used to fit the precision tree in the sequence of events (Wright et al. 2005). Probabilities are multiplied along branches to calculate the overall P-value that the BC agent will attack a non-target species. The multiplication of the overall P-value by the density of the non-target species should provide an estimate of the extent of that impact on a population of the non-target species.

When PRA is conducted on a preferred or target host in a non-target habitat, the results will only indicate that the BC agent is present and able to forage for the target hosts but can not be used to infer the magnitude of effects on a non-target host. However, if the effects assessment part is carried out with non-target hosts or surrogate species that are ecological and phylogenetically close to the non-target species, in a similar habitat then results of the risk assessment will approximate the probability that the agent will actually parasitize the non-target host in a specific type of habitat.

Uncertainty analysis

In this step the uncertainty in the prediction resulting from the uncertain data used is assessed. Data used in any risk assessment are subject to uncertainties from different sources. Therefore, the decision maker needs to understand and quantify the uncertainties associated with the scientific information on which the decision will be based (Suter,

1993). Sources of uncertainty in risk assessment may arise from 1) inherent variability of the data (stochasticity); 2) data gaps and 3) mistakes in the assessment activities (investigator error). Uncertainty originating from data gaps and investigator error can be reduced but uncertainty originating from variability of the data can only be described and estimated, but not reduced (Suter 1993).

Uncertainty is modeled by using Monte Carlo uncertainty analysis. Uncertainty analyses (Monte Carlo simulations) can be performed with computer software such as Crystal Ball (Decisioneering 2005) or @Risk (Palisade Corporation 2002).Monte Carlo (MC) analysis is a type of probabilistic approach used to quantify the change in model outputs as a function based on probability distributions of each of the uncertain parameters of the model. The basic steps for Monte Carlo simulation analysis are the following (Suter, 1993): 1) defining the statistical distributions of the input parameters, 2) randomly sampling from these distributions, 3) performing repeated model simulations using the randomly selected set of parameters, and 4) analyzing the output. The outputs of the analysis are presented in a form of statistical distribution (uncertainty distribution) representing the entire range of possible outcomes (e.g. impact on a non-target species) and the likelihood of each outcome being realized.

Uncertainty analysis provides the basis for efficient data collection, and application of refined methods (USEPA 1998) or justifies a degree of conservatism in the predicted outcomes in the face of uncertainty (Suter 1993).Uncertainty analysis can therefore increase the confidence in a decision.

Validation of the probabilistic risk assessment methodology

This section presents the procedures used to answer the four main questions stated in the introduction, regarding the validation of the PRA.

Question 1: Would it have been possible to predict that any of the three parasitoid species will attack U. stellata based on literature data, and would have been possible to predict their level of impact on this non-target species from literature data using PRA?

In order to address the first part of the question, whether records in the literature would have given and indication that any of the three parasitoid species would attack *U. stellata*, reports on parasitism by *C. marginiventris*, *M. laphygmae* and *T. flavoorbitalis* were searched in older literature records (from 1913 to date of introduction or first record) were assessed by consulting the printed copies of the abstract journal The Review of Applied Entomology. The Thompson catalogues of host-parasitoid associations (Thompson 1953, 1957) were also consulted (similar procedure described by De Nardo and Hopper 2004, Sands and van Driesche 2004).

The second part of the question, whether it would have been possible to predict their level of impact on *U. stellata* based on literature data using PRA, validates a probabilistic risk assessment approach (Wright et al. 2005) based on the use of precision trees. As far as possible, probabilistic methods were applied by deriving probabilities from available data that could be gathered on the biology and ecology of the species being examined, from published literature as if it would have been available prior the "introduction" of the species of interest. These data form the basis for predictions (hypotheses) available at the 'time of introduction' and provide the first step for the

validation. As actual non-target impacts were measured during the course of this study (chapters 1-4), it should be possible to corroborate the probabilistic model "predictions" with quantitative field data (presented as probability distributions). The validation was made by developing precision trees based on literature data compared to actual impacts measured in the field.

The parasitoid species used for the validation section were: *Cotesia marginiventris, Meteorus laphygmae*and *Trathala flavoorbitalis*with *U. stellata* as the non-target organism. Validation was not performed for the other species: *Diadegma blackburnii, Pristomerus hawaiiensis, Casinaria infesta* and *Triclistus* nr. *aitkeni* since only little information was available in the literature on the latter four.

Problem formulation

The non-target host *Udea stellata* (Butler)

The genus *Udea* (Lepidoptera: Crambidae) is a very large group that occurs in the Americas, Eurasia and into the Pacific (Zimmerman 1958). Hawaii has 44 endemic species in this genus (Nishida, 2002). *Udea stellata* was first described by Butler in 1883. *Udea stellata* (Lepidoptera: Crambidae) is a multivoltine species that undergoes six larval stages. The larvae feed on endemic host plants in the genus *Pipturus* (Urticacea, common name "mamaki"). All six larval stages are susceptible to parasitism by at least one of seven introduced parasitoid species associated with them (Chapter 2).

Habitats supporting the non-target species *U. stellata*

The endemic host plants of *U. stellata*, *Pipturus* spp. are typically found in mesic forests. They occur across a moderate elevation gradient (from sea level to 1,400 m). Most *Pipturus* populations in Hawaii occur in habitats with various levels of disturbance by alien plant species. *Pipturus* habitats in Hawaii were classified based on landuse/landcover (LULC, State of Hawaii Office of Planning) and they fall into two categories: shrub and rangeland and evergreen forests. Even though *Pipturus* spp. do not occur in grassland habitats *per se*, they do occur in scrubland and shrubland areas at the margins of grasslands. Elevation was also used to classify habitats where *Pipturus* spp. occur, since it is one of the ecological factors found to significantly influence the parasitoid assemblage associated with *U. stellata* (Chapter 4). Non-target habitats below 500m were considered low elevation, habitats between 500 and 900m were considered as medium elevation and habitats above 900m were considered as high elevations.

Cotesia marginiventris (Cresson) (Hymenoptera: Braconidae)

It is a solitary endoparasitoid native to the West Indies (Muesbeck 1921). Eggs of *C. marginiventris* are oval. Boling and Pitre (1970) studied the biology of this species in the hosts *Tricoplusia ni* (Hübner), *Pseudoplusia includens*(Walker) and *Heliothis virescens* (Fabricius). *Cotesia marginiventris* undergoes three larval stages. Soon after molting to the third instar, the parasitoid larva exits its host to spin a white cocoon and pupates inside it. The host dies shortly after parasitoid emergence. Females bear short ovipositors and have a preference to oviposit in early instar larvae.

This parasitoid is currently distributed in Asia (India), Africa (Cape Verde, Egypt), Oceania (Australia) and North America (Bermuda, Canada, Mexico, in the United States: Alabama, Arkansas, California, Florida, Georgia, Hawaii, Illinois, Iowa, Kentucky, Louisiana, Mississippi, North Carolina, Ohio, Oklahoma, South Carolina, Texas and Virginia), Central America (Barbados, Cuba, Honduras and Trinidad and Tobago) and South America (Argentina, Bolivia, Brazil, Uruguay, Venezuela) (Crop Protection Compendium, CAB International, 2006).

Cotesia marginiventris has been purposely introduced to Cape Verde (Africa), Karnakata (India), Trinidad and Tobago (Central America), Australia (Oceania) and Hawaii (North America). Mr. Fred Bianchi, assistant entomologist of the Hawaiian Sugar Planters' Association (H.S.P.A) Experimental Station, introduced this parasitoid to Hawaii from Brownsville (Texas) to control the sugar cane pest *Spodoptera exempta* (Bianchi 1944, Pemberton 1948a, 1948b). The introduction was made during the last half of 1942. A total of 4,277 adults were distributed to Kauai, Oahu, Maui and Hawaii. It became quickly established in the Islands. At the time of the introduction there was no concern about possible non-target effects. Funsaki et al. (1988) and Henneman and Memmott (2001), report this species attacking other exotic species as well as native insects in Hawaii (Table 5.1). Retrospective studies on the non-target host *U. stellata* have shown that *C. marginiventris* can parasitize second, third and fourth instars and emerges from the host when it is either fifth of sixth instar (Chapter 2).

Meteorus laphygmae (Viereck) (Hymenoptera: Braconidae)

It is a nocturnal solitary koinobiont endoparasitoid (Fernández and Terán 1990b,Ortegón et al. 1988). Fernández and Terán (1990a) studied the biology of *M. laphygmae* in the host *Spodoptera frugiperda* (J. E. Smith). Eggs are oval with a pedicel that is thought to be used as a hook to adhere to internal organs of the host. *Meteorus laphygmae* undergoes three larval stages, the first two develop inside the host and soon after molting to the third instar the larva emerges from of the host to immediately start spinning a cocoon, inside which it metamorphoses to the pupal stage. The pupal cocoon is suspended from a thread anchored on foliage, as a protection from natural enemies (Bianchi 1944).

This parasitoid is currently distributed in many states in the continental United States as well as Hawaii Mexico, Central America (Trinidad and Tobago) and South America (Colombia and Venezuela) (Crop Protection Compendium, CAB International 2006). This parasitoid was purposely introduced to Hawaii by Bianchi, from Brownsville Texas to control the sugar cane pest *Spodoptera exempta*. The introduction process was done from June to November of 1942. A total of 3,900 wasps were distributed in various fields of Oahu, Maui, Kauai and Hawaii (Bianchi, 1944, Pemberton, 1948b).
At the time of the introduction there was no concern about possible non-target effects. Funasaki et al. (1988) and Henneman and Memmott (2001) reported *M. laphygmae* parasitizing other exotic species as well nontarget native in Hawaii (Table 5.2). Retrospective studies on the non-target host *U. stellata* have shown that *M. laphygmae* can parasitize second to fifth instar larvae and emerges from the host at sixth instar (Chapter 2).

Trathala flavoorbitalis(Cameron) (Hymenoptera: Ichneumonidae)

It is a solitary, parthenogenetic endoparasitoid (Sandanayake and Edirisinghe 1993). The literature reports that this parasitoid is a larval pupal parasitoid (Sandanayake and Edirisinghe 1993) but it was never observed emerging from pupa of *U. stellata* in Hawaii (Chapter 2).

Trathala flavoorbitalis is distributed in Asia (China, India, Indonesia, Japan, Myanmar, Philippines, Singapore, Sri Lanka), Europe (former USSR, Russian Federation), Hawaii and Australia. This parasitoid has not been purposely introduced anywhere in the world. It is considered an important biological control agent of many Lepidopteran pests in Asia (Sandanayake and Edirisinghe 1993). *Trathala flavoorbitalis*is an adventive species to Hawaii, first detected in 1910 (Swezey 1919) and for the purpose of this chapter it is considered as if it was intentionally introduced for the sake of risk modeling. This species has been reared from many adventive and endemic species in Hawaii (Table 5.3). Retrospective studies on the non-target host *U. stellata* have shown that *T. flavoorbitalis* can parasitize first to fifth instar larvae and emerges from the host at sixth instar (Chapter 2).

Analysis

For the purpose of the validation, *U. stellata* is considered to be the only non-target species of concern for each of the three parasitoids. In order to explore ways to express uncertainty created by the dearth of data available, two different scenarios were modeled: the average and the worst case scenario. Average scenarios were modeled based strictly on the information found in the literature; whereas the worst case scenario

was based on the assumption that data in the literature were not complete and that hypothetically the agents could successfully overlap, locate and parasitize susceptible stages of *U. stellata* at same rates as the target host.

Exposure assessment

Spatial overlap: Literature reports on parasitism were gathered for the three different parasitoids. Information on elevation and habitat type (land use and land cover: LULC) reported in the literature consulted was used to predict potential spatial overlap. The average case scenario for the three parasitoids assumed no spatial overlap (P = 0) with habitats that maintain populations of *U. stellata* (evergreen forests, and scrubland/shubland sites)at low (below 500 m), medium (between 500 and 900 m) and high elevation (above 900 m) sites based on the fact that literature data only report parasitism in agricultural and grassland areas in their areas of distribution (Alvarado-Rodriguez 1987, Karla 1989, Molina-Ochoa et al. 2004, Tandon and Lal 1983). The worst case scenario assumed that data in the literature were biased by habitat type and that the three parasitoids will be able to occur in other habitats such as forest and shrub land areas at the three elevation ranges, therefore overlap spatially with *U. stellata* populations (P = 1.0).

Temporal overlap: The non-target species, *U. stellata,* is perennially present. Because of the environmental conditions in Hawaii, it is assumed that the three parasitoids will also be perennially active. Therefore full synchronization with susceptible stages of *U. stellata* is considered to be the most likely scenario for all three cases (probability of temporal overlap = 1).

Effects assessment

The effects assessment phase was based on literature records of parasitism for each of the three parasitoid species. Parasitism data obtained from the literature were used to construct probability distributions for each elevation level (low, medium and high).

The standard case scenario assumed that each of the parasitoids will attack only hosts in the families with records of parasitism obtained from literature data. The worst case scenario will assume that hosts in the family Crambidae (specifically *U. stellata*) will also be subject to parasitism.

For *C. marginiventris* and *M. laphygmae*, parasitism data were obtained for the target hosts *S. frugiperda* and *S. exiguaat* low, medium, and high elevations(Alvarado-Rodriguez 1987, Molina-Ochoa et al. 2004) in different states in Mexico (sites at similar latitude to Hawaii). For *T. flavoorbitalis*, parasitism rates were obtained for the target pest *Antigastra cautalaunalis* in India at only low elevation sites (below 500 m) (Karla 1989, Tandon and Lal 1983)(Latitude: 29 and 23 degrees north, respectively).

Risk characterization

Data from the exposure and effects assessment from the analysis section were used to construct the precision tree to calculate an overall probability distribution that effects might occur for each of the given scenarios. Figure 5.2 shows the precision tree for the worst case scenario

Data on apparent mortality (chapter 2) were used to construct probability distributions and validate the results obtained from the precision trees (based on literature information).

Uncertainty analyses (MC simulations) were conducted in Crystal Ball® (Decisioneering 2005) and run with 2000 trials each. Probability distributions were also generated for the observed parasitism rates in Hawaii. Results of the MC simulations are presented graphically as probability/frequency distributions of all possible outcomes. Data generated during the simulations were extracted and analyzed using nonparametric statistics (does not assume normality or equal variance). The Mann-Whitney Rank Sum Test was used to test for significant differences between two groups, and the Kruskal-Wallis ANOVA on Ranks was used to compare more than two groups. The statistical analyses were done using SigmaStat.

Question 2: 'Does using single point estimates as opposed to probability distributions to estimate exposure significantly impact the final risk assessment?

To answer this question a number of hypothetical scenarios were created. Eighteen random numbers were chosen to reach a mean of 0.5333, and this was repeated 10 times in order to generate simulated data sets each with a different variance (Table 5.6). Probability distributions were created for each of the 10 hypothetical data sets and MC simulations run with 2000 trials each. The data generated in the MC simulations for the10data sets were extracted and compared statistically using the Kruskal-Wallis ANOVA on Ranks test.

Question 3: How useful are estimates of marginal attack rates versus apparent mortality (and measures of uncertainty in both) in conducting probabilistic risk assessment?

Values of marginal attack rates (from life table studies, Chapter 3) and apparent mortality rates (field parasitism, Chapter 2) of *U. stellata* were used for this analysis, for each of the three parasitoid species in order to build probability distributions. Data from the life table studies reported in Chapter 3 and from field surveys of apparent mortality (Chapter 2) were pooled across exposure trials and surveys respectively to permit the development of probability distributions. Probability distributions of marginal attack rate and apparent mortality were used as input variables in probabilistic models (run in Crystal Ball®) keeping all other variables of the model constant (spatial and temporal overlap) to run the simulations. This provided estimates of mean mortality as if run in a risk-analysis using measures of apparent and marginal mortality. Data generated during the simulations were extracted. The Mann-Whitney Rank Sum Test was used to test for significant differences between the two groups. In order to test the overall impact of using apparent or marginal mortality in combination with other factors varying, the same analysis was run but this time having another input variable as probability distribution ('spatial overlap').Data sets 3, 4 and 10 from Question 2 (Table 5.6) were used to generate the probability distributions for 'spatial overlap'.

Question 4: Are there any key ecological variables that would be worth considering in the hypothetical case that any of the species will be considered for introduction in another place or are worth considering in any risk assessment?

The results of the comparison of the prediction model and actual field parasitism in Hawaii (Question 1) will indicate which aspects of a potential biological control agents' biology and ecology need to be investigated to provide a robust estimate of their non-target potential.

Results and Discussion

Question 1: Would it have been possible to predict that any of the three parasitoid species will attack *U. stellata* based on literature data, and would have been possible to predict their level of impact on this non-target species using PRA?

Cotesia marginiventris

Table 5.4 presents the list of known hosts before it was introduced to Hawaii in 1942. Most records of parasitism by *C. marginiventris* associate this parasitoid with Noctuidae hosts. Nevertheless, the literature also reports parasitism on species in the family Sphingidae and Crambidae. Within the family Crambidae, *C. marginiventris* is known to parasitize *Udea rubigalis* (Table 5.4), a species native to continental North America and pest of celery, also known as the celery leaf-tyer. This is a direct indication that other species in that genus, such as *U. stellata*, could have been predicted as potential non-target hosts (Hawaii has 44 described endemic representatives in this genus). Records following introduction in Hawaii show that besides attacking *U. stellata*, *C. marginiventris* also parasitize the endemic species *U. pyranthes* as well as other endemic non-target hosts in the families Crambidae, Tortricidae, Geometridae, Oecophoridae as well as Noctuidae (Table 5.1).

Based on data gathered from the literature it was also predicted that *C. marginiventris* would only occur in agricultural and grassland areas at low to high elevation sites. Figure 5.3 shows the results of the PRA based on parasitism reported in the literature on the target pests *Spodoptera exigua* and *S. frugiperda* in agricultural and grassland areas at low, medium and high elevation sites. The data used were from an extensive field survey for parasitoids associated with those pests in Mexico (sites at similar latitude to Hawaii). Results of the PRA are presented in graphs, which are probability/frequency distributions of all possible parasitism rates (expressed as proportions on the x-axis) based on records of parasitism. They represent the worst case scenario which assumes spatial overlap and temporal overlap ($P = 1$ for both factors) with populations of *U. stellata* and that parasitism rates on *U. stellata* will be similar to those recorded in the literature for its target hosts *Spodoptera exigua and S. frugiperda.* The standard case scenario leads to an overall probability of zero (therefore no probability distribution could be built) since it assumed successful temporal overlap ($P = 1$) but no spatial overlap ($P = 0$).

Retrospective studies in Hawaii have shown that *C. marginiventris* can occur in forest and scrubland/shrubland areas, and therefore overlap with populations of *U. stellata*, which coincides with the assumptions in the worst case scenario. Figure 5.4 shows the probability distribution of the observed parasitism rates of *U. stellata* in Hawaii at high elevation sites. Parasitism of *U. stellata* by *C. marginiventris* was not recorded at low and medium elevations sites even though the standard case scenario predicted that this species could occur also at low and medium elevations.

The Kruskal-Wallis ANOVA on Ranks was used to detect significant differences in means and standard deviations from the statistical reports of the Monte Carlo (MC) simulations analyses with data gathered from the literature at the three elevations. The Mann-Whitney Rank Sum Test was used to detect significant differences between results of the MC analysis with data from the literature and with data from the retrospective study.

Based on the results of the statistical analyses, there were significant differences in parasitism rates by *C. marginiventris* reported in the literature at the three elevation sites ($H = 2255.2$; $P < 0.001$). When results of the MC analysis with parasitism rates recorded at high elevations in Hawaii was compared statistically with results of the MC simulation analysis with parasitism from literature data at low, medium and high elevations, field parasitism of *U. stellata* at high elevations in Hawaii was significantly higher than parasitism of *S. exempta* at low, medium and high elevation sites ($P < 0.001$). Based on the results of the simulation with literature data (which represents the worst case scenario), parasitism rates of *U. stellata* by *C. marginiventris* in Hawaii were expected to be higher at medium and low elevation sites and minimal at high elevation sites. Nevertheless, the quantitative data from field studies in Hawaii show that all the parasitism of *U. stellata* by *C. maginiventris* was inflicted at high elevations and none at low and medium elevations. The target hosts *S. exempta* and *S. frugiperda*, are distributed from low to high elevation sites in the area where data on parasitism rates by *C. marginiventris* were gathered from (Mexico). In Hawaii the main target hosts *S. exigua* (sugar cane pest) and *S. mauritia* (common pest of turf grasses) occur mainly at low and maybe medium elevation sites, but not at high elevation sites, which might explain why

parasitism of *U. stellata* by *C. marginiventris* was only observed at elevations where there is absence of the preferred host.

Based on records in the literature, it would have been possible to predict that *U. stellata* would be within the physiological host range of *C. marginiventris*. It would have also been possible to predict that this species should be able to persist across a wide elevation gradient. However, literature records did not provide any indication that this species will be able to occupy areas other than agricultural lands and grasslands. Predictions of parasitism rates based on literature records would have been further limited since no parasitism records were found on a closely related species such as *U. rubigalis* (reported as a host but no records of parasitism rates were found in the literature).

Meteorus laphygmae

Table 5.5 shows the list of known hosts of *M. laphygmae* prior its introduction to Hawaii. Most literature records associate *M. laphygmae* with Noctuidae pests in agricultural systems. Based on the records there was no direct indication that this species would have been able to attack species in the family Crambidae at the time of introduction. Records of non-target parasitism in Hawaii have shown that this species can attack hosts in the families Crambidae, Oecophoridae, Tortricidae and Heliconiidae, besides non-target hosts in the families Noctuidae and Geometridae (Table 5.2).

As with *C. marginiventris*, *M. laphygmae* was predicted to occur only in grassland and agricultural areas. The worst case scenario assumed that *M. laphygmae* could occupy habitats of *U. stellata* and parasitize hosts in the family Crambidae at the same parasitism rates as their target hosts *S. exigua* and *S. frugiperda* in Mexico.

Retrospective studies in Hawaii have shown that *M. laphygmae* can occupy habitats of *U. stellata* and successfully parasitize this non-target species. Figure 5.5 shows results of the PRA analysis using data from the literature for low, medium and high elevation sites. Figure 5.6 shows the probability distributions for the observed parasitism rates on *U. stellata* in Hawaii at medium and high elevation sites. *Meteorus laphygmae* was not found parasitizing larvae of *U. stellata* at low elevation sites.

Based on the results of the statistical analyses, there were significant differences in parasitism rates by *M. laphygmae* reported in the literature at the three elevation sites (H =1725.8; $P < 0.001$).When results of the simulation analysis with retrospective data from Hawaii at medium and high elevation sites were compared statistically; parasitism rates at high elevations were significantly higher than medium elevation sites (T = 4978341; $P < 0.001$). There was no significant statistical difference between the results of the simulation analysis with parasitism data from literature at low elevations and results of the simulation analysis with parasitism on *U. stellata* in Hawaii at high elevation sites(T = 3808532; $P = 0.444$). No significant differences were found between results of the simulation with data from the literature at medium elevation and results with retrospective data from Hawaii at medium elevations (T = 3850017; $P = 0.886$). *Meteorus laphygmae* can inflict as much parasitism on *U. stellata* as on its target hosts, nevertheless this parasitism occurs at altitudes where the target hosts are not present.

Based on literature records it would have been unlikely to predict that *U. stellata* will be in *M. laphygmae*'s physiological host range since there was no indication that this species would attack hosts in the family Crambidae. Nevertheless it would have been possible to predict that this species could occur across a wide range of elevations. The

expectation that *U. stellata* will have similar trends in parasitism level as on its preferred hosts in the area of distribution did not hold valid; it would have been predicted that higher parasitism rates should be inflicted at low elevation sites rather than medium and high elevations. Nevertheless, no parasitism by *M. laphygmae* was detected at low elevations, but at medium and mostly high elevations. As in the case of *C. marginiventris*, the target hosts of *M. laphygmae* in Hawaii such as *Herpetogramma licarsisalis*, *S. mauritia* (pests of turf grasses), *S. exempta* (pest of sugar cane and grasses) and *S. exigua* (common pest of crucifer crops) predominate at low and medium elevations, which may suggests that degree of non-target parasitism might be mediated by availability of preferred hosts. Duan and Messing (1998) reported similar results from Hawaii, percentage parasitism of the lantana gall fly (a purposely introduced non-target species), was more heavily parasitized by the fruit fly parasitoid *Dichasmimorpha tryoni* in upland forest habitats rather than in low-land pastures and mid-elevation sugar cane fields.

Trathala flavoorbitalis

No host records of known hosts could be obtained for *T. flavoorbitalis* prior to the year that it was first observed in Hawaii ('introduction'). The first records found in the literature correspond to hosts in Hawaii. Table 5.3 shows the known hosts in Hawaii.

This species was found occupying *U. stellata* habitats at low, medium and high elevation sites, even though literature records consulted only report parasitism in low elevation agricultural areas. The worst case scenario assumed that this species would occupy *U. stellata* habitats and attack this non-target species at the same rates as its

known target host, *Antigastra catalaunalis* (Lepidoptera: Crambidae), in their area of redistribution (India). Figure 5.7 shows the results of the probabilistic analysis with data from the literature at low elevation sites. Figure 5.8 shows the results of the MC analysis with field parasitism rates of *U. stellata* at low, medium and high elevation sites in Hawaii. When results of the simulation analysis using literature data at low elevation were compared statistically with results of the simulation analysis with field data in Hawaii at low elevation sites, field parasitism in Hawaii was significantly higher ($T = 5418198.5$; $P < 0.001$); in other words *U. stellata* parasitism rates at low elevation sites in Hawaii were significantly higher than parasitism rates on *A. cautalaunalis* reported in the literature at low elevation sites in its area of distribution. When results of the simulation analysis with retrospective data from Hawaii at the three elevations were compared statistically using the Kruskal-Wallis ANOVA on ranks, significant differences were found ($H = 2517.7$; $P < 0.001$), parasitism rates at low and medium elevations were significantly higher than parasitism rates at high elevation sites ($P < 0.05$).

Based on literature records it would have been unlikely to predict that *T. flavoorbitalis* would occur at medium and high elevations. Known target hosts from its area of distribution (*Spoladea recurvalis* and *Herpetogramma licarsisalis*) are also present at low elevations in Hawaii, nevertheless, significantly higher parasitism rates on *U. stellata* by *T. flavoorbitalis* is inflicted at low and medium elevations than at high elevations, which contrasts with the pattern seen with *C. marginiventris* and *M. laphygmae*. Perhaps this species performs better under lower elevations and/or the known hosts in the area of introduction are not preferred hosts.

Question 2: 'Does using single point estimates as opposed to probability distributions to estimate exposure significantly impact the final risk assessment?

Results of the ANOVA showed that there were significant differences among the 10data sets that have the same mean (H = 1711.1; $P < 0.001$). Therefore different data sets with the same mean but different variances will have a different set of possible outcomes that may significantly influence the estimates of risk (Figure 5.9). Single point estimates ignore variation of a data set, and provide a false sense of precision that leads to what is called "analysis paralysis" (Decisioneering, Crystal Ball User Manual 7.3 2007). Probabilistic analysis provides the capacity to forecast all possible outcomes and a tool for quantifying certainty in achieving a particular result, and therefore leads to more acute estimates of risk posed by a particular scenario. By replacing single points with probability distributions it will be possible to answer question such as 'what are the chances that parasitism rates will be higher than 40% in a certain habitat?', or 'what are the chances that parasitism rates will be lower than 20%'? This becomes important in situations where in order to take the decision to release a biological control agent one needs to be 90% certain that field parasitism rates will be lower than 20% in a certain non-target species in a certain habitat, or any other combination of certainties. Figures 5.10, 5.11 and 5.12 show the results of 3 of 15 MC simulation analyses (data sets 3, 4 and 10, respectively) to illustrate this point. Figure 5.10 shows the range of all possible outcomes given the uncertainty within the data, and specifically predicts that there is 38.44% certainty that parasitism rates will be over 0.40 (or 40%) under the circumstances in the model. Figure 5.11 and 5.12 forecast that there is 60.92% and 96.29% chance that parasitism rates will be over 40%, respectively for the scenario analyzed.

Question 3: How useful are estimates of marginal attack rates versus apparent mortality (and measures of uncertainty in both) in conducting probabilistic risk assessment?

No probability distributions could be built for *C. marginiventris* and *M. laphygmae* marginal attack rates since 17 of the 18 values were zeros and those values were extremely low (Tables 5.7, 5.8). Figure 5.13 shows a graphical summary of the output of the Monte Carlo simulations for *T. flavoorbitalis* for apparent mortality and marginal attack rates. The range of possible apparent mortality rates vary from zero to 59%, whereas the range of possible outcomes for marginal attack rate ranged from zero to 15%. Results of the Mann-Whitney Rank Sum Test showed that there were significant statistical differences (T = 1960261; $P < 0.001$) in the outputs of the simulation analysis. Results of the simulations using apparent mortality significantly increased the estimates of risk. Results of the MC analyses with apparent mortality data were still significantly higher than results with marginal mortality data when a second uncertainty factor (representing 'spatial overlap') was added to the analysis ($P < 0.00$; using sets 3, 4 and 10 in Table 5.6). Figure 5.14 shows the range of all possible values for *T. flavoorbitalis* apparent mortality and specifically indicating a 28.21 % probability that values of apparent mortality will fall above 40%. Hawkins and Cornell (1994) suggest that in most cases parasitism rates above 40 % are necessary to cause a population level impact. Nevertheless, in this system, life table studies have shown that parasitism by *T. flavoorbitalis* (which can exceed 40%) has a minimal contribution to total mortality of *U. stellata* (Chapter 3).

The use of marginal attack rates derived from life table studies should be encouraged whenever possible, as they provide a more realistic estimate of the non-target risk as they indicate the level of mortality over a generation, whereas apparent mortality does not have population impact context and can potentially overestimate or underestimate the actual level of mortality in situations where susceptible stages are over or under-sampled (Simmonds 1949, Van Driesche 1983, Van Driesche et al.1991).

Question 4: Are there any key ecological variables that would be worth considering in the hypothetical case that any of the species will be considered for introduction in another place or are worth considering in any risk assessment?

Environmental context clearly influenced the level of non-target parasitism in this study system (*U. stellata* – parasitoid species). Results of this and previous chapters have shown that parasitism levels vary at low, medium and high elevation. In the case of *T. flavorbitalis*, parasitism rates on *U. stellata* at low and medium elevations sites were significantly higher than at high elevation sites. Non-target parasitism of *U. stellata* by *C. marginiventris* and *M. laphygmae* was significantly higher at high elevations than at medium or low elevations. If identified non-target species occur in multiple habitats or across a wide range of environmental conditions, such as, in this particular study elevation and level of disturbance by alien plants (Chapter 4), they should be incorporated in RA.

Even though it has been acknowledged that non-target parasitism varies among habitats (Barratt et al. 2007, Benson et al. 2003, Follett et al. 2000b, Johnson et al. 2005, Oboyski et al. 2004), most risk assessment studies give emphasis to assessing host range

or level of non-target parasitism but not to assessing how habitat and/or environmental/ecological gradients may mediate those levels of non-target parasitism. By incorporating spatial and ecological context in risk assessment, it will be possible to detect habitats that might be at higher risk for adverse effects, and use that information for regulation. For example if parasitism rates on a non-target species are expected to occur or be above an acceptable level in habitats in conservation areas, the introduction of the BC agent might not be justified. But if non-target parasitism is expected in areas that do not have conservation priority, the decision might depend on the level of confidence that parasitism levels will be below a certain acceptable level (which will not cause an impact at the population level). Results from this type of RA can also provide a basis for establishment of possible management programs. For example when considering augmentative biological control agents, depending on the habitat and its associated level of parasitism, it could be possible to restrict releases to areas that have low levels of risk or acceptable parasitism rates. Furthermore, by incorporating data based on seasonal effects it could be possible to plan releases at times in the year when they have lower levels of risk.

Another component that likely influenced the degree of non-target host use in this study system was the availability of preferred hosts. In the case of oligophagous/polyphagous species, presence of preferred and/or alternative hosts can play an important role in the level of non-target attack (Briese et al. 2002, Briese et al.2005, Louda et al. 2003a). *Cotesia marginiventris* and *M. laphygmae* can occur in a wide range of ecological conditions (Molina-Ochoa et al. 2004, Peck et al. 2008); nevertheless their association with *U. stellata* is mainly in less disturbed, high altitude

sites in Hawaii, which are dominated by native species. The fact that they are associated with *U. stellata* at high altitude sites does not necessarily mean that they prefer those habitats, but perhaps that at those sites they are more dependent on endemic non-target species due to the absence of the preferred hosts. It is therefore important to not only describe the environment where the BC candidate and non-target species occupy but also describing the environment that the target hosts will occupy in the area of introduction since BC agents can disperse beyond habitats of the target hosts (Johnson et al. 2005).

General Discussion

The major advantage of the PRA framework is that it considers effects to non-target species individually at a spatial scale and evaluates the risk posed by new introductions in a more meaningful way than procedures that rely on single point estimates and that do not take into account spatial variability. Nevertheless more realistic and meaningful ways are not always the simplest to apply. PRA will require more data than other proposed approaches (van Lenteren et al. 2003, 2006), which may not always be available for decision makers. Even though in order to conduct PRA it is not necessary to use probability distributions for all input variables, the effectiveness and reliability of PRA will depend on the incorporation of probability distributions for those variables that are known to greatly influence the overall estimate risk to adequately provide a description of all possible outcomes. Levins (1966) suggested that there is an unavoidable tradeoff between precision, generality and realism in ecological models. A precise and realistic model will necessary be limited to a few applications, whereas a very general approach applicable to many situations will necessary be limited in precision.

Not all non-target species are affected equally, and the same non-target species may not be affected equally at different environmental conditions (Barratt et al. 2007, Follett at al. 2000b, Johnson et al. 2005).Comprehensive risk assessment requires a comprehensive understanding of the ecology of the biological control agent, as well as the ecology of the target and non-target species (Louda et al. 2003a). Observations on the ecology of the BC agent in other areas (including the area of origin) and how parasitism rates are mediated in those areas under different environmental/ecological conditions can provide useful baseline information for predictions.

Biological control practitioners as well as regulators should prefer the release of agents with narrow host ranges (Louda et al. 2003b). Nevertheless, when faced with situations where agents with great potential to control the target pest and may attack some native and beneficial species, careful characterization of the risk should be done to estimate the relative impact of the proposed introduction on populations of the non-target hosts and weighted against the potential benefits (Delfosse 2005, Jetter 2005). In those situations the use of PRA could be a tool for better decision-making. Some retrospective studies on polyphagous BC species have shown that they cause minimal impact on the non-target species of concern (Barron et al. 2003, Johnson et al. 2005), and it will be valuable to weigh the environmental benefits attributable to those releases, to determine if the non-target parasitism on endemic species, even if minimal, was justified. Collective negative impacts such as habitat destruction and mortality for various sources may however result in even minimal non-target mortality becoming important; therefore it is also important to assess impacts of BC introductions in context to other current sources of mortality (Follett et. al. 2000b).

Forecasts from risk assessment analysis may be used to make decisions to either accept or reject a proposed introduction. As far as possible quantitative methods which incorporate a measure of uncertainty should be preferred. Use of uncertainty in probabilistic analysis can be used to set standards based on the probability that some threshold for unacceptable effects will be exceeded (Suter, 1990), therefore not only provide information for the risk characterization phase but can play an important role in benefit risk analysis phase. The use of probability distributions in risk assessment can clarify the relationship between decision making and uncertainty and justify additional studies.

Not all proposed introductions will need PRA, obviously safe BC agents probably will not require such assessment. The environmental risk assessment proposed by van Lenteren et al. 2003, 2006 is a procedure can be used as a first step to encourage the screening of safe biological control agents to all potential non-target species using pre-determined qualitative and quantitative rankings. When promising BC agents are found to attack non-target species, PRA can provide an effective means to characterize the risk to selected non-target species of concern which can be used to provide levels of confidence that a certain level of non-target effects will not be exceeded, and could further be implemented in risk benefit analysis. Non-target effects from biological control introductions is not desirable, but if effects are predicted with high levels of confidence and are acceptable (not causing an impact a population level) the decision to accept or reject a BC agent can be knowledge driven rather than fear based (Briese 2005).

Table 5.1. List of known hosts of *C. marginiventris* in Hawaii (adventive = accidental introduction)

Species	Family	Origin	Reference
Agrotis hephaestaea (Meyrick)	Noctuidae	Endemic	1
Ethmia nigroapicella (Saalmuller)	Oecophoridae	Adventive	2
Eudonia sp.	Crambidae	Endemic	1
Eupithecia monticolens Butler	Geometridae	Endemic	1
Fletcherana leucoxyla Meyrick	Geometridae	Endemic	1
Haliophyle euclidias Meyrick	Noctuidae	Endemic	1
Scotorythra arboricolans Butler	Geometridae	Endemic	1
Scotorytorythra caryopis Meyrick	Geometridae	Endemic	2
Scotorythra hecataea Meyrick	Geometridae	Endemic	1
Scotorythra pauludicola (Butler)	Geometridae	Endemic	2
Scotorythra rara Butler	Geometridae	Endemic	1
Scotorythra trapezias Meyrick	Geometridae	Endemic	2
Scotorythra spp.	Geometridae	Endemic	1
Spodalea recurvalis (Fabricius)	Crambidae	Adventive	2
Pseudaletia unipuncta (Haworth)	Noctuidae	Adventive	2
Spodoptera exempta (Walker)	Noctuidae	Adventive*	2
Spodoptera mauritia (Boisduval)	Noctuidae	Adventive	2
Spheretista pleonectes Walsingham	Tortricidae	Endemic	1
Udea stellata (Butler)	Crambidae	Endemic	3

[1] http://www.umwestern.edu/shares/envirosci_share/laurie/lepidoptera/welcome.htm
[2] Funasaki et al. 1988
[3] Kaufman and Wright, submitted
* Target host

Table 5.2. List of known hosts of *M. laphygmae* in Hawaii (adventive = accidental introduction, Introduced = purposely introduced)

Species	Family	Origin	Reference
Agraulis vanillae (Linnaeus)	Nymphalidae	Adventive	2
Agrotis hephaestaea (Meyrick)	Noctuidae	Endemic	1
Agrotis ipsilon (Hufnagel)	Noctuidae	Adventive*	2
Amorbia emigratella Busck	Tortricidae	Adventive	1
Amyna natalis (Walker)	Noctuidae	Adventive	2
Autegumia ebulealis Guenee	Crambidae	Introduced	2
Elaphria nucicolora (Guenee)	Noctuidae	Adventive	2
Eudonia sp.	Crambidae	Endemic	1
Eupithecia monticolens Butler	Geometridae	Endemic	1
Fletcherana leucoxyla Meyrick	Geometridae	Endemic	1
Haliophyle euclidias Meyrick	Noctuidae	Endemic	1
Helicoverpa zea (Boddie)	Noctuidae	Adventive	2
Herpetogramma licarsisalis (Walker)	Crambidae	Adventive	2
Melipotis indomita (Walker)	Noctuidae	Adventive	2
Omiodes accepta (Butler)	Crambidae	Endemic	2
Rynchopalpus brunellus Hampson	Noctuidae	Introduced	2
Scotorythra spp.	Geometridae	Endemic	1
Scotorythra apicalis Swezey	Geometridae	Endemic	1
Scotorythra hecataea Meyrick	Geometridae	Endemic	1
Scotorythra ortharcha Meyrick	Geometridae	Endemic	1
Scotorythra rara Butler	Geometridae	Endemic	1
Scotorythra paludicola (Butler)	Geometridae	Endemic	2
Scotorythra trapezias (Meyrick)	Geometridae	Endemic	2
Spodoptera exempta (Walker)	Noctuidae	Adventive*	2
Spodoptera exigua (Hubner)	Noctuidae	Adventive*	2
Spodoptera mauritia (Boisduval)	Noctuidae	Adventive*	2
Thyracopa spp.	Oecophoridae	Endemic	1
Udea stellata (Butler)	Crambidae	Endemic	2
Udea pyranthes (Meyrick)	Crambidae	Endemic	1

[1] http://www.umwestern.edu/shares/envirosci_share/laurie/lepidoptera/welcome.htm
[2] Funasaki et al. 1988
* Target host

Table 5.3. List of known hosts of *T. flavoorbitalis* in Hawaii (adventive = accidental introduction, Introduced = purposely introduced) (Swezey 1929)

Species	Family	Origin
Agonoxena argaula Meyrick	Agonoxenidae	Adventive
Asymphorodes dimorpha (Busck)	Asymphorodes	Aventive
Bradleyella metallurgica (Walsingham)	Tortricidae	Endemic
Bracta straminea (Butler)	Tortricidae	Adventive ?
Bracta venosana (Zeller)	Tortricidae	Introduced
Carposina gramicolor (Walsingham)	Carposinidae	Endemic
Chedra microstigma (Walsingham)	Batrachedridae	Adventive
Chilo supressalis (Walker)	Crambidae	Adventive
Crocidosema blackburni (Butler)	Tortricidae	Endemic ?
Crocidosema marcidella (Walsingham)	Tortricidae	Endemic ?
Crocidosema lantana Busck	Tortricidae	Introduced
Cryptophlebia illepida (Butler)	Tortricidae	Adventive
Erechthias minuscula (Walsingham)	Tineidae	Adventive
Herpetogramma licarsisalis (Walker)	Crambidae	Adventive
Omiodes accepta (Butler)	Crambidae	Endemic
Omiodes blackburni (Butler)	Crambidae	Endemic
Omiodes localis (Butler)	Crambidae	Endemic
Omiodes meyricki Swezey	Crambidae	Endemic
Omiodes monogramma Meyryck	Crambidae	Endemic
Omiodes muniscola Swezey	Crambidae	Endemic
Pyroderces rileyi (Walsingham)	Cosmopterigidae	Adventive
Spheterista infaustana (Walsingham)	Tortricidae	Endemic
Spheterista reynoldsiana (Swezey)	Tortricidae	Endemic
Spodalea recurvalis (Fabricius)	Crambidae	Adventive
Thyrocopa spp.	Oecophoridae	Endemic
Udea chalcophanes (Meyrick)	Crambidae	Endemic
Udea micacea (Butler)	Crambidae	Endemic
Udea platyleuca (Meyrick)	Crambidae	Endemic
Udea stellata (Butler)	Crambidae	Endemic
Udea violae (Swezey)	Crambidae	Endemic

Table 5.4. List of known hosts of *C. marginiventris* prior to its introduction to Hawaii in 1942.

Species	Family	Reference
Pseudaletia latiuscula (Herrich-Schaffer)	Noctuidae	Jones and Wolcott 1922
Spoladea recurvalis (Fabricious)	Crambidae	Poost 1927
Spodoptera exigua(Hübner)	Noctuidae	Wilson 1932
Spodoptera frugiperda(J. E. Smith)	Noctuidae	Luginbill 1928
Spodoptera praefica (Grote)	Noctuidae	Blanchard and Conger 1932
Udea rubigalis (Guenee)	Crambidae	Ball et al. 1935

Table 5.5. List of known hosts of *M. laphygmae*previous its introduction to Hawaii in 1942.

Species	Family	Reference
Agrotis subterranea (Fabricius)	Noctuidae	Muesebeck 1923
Helicoverpa zea (Boddie)	Noctuidae	Muesebeck 1923
Monodes spp.	Noctuidae	Muesebeck 1923
Pseudaletia unipuncta (Haworth)	Noctuidae	Vickery 1915
Spodoptera spp.	Noctuidae	Muesebeck 1923
Spodoptera praefica (Grote)	Noctuidae	Blanchard and Conger 1932
Spodoptera frugiperda(J. E. Smith)	Noctuidae	Muesebeck 1923
Spodoptera exigua(Hübner)	Noctuidae	Muesebeck 1923
Colias eurytheme (Boisduval)	Pieridae	Muesebeck 1923

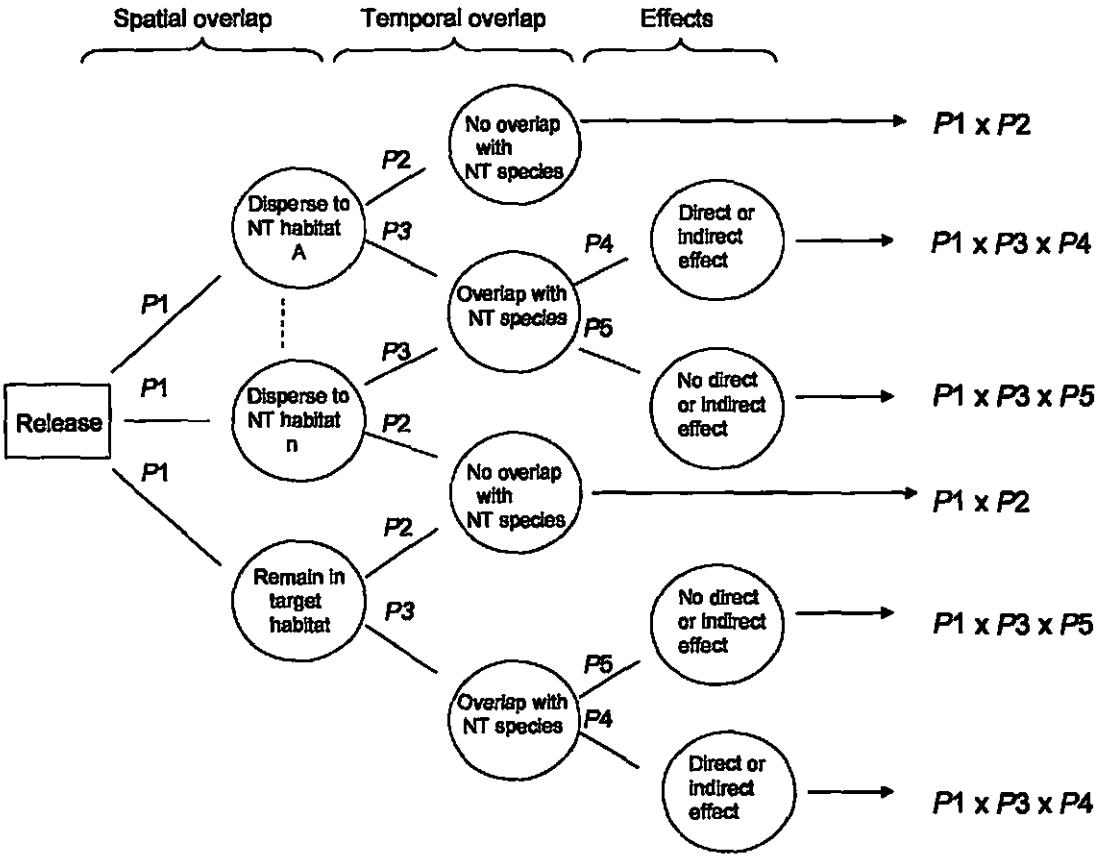

Figure 5.1. Conceptual precision tree. Circles contain contingencies (spatial overlap, temporal overlap and effects), probability of each occurring is given in connecting lines. The overall probability of impact is estimated by multiplying the *P* values along each branch.

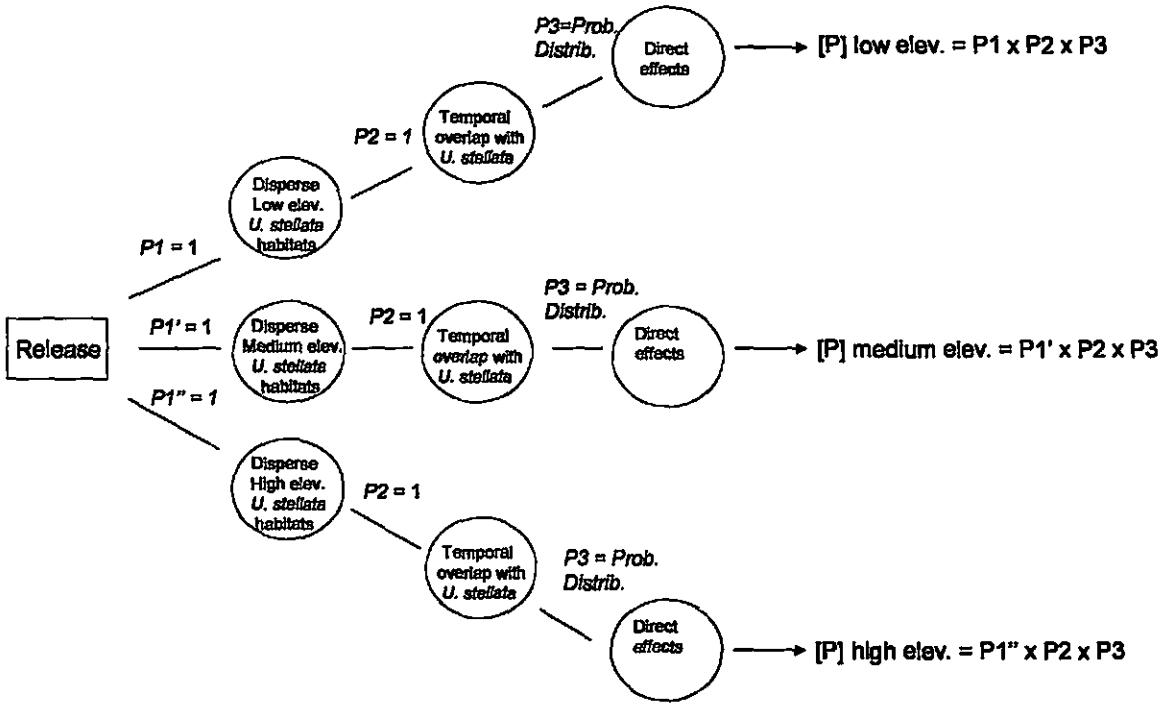

Figure 5.2. General precision tree for the worst case scenario. *P*1 represents the probability for spatial overlap, *P*2 represents the probability for temporal overlap and *P*3 represents the probability distribution of all possible outcomes for parasitism (effects on a non-target species).

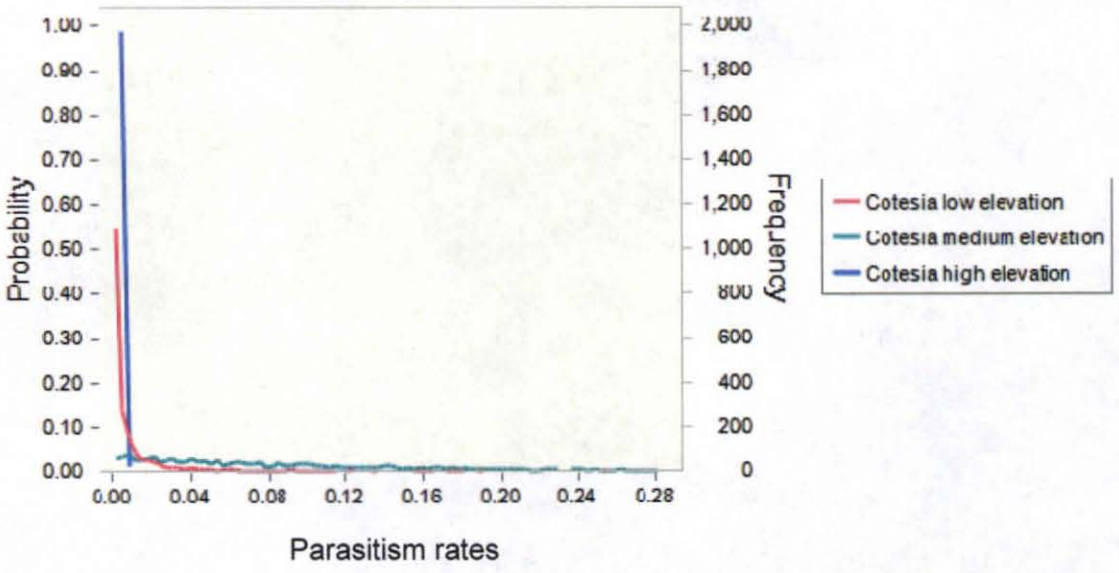

Figure 5.3. Overlay chart showing forecast of expected parasitism rates (as proportion on the x-axis) for *C. marginiventris* at low, medium and high elevation sites using data from the literature.

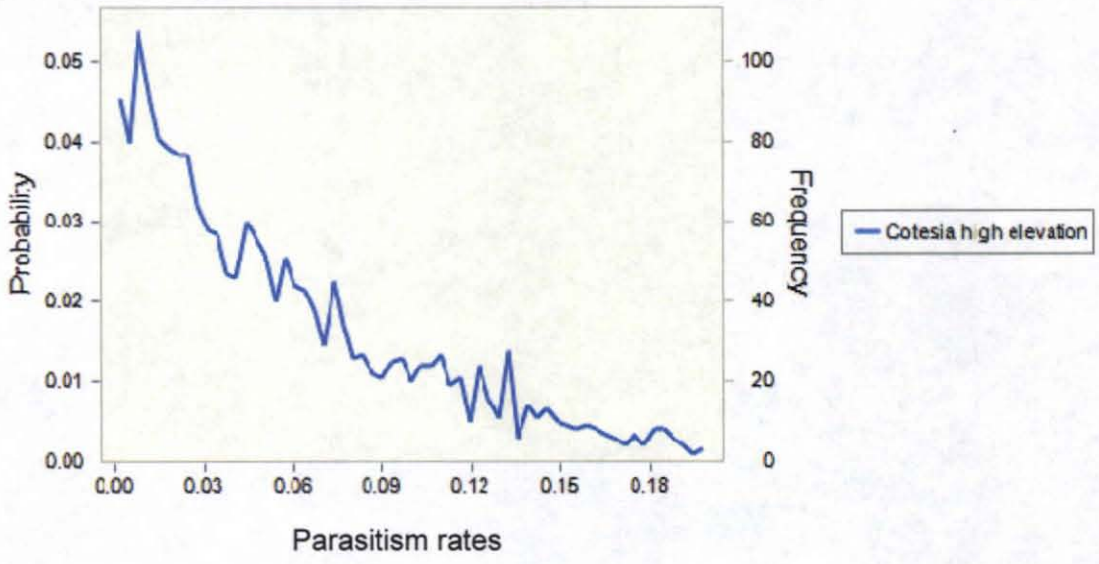

Figure 5.4. Forecast of all possible parasitism rates (as proportion on the x-axis) for *C. marginiventris* at high elevation sites using field collected data from retrospective studies in Hawaii.

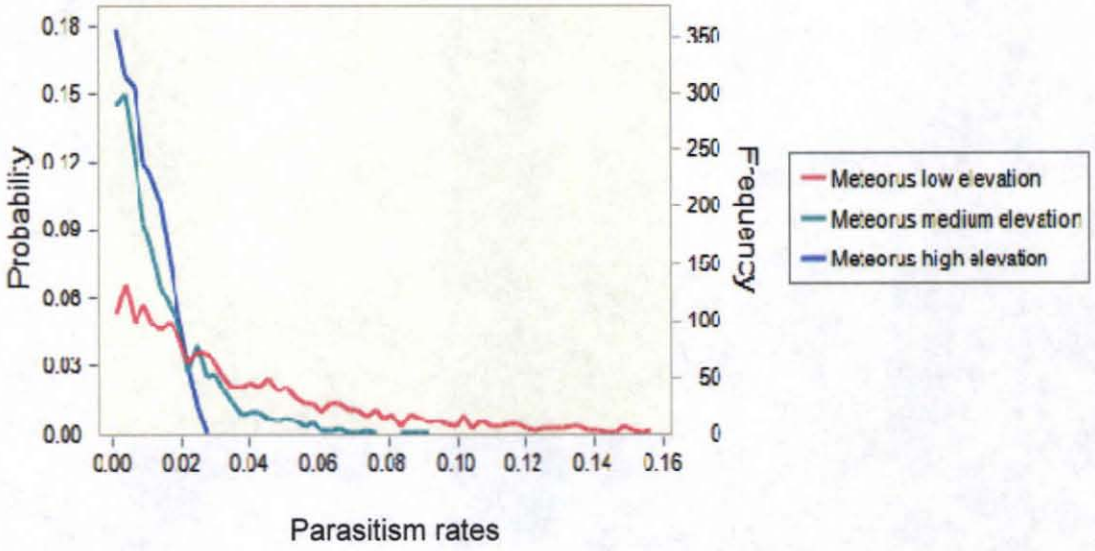

Figure 5.5. Overlay chart showing forecast of all possible parasitism rates (as proportion on the x-axis) for *M. laphygmae* at low, medium and high elevation sites using data from the literature.

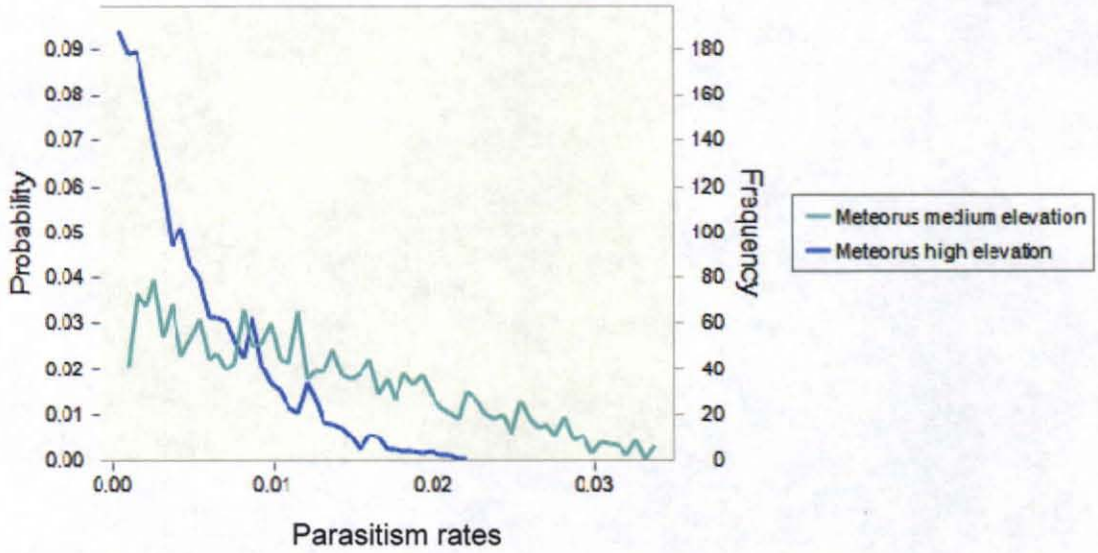

Figure 5.6. Overlay chart showing forecast of all possible parasitism levels (as proportion on the x-axis) for *M. laphygmae* at medium and high elevation sites using data from the literature.

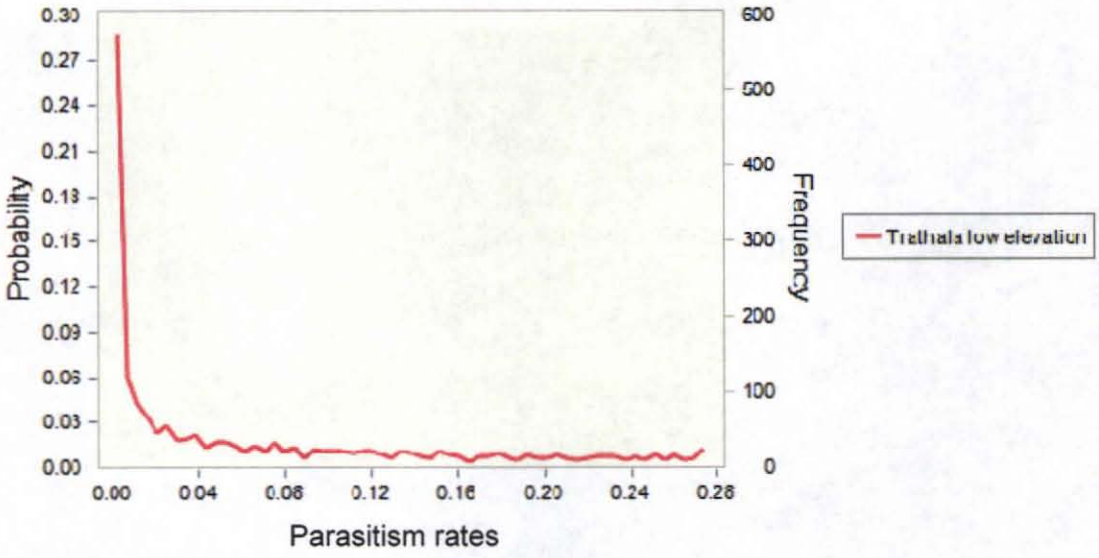

Figure 5.7. Forecast of all possible parasitism rates (as proportion on the x-axis) for *T. flavoorbitalis* at low elevation sites using data from the literature.

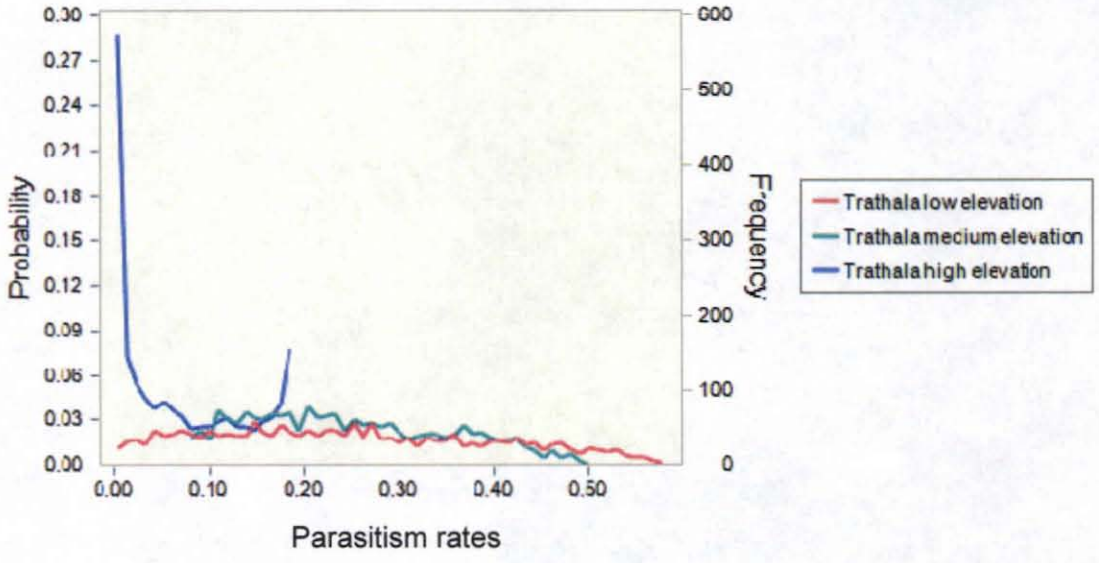

Figure 5.8. Overlay chart showing forecast of all possible parasitism rates (as proportion on the x-axis)for *T. flavoorbitalis* at low, medium and high elevation sites using data from retrospective studies in Hawaii.

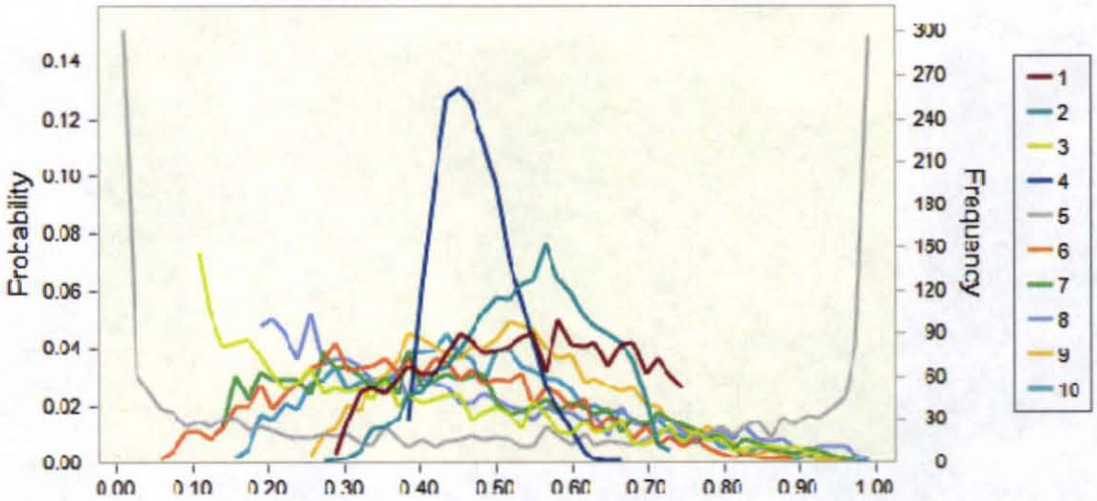

Figure 5.9. Overlay chart showing distribution of all possible outcomes using sets 1 to 10.

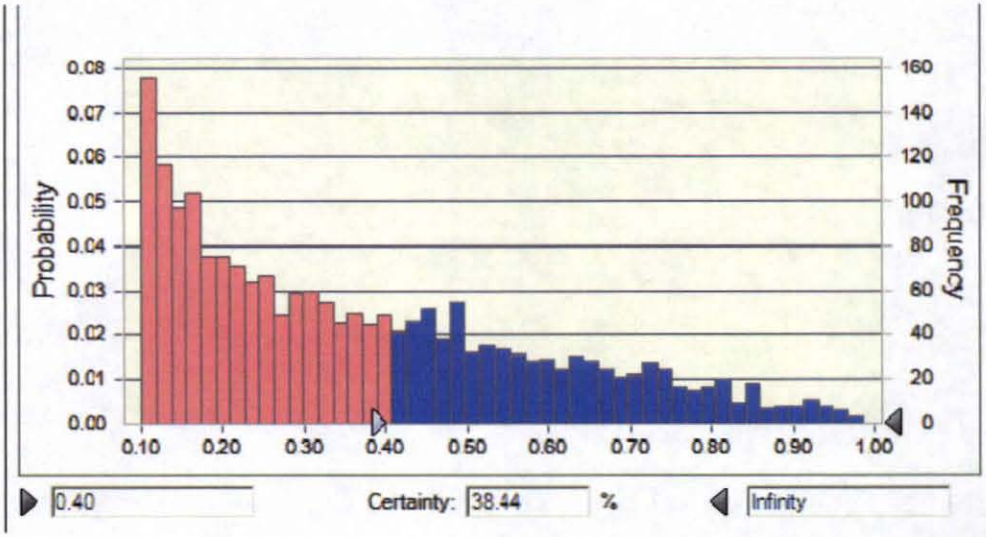

Figure 5.10. Distribution of all possible outcomes using set 3 from Appendix 5.1 and specifically indicating a 38.44% probability that expected values will fall above 0.40

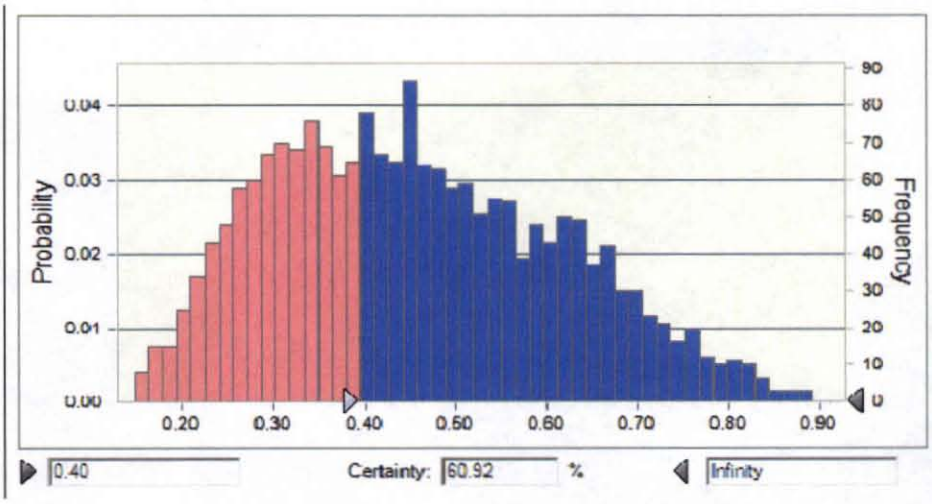

Figure 5.11. Distribution of all possible outcomes using set 10 from Appendix 5.1 and specifically indicating a 60.92 % probability that expected values will fall above 0.40

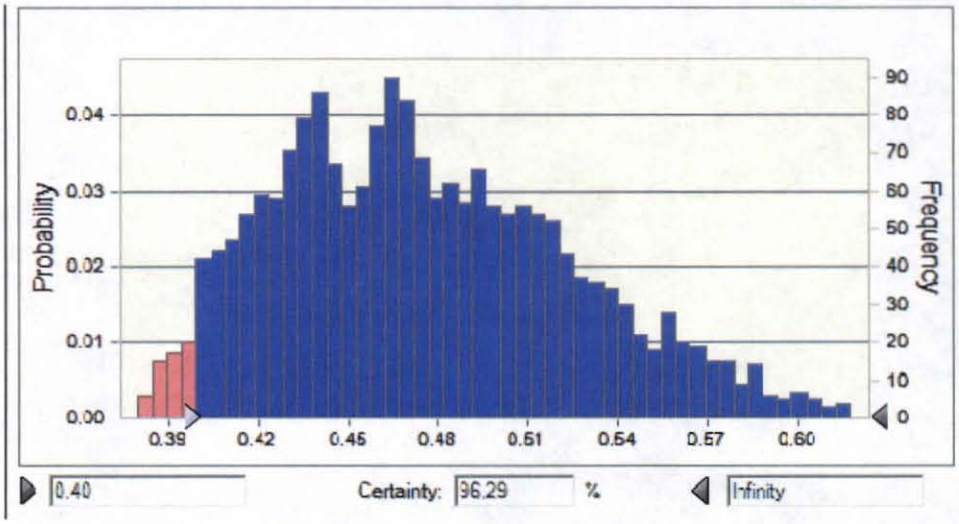

Figure 5.12. Distribution of all possible outcomes using set 4 from Appendix 5.1, specifically indicating a 96.29 % probability that expected values will fall above 0.40

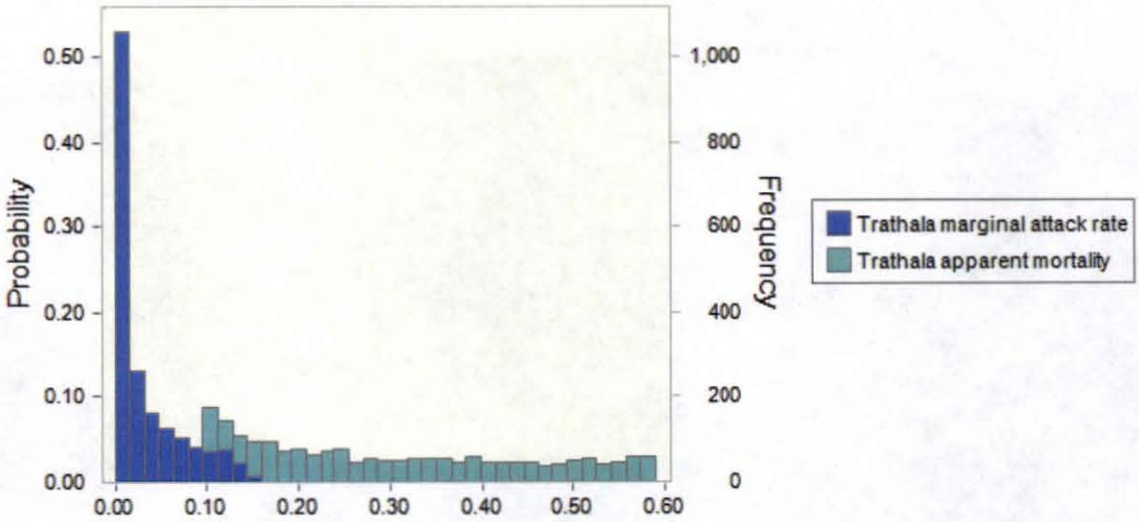

Figure 5.13. Overlay chart showing the probability distributions of marginal attack rate and apparent mortality (expressed as proportions on x-axis) for *T. flavoorbitalis*.

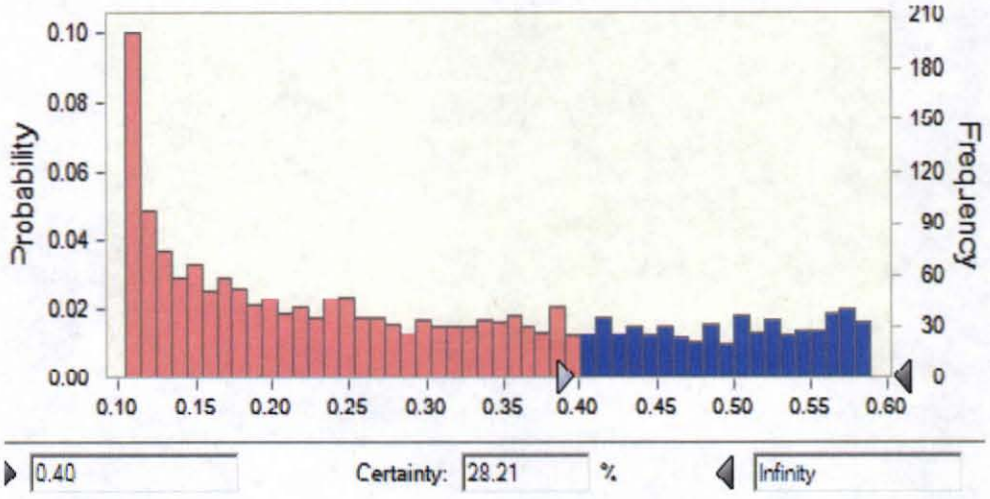

Figure 5.14. Distribution of all possible values of apparent mortality rate for *T. flavoorbitalis*, specifically indicating a 28.21 % probability that expected values will fall above 0.40

Table 5.6. Simulated data sets used to address Question 2.

	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9	Set 10
1	0.5	0.7	1.0	0.5	1.0	1.0	1.0	1.0	0.8	0.7
2	0.4	0.4	0.5	0.5	0.1	0.5	0.5	0.2	0.4	0.5
3	0.5	0.5	0.4	0.5	0.1	0.6	0.6	0.4	0.3	0.6
4	0.3	0.4	0.5	0.5	0.0	0.4	0.4	0.5	0.5	0.4
5	0.5	0.5	0.3	0.5	1.0	0.5	0.5	0.3	0.3	0.6
6	0.7	0.7	0.3	0.6	0.9	0.3	0.3	0.3	0.5	0.3
7	0.6	0.6	0.5	0.6	0.1	0.5	0.5	0.5	0.5	0.5
8	0.6	0.6	0.4	0.5	0.8	0.6	0.6	0.4	0.6	0.6
9	0.7	0.6	0.3	0.7	0.9	0.2	0.2	0.5	0.5	0.3
10	0.4	0.5	1.0	0.4	0.0	0.8	0.9	1.0	0.7	0.9
11	0.5	0.5	0.5	0.5	0.1	0.5	0.4	0.5	0.5	0.5
12	0.7	0.6	0.4	0.7	1.0	0.2	0.4	0.4	0.4	0.5
13	0.4	0.4	0.5	0.5	0.0	0.5	0.5	0.7	0.9	0.5
14	0.6	0.5	0.9	0.6	0.9	1.0	0.9	0.9	0.5	0.8
15	0.7	0.7	0.5	0.6	0.9	0.5	0.5	0.4	0.7	0.5
16	0.5	0.5	0.5	0.5	0.9	0.5	0.3	0.5	0.5	0.3
17	0.6	0.6	1.0	0.5	0.9	0.9	1.0	1.0	0.7	0.9
18	0.4	0.3	0.1	0.4	0.0	0.1	0.1	0.1	0.3	0.2
Mean	0.5333	0.5333	0.5333	0.5333	0.5333	0.5333	0.5333	0.5333	0.5333	0.5333
Std Dev	0.0291	0.0268	0.0626	0.0198	0.1057	0.0610	0.0621	0.0652	0.0404	0.0471

Table 5.7. Apparent mortality data used in simulations for Question 3. (Data from Chapter 2).

	Trathala	*Meteorus*	*Cotesia*
1	0.106	0.070	0.088
2	0.141	0.001	0.001
3	0.303	0.059	0.176
4	0.122	0.001	0.001
5	0.489	0.001	0.083
6	0.510	0.001	0.001
7	0.243	0.001	0.001
8	0.585	0.001	0.001
9	0.338	0.001	0.001
10	0.222	0.001	0.001
11	0.411	0.001	0.001
12	0.508	0.001	0.001
13	0.295	0.030	0.091
14	0.373	0.001	0.001
15	0.174	0.001	0.071
16	0.538	0.001	0.001
17	0.333	0.001	0.001
18	0.146	0.001	0.001
19	0.430	0.001	0.001
20	0.286	0.001	0.026
21	0.123	0.001	0.001
22	0.103	0.014	0.014
23	0.152	0.001	0.001
24	0.144	0.001	0.266
25	0.193	0.001	0.302

Table 5.8. Marginal attack rate data used for simulations in Question 3. (Data from Chapter 3)

	Trathala	*Meteorus*	*Cotesia*
1	0.006	0.006	0.000
2	0.040	0.000	0.000
3	0.015	0.000	0.000
4	0.001	0.000	0.000
5	0.001	0.000	0.017
6	0.053	0.000	0.000
7	0.001	0.000	0.000
8	0.001	0.000	0.000
9	0.001	0.000	0.000
10	0.128	0.000	0.000
11	0.037	0.000	0.000
12	0.035	0.000	0.000
13	0.080	0.000	0.000
14	0.009	0.000	0.000
15	0.001	0.000	0.000
16	0.017	0.000	0.000
17	0.141	0.000	0.000
18	0.031	0.000	0.000

GENERAL CONCLUSIONS

Retrospective studies on biological control introductions provide an important tool in the prediction of potential non-target effects of future biological control programs. Retrospective studies build case histories and reveal patterns to help identify key biological and ecological factors that need to be investigated to provide robust estimates of candidate biological control agent's non-target impact potential (Louda et al. 2003). This dissertation is entitled "Non-target impacts of introduced parasitoids, and validation of probabilistic risk assessment for biological control introductions." The non-target subject of this dissertation was the endemic Hawaiian moth *Udea stellata* (Butler). *Udea stellata* is not a species of special concern, but one distributed across a wide range of elevations and anthropogenic disturbance, which offers the opportunity to examine the impacts of introduced and adventive parasitoids species in a range of circumstances. The overall aims of this dissertation were to: 1) Assess current impacts some alien parasitoids on the endemic moth *U. stellata*

2) Refine and validate a probabilistic risk assessment approach proposed by Wright et al. (2005).

In Chapter 1, the life history and phenology of *U. stellata* were described, providing a basis for understanding how ecology and biology of this species may influence its susceptibility to introduced biocontrol agents. *Udea stellata* is a multivoltine species that undergoes six larval stages, as determined by measuring head capsule widths over a generation. It was found to occur throughout the Hawaiian Islands at elevations between 240 and 1300 m. The parasitoid assemblage associated with *U. stellata* larval

stages comprised seven species. *Trathala flavoorbitalis*, *Casinaria infesta* and *Triclistus* nr. *aitkeni* are of adventive origin, *Cotesia marginiventris* and *Meteorus laphygmae* were purposely introduced to Hawaii, and *Diadegma blackburni* and *Pristomerus hawaiiensis* are of uncertain origin.

Parasitism levels of wild populations of *U. stellata* at different sites were assessed from July 2004 to July 2006 (Chapter 2). Adventive parasitoids species, especially *T. flavoorbitalis*, were responsible for most of the parasitism in this study system. Parasitism by purposely introduced biological control agents was minimal (less than 10%) and restricted to medium-high elevation relatively undisturbed sites.

Since field parasitism does not often provide an effective measure of parasitoid impact at the host population level, life-table studies were conducted (Chapter 3) to evaluate the relative contribution of parasitism to total mortality and with respect to other sources of mortality. Larvae were exposed under field conditions in open and exclusion treatments. Disappearance was the major mortality factor in the open treatment followed by death due to unknown reasons and parasitism, respectively. The open treatment had significantly higher larval disappearance than the exclusion treatment, which suggest that in large part disappearance was the result of predation. Adventive parasitoids inflicted greater total larval mortality than purposely introduced parasitoids. Results from field surveys (Chapter 2) contrasted with results in life table studies (Chapter 3) in that *T. flavoorbitalis* was not the species that contributed the most to mortality due to parasitism, but *T.* nr. *aitkeni* (field surveys showed that *T. flavoorbitalis* was the dominat parasitoid). This was possibly due to over and under representation of certain larval stages during field surveys. This emphasizes the importance of interpreting results of field surveys with

caution, since they can potentially overestimate or underestimate the actual level of mortality in situations where susceptible stages are over or under-sampled.

Chapters II and III showed that the larval parasitoid assemblage and parasitism rates vary by locality. Chapter 4 aimed at identifying ecological factors that might play a role in determining the structure of the parasitoid assemblage associated with *U. stellata* larval stages by using multivariate analyses, specifically Principal Component Analysis (PCA) and partial Redundancy Analysis (RDA). Results of the RDA analysis showed that only three of the 14 measured environmental variables (*U. stellata* density, elevation, and level of habitat disturbance) significantly explained variability in the parasitoid assemblage among sites. Adventive parasitoids occurred across all environmental gradients, and were most strongly associated with moderately disturbed habitats. Purposely introduced parasitoids were frequently associated with less disturbed habitats.

In Chapter 5, a refinement and validation of a probabilistic risk assessment (PRA) approach (Wright et al. 2005) was presented. The refinement included incorporation of spatial context to PRA as well as incorporation of uncertainty analysis. Published literature as well as information collected in previous chapters were used to validate the PRA approach. By comparing the use of single point estimates versus probability distributions in quantitative risk assessment, it was demonstrated that the use of point estimates can hide important variability and significantly impact the estimates of risk. It was also demonstrated that, at least in this study system, the use of apparent mortality significantly increased the estimate of risk compared to the use of marginal attack rate.

Putting results obtained in this dissertation in context to results found by research in other systems, the following findings are supported:

- Non-target feeding is different than non-target impact (Barron et al 2003, Follett et al. 2000a, Johnson et al. 2005, van Lenteren et al. 2003). Non-target impact meaning having an effect at the population level.
- Non-target effects by adventive species can be more substantial than effects by introduced species (Barron et al. 2003, Duan and Messing 1996, Johnson et al. 2005)
- Non-target parasitism varies with type of habitat (Barratt et al. 1997, Barratt et al. 2007, Benson et al. 2003, Duan and Messing 1998, Follett et al. 2000, Johnson et al. 2005, Le Corff et al. 2000).
- Biological control agents can disperse beyond the habitat range of the target hosts (Follett et al. 2000, Henneman and Memmott 2001, Johnson et al. 2005, Louda et al. 2003).

Contributions and consideration for risk assessment:

- When non-target species, such as *U. stellata*, occur in different habitats, incorporation of spatial and habitat context in risk assessment will provide a better characterization of the risk. In this particular case, level of disturbance of areas in which indigenous species persist was found to have a significant contribution in explaining parasitoid species assemblage and their relative abundance, therefore it is an important factor to consider to provide habitat context in risk assessments.

Whether habitat disturbance is a factor that typically predisposes habitats to invasion by parasitoids deserves further investigation.

- Replacing a single value by a probability distribution (for important uncertain variables) permits analysis of uncertainty. By conducting analysis of uncertainty it will possible to quantify the probability of different outcomes that mjay occur under various conditions, thus enhancing decision making capacity.
- The use of apparent mortality in quantitative risk assessment can significantly overestimate the risk. Therefore the use of marginal attack rate should be encouraged whenever possible.
- Last but not least, this research provided basic information on an endemic species that, although it was described more than a century ago, little was known about its basic biology and ecology.

Non-target effects from biological control introductions are not desirable, but if these non-target effects can be predicted with high levels of confidence, and are acceptable (e.g. not causing an impact a population level, of if the benefits outweigh the risk) the decision to accept or reject a BC agent can be knowledge-driven rather than fear based (Briese 2005). It is proposed here that when candidate biological control agents are found to be effective in controlling the target pest, but at the same time are able to use non-target species, probabilistic risk assessment can be a useful tool to effectively characterize the risk.

PRA for biological control introductions would certainly benefit from further refinement and validation. Future aspects to be investigated are the applicability of PRA in the risk benefit analysis phase and the management phase. Retrospective studies on polyphagous biological control agents that have been shown to cause minimal impacts on non-target species of concern can provide valuable testing grounds to weigh the environmental benefits attributable to those releases, and to determine if the effects on non-target species, even if minimal, were justified.

More studies can be done to elucidate how availability of preferred hosts may mediate *U. stellata* parasitism by *C. marginiventris* and *M. laphygmae* (as suggested in Chapters IV and V). This can be done by offering target hosts in the presence of non-target hosts at low, medium and high elevations.

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