Chapter 5: Research Activities

With the exception of section 5.4 (*Drosophila* surveys), this chapter describes the status and outcome of actions proposed in the OANRP 2005-2006 (6.1-6.13) and carried out under the direction of the program's Research Specialist (RS). The OANRP 2005-2006 included many planned projects initiated by the RS. Its purpose was to introduce the research program, then in its first year. This chapter, by contrast, includes only brief descriptions of future work in order to focus on completed and on-going work. Of necessity, some information, from the OANRP 2005-2006 is restated here in order to better understand results. Otherwise, justification, background and basic information on research projects can be found in the OANRP 2005-2006 (6.1-6.13).

Program objectives outlined in 2005-2006 centered on improving control methods for slugs (Mollusca: Gastropoda) and the black twig borer (*Xylosandrus compactus*). Work summaries are organized by actions proposed in 2005-2006 and executed in 2007. Findings from completed research projects, when available, are provided separately following the general summary. Future work appears at the end of each section. All statistical analyses were performed with Minitab® Release 14 software of Minitab Inc. (Ryan *et al*. 2005). Significance during hypothesis testing was characterized by p-values less than 0.05. Nonparametric statistical methods were used to analyze datasets with non-normally distributed residuals and dissimilar variation between groups, otherwise parametric methods were used.

Chapter 5.1 Black Twig Borer Repellent Study

Proposed action: The anti-aggregation pheromone Verbenone® (Phero Tech Inc., Point Roberts, WA) successfully deters *X. compactus*, from entering traps baited with an ethanol lure (Dudley *et al.* 2006). Whether it similarly repels *X. compactus* from damaging target tree species is unknown. With cooperators Nick Dudley (Forester, Hawaiʻi Agriculture Research Center Forester) and Nancy Gillette, (Entomologist, U.S. Forest Service) test Verbenone's ability to reduce damage caused by *X. compactus* on outplanted *Flueggea neowawraea* in Kahanhāiki Management Unit (KMU).

Status: This project is currently delayed. It was postponed after National Tropical Botanical (NTBG) Staff observed a slight *increase* in *X. compactus* activity around, and damage to, *F. neowawraea* trees following Verbenone deployment (William Hunt, *pers. comm.*). Regrettably, few to no *X. compactus* were recorded in passive, unbaited, traps deployed throughout the study area (N. Gillette, *pers. comm.*). Because traps were the primary means by which researchers hoped to evaluate Verbenone performance, conclusions as to its efficacy as a repellent (or attractant) remain speculative. Nonetheless, this study made clear Verbenone is not likely to be the panacea hoped for by resource managers. In light of this, NRS is looking into alternative control strategies which might work in lieu of, or in conjunction with, Verbenone, to protect rare plants.

Future research: The distressing possibility that Verbenone may attract *X. compactus* has led to the postponement of *F. neowawraea* tests until *X. compactus* behavior is better understood. *Xylosandrus compactus* response to Verbenone might be more safely tested in a less vulnerable plant population, such as coffee. If such a trial takes place and results show no risk of increased plant damage, NRS plans to resume testing on *F. neowawraea*. In the meantime, one strategy currently under investigation by NRS is whether saturation of an area with baited traps can reduce damage to *F. neowawraea* by acting as a sink for *X. compactus*. A field trial testing the efficacy of this method is currently underway. Rings of traps have been placed around randomly selected *F. neowawraea* (Fig. 5.1.1) and damage to these trees compared against trees without traps. High release ethanol baits (Aptiv Inc. Marylhurst, OR) are being used as lures in this study. They are effective for 45 days.

Figure 5.1.1 Ring of traps placed around a *Flueggea neowawraea* in an effort to capture *Xylosandrus compactus* prior to gallery construction.

Chapter 5.2 Black Twig Borer Population Monitoring

Proposed action: Effective control is hampered by a lack of basic methodology needed to evaluate the efficacy of potential treatments. This lack was made clear in the difficulties encountered by researchers at NTBG. NRS seeks to improve sampling protocol by clarifying the relationship between *X. compactus* found in traps and numbers of new galleries recorded on *F. neowawraea*. If closely associated, NRS plans to discontinue damage assessments which are both more labor intensive and prone to observer bias than traps. Little is known regarding *X. compactus* densities or population response to seasonal cues. NRS will monitor *X. compactus* abundance using both methods and identify patterns, if any.

Status: A year long monitoring program using both traps and damage assessments has provided insights into *X. compactus* behavior and identified potential seasonal triggers. Baseline damage rates have also been established for *F. neowawraea* outplanted in the KMU. Whether damage rates will decline, increase or remain static in the upcoming year can only be determined through continued monitoring... The results and conclusions presented below are preliminary.

Study: Damage caused to *F. neowawraea* by *X. compactus* at two sites and seasonal changes in pest abundance for the year 2007

Methods: Work took place at two *F. neowawraea* stands, 250 m apart, located within the KMU at an elevation of 2000 ft (Fig. 5.2.1). The two sites, referred to here as Up Gulch (UG) and Down Gulch (DG), provide habitat for 37 and 24 trees respectively. Trees were reared in the greenhouse and planted by NRS on 2/17/2005, 2/22/2006 (UG) and 1/2/2007 (DG). DG contains 24 trees, seven of which were transplanted from a nearby site, Pteralyxia Gulch (PG), where they had been doing poorly. These seven, plus an additional 19 plants were originally planted at PG on 12/10/2003. UG monitoring commenced August 2006. Monitoring DG did not occur until January 2007, when planting occurred. Comparisons between the UG and DG sites, therefore, include data collected UG only *after* 1/1/2007.

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available upon request

Figure 5.2.1 *Flueggea neowawraea* stands with number of trees listed

Xylosandrus compactus were lured to Japanese beetle traps (Trece Inc., Adair, OK) using ethanol. One fl. oz. of 100% ethanol was dispensed into a vial plugged with cotton mounted on the trap. An insecticidal strip (Vaportape II™, Hercon® Environmental, Emigsville, PA) in the collection cup killed any insect entering the trap. Trapped *X. compactus* were counted weekly and ethanol replenished. This type of trap has been used successfully elsewhere in Hawai‘i (Dudley *et al*., 2006) and is the same one pictured in Figure 5.1a with the exception of the type of lure. Six traps were deployed UG on 10/12/2006 and six DG on 12/24/2006. When intra-trap variation proved high, three traps were added to each site on 6/26/2007. Subsequent data analysis made use of the mean rather than absolute number of *X. compactus* per trap. Here, we refer to the former as “trap catch” and represent it with the notation $\bar{X}$ BTB/trap.

As in a previous study (Gillette *et al*. 2006) *X. compactus* entry holes were counted and marked on *F. neowawraea* trees to determine attack rates. Damage was measured using the following formula:

$$o/h/t$$

Where *o* is the number of new (unmarked) holes (+0.0001, see below), *h* is the height of the tree (m) and *t* is the time elapsed (days) since holes were last counted and marked. In order to eliminate zeros +0.0001 holes were added to each damage assessment at 30 day intervals.

Analysis: Damage was assessed approximately once a month while traps were counted much more frequently. Rainfall measurements were recorded approximately 15 times per month using a gauge stationed at the Nike greenhouse (Fig. 5.2.1). Frequently collected data, such as rainfall, was averaged prior to comparison with smaller datasets such as damage. Regression and correlation analyses were used to identify associations between variables.

Results and discussion: Figure 5.2.2 shows damage over time at both sites. When grouped by site, data was not normal and variance was dissimilar between groups. Though it would have been desirable to look at the effects of both time and site on damage, only site is considered here and all values between 1/2007 and 9/2007 are pooled. Transformation was attempted, but data remained in violation of assumptions intrinsic to parametric two-factor statistics. The RS continues to investigate whether a nonparametric alternative exists to the two-way ANOVA or repeated measures analysis.

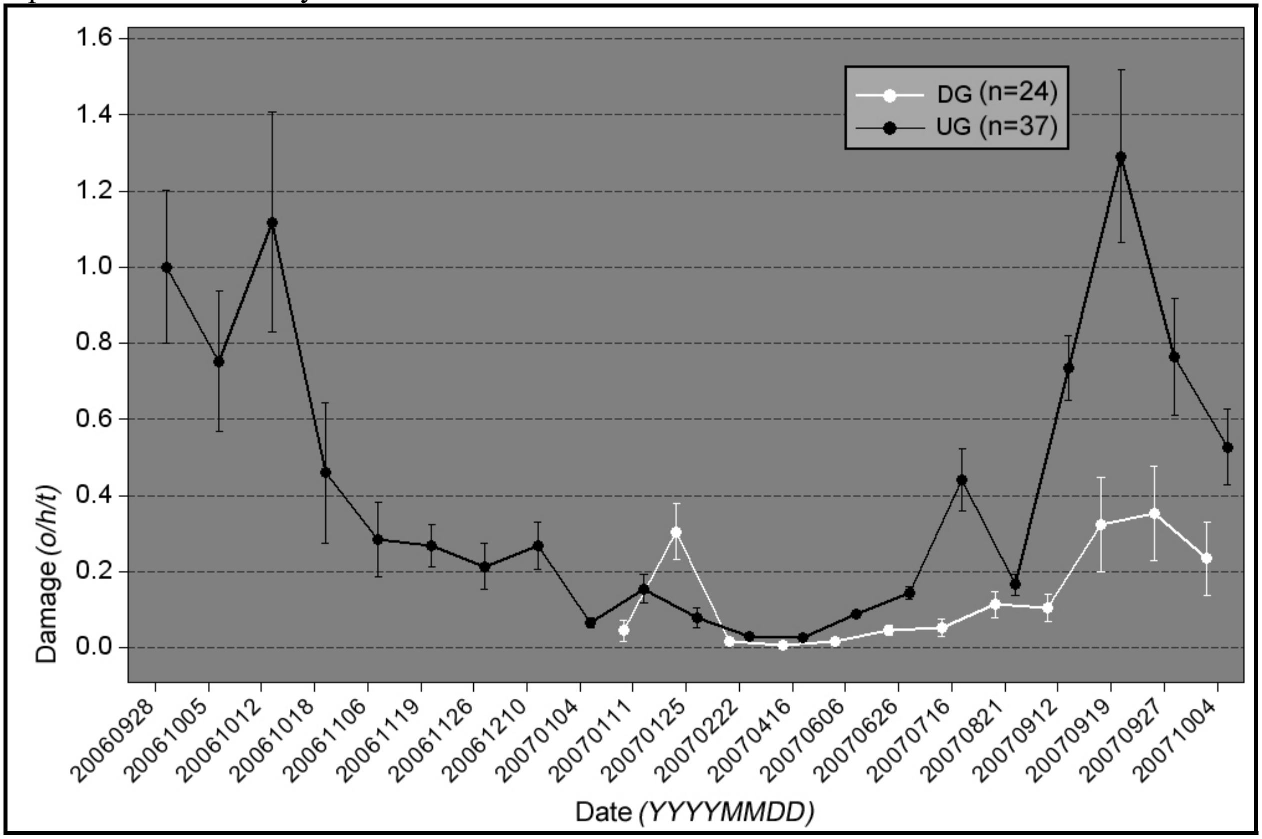

Figure 5.2.2 Damage to *Flueggea neowawraea* (n = number of trees) over time by site. Bars are ± 1 SEM.

Trees UG sustained significantly higher levels of damage than those DG (Mann-Whitney U Test: $p < 0.0001$). Possible reasons for this discrepancy may include factors intrinsic to the tree itself (*e.g.*, age, health) and/or extrinsic factors (*e.g.* tree spacing, proximity to other *X. compactus* host plant species). The tree population UG is both larger (*e.g.* more numerous), and, on average, older than that DG. Possibly, *X. compactus* has reached its carrying capacity UG while the population DG is yet expanding. In addition, the role initial level of infestation plays in subsequent damage accumulation has not been adequately controlled for in this study. For example, a tree with a high number of holes at the outset may have a greater chance of gaining even more holes in the future relative to a tree with few nor no galleries. The number of *F. neowawraea* at each site may prove important as well, especially if *X. compactus* is unable to disperse long distances. Just as a large, densely packed population facilitates disease transmission, the number and spacing of trees may encourage or hinder *X. compactus* colonization.

A seasonal pattern of damage is evident at both sites (Fig. 5.2.2) with relatively low levels prevailing between January and July and increasing thereafter. Damage at the UG site peaked in late September in both 2006 and 2007. Such symmetry only makes sense if *X. compactus* activity is driven by seasonal factors. Of note is the anomalous spike in attacks witnessed among the DG trees two weeks after planting. It was the only time damage DG surpassed damage UG. Among the possible explanations for this spike, three are immediately apparent. First, it may result from *X. compactus* taking advantage of a new resource. Second, recently planted *F. neowawraea* may have fewer defenses relative to older outplantings. This deficiency is sometimes caused by stress associated with transplanting or by greenhouse conditions that fail to prepare the plant adequately for harsh conditions encountered in the wild. The roughly, similar levels of damage at both sites over time suggest a third possibility. When compared to those observed UG, fluctuations are generally later and less pronounced in the DG population. This may result when slight changes to temperature, humidly, or rainfall produce favorable conditions for *X. compactus* at one site but not at another until later in the season. Based on anecdotal observations, DG is sunnier, warmer and drier with more native canopy trees than the area UG. Also notable, is the abundance of heavily infested *Buddleia asiatica* at the latter site, which appear to thrive despite attack by *X. compactus*. Any or all of these factors may explain the observed discrepancies between sites.

The trend in mean number of *X. compactus* per trap was remarkably similar between sites (Fig. 5.2.3). As with damage, trap catch was slightly higher UG. Unlike damage, changes in trap catch occurred earlier DG than UG, and, when pooled did not differ significantly between sites (Mann-Whitney U Test: $p = 0.5368$).

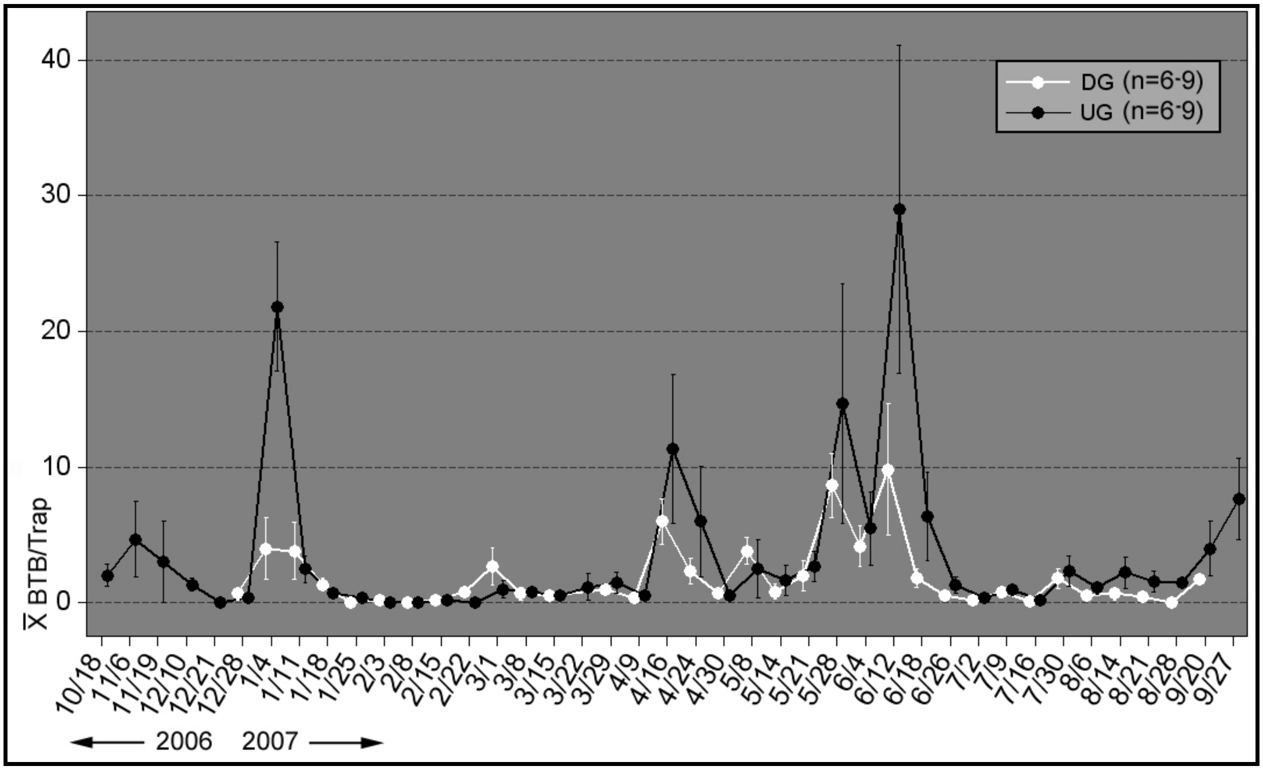

Figure 5.2.3 Average number of *X. compactus* found per trap by site (n = total number of traps before and after 6/26/07). Bars are ± 1 SEM.

The relationship between trap catch and damage was unclear and no significant association was found (Pearson correlation: p = 0.895). Despite this failure, the higher overall values of both factors at the UG site relative to the DG site, as well as consistent changes over time regardless of site, are suggestive. Perhaps both are tied to *X. compactus* population size, but each measures a different behavior with a different environmental trigger. For example, perhaps trap catch better explains *X. compactus* dispersal behavior while damage assessment is a better measure of activities related to nest building, or egg laying. Interestingly damage appeared to track rainfall, but again, this relationship was not significant (Fig. 5.2.4). Further analysis of the data using multivariate statistics should shed light on the relationship between trap catch, site, rainfall and damage.

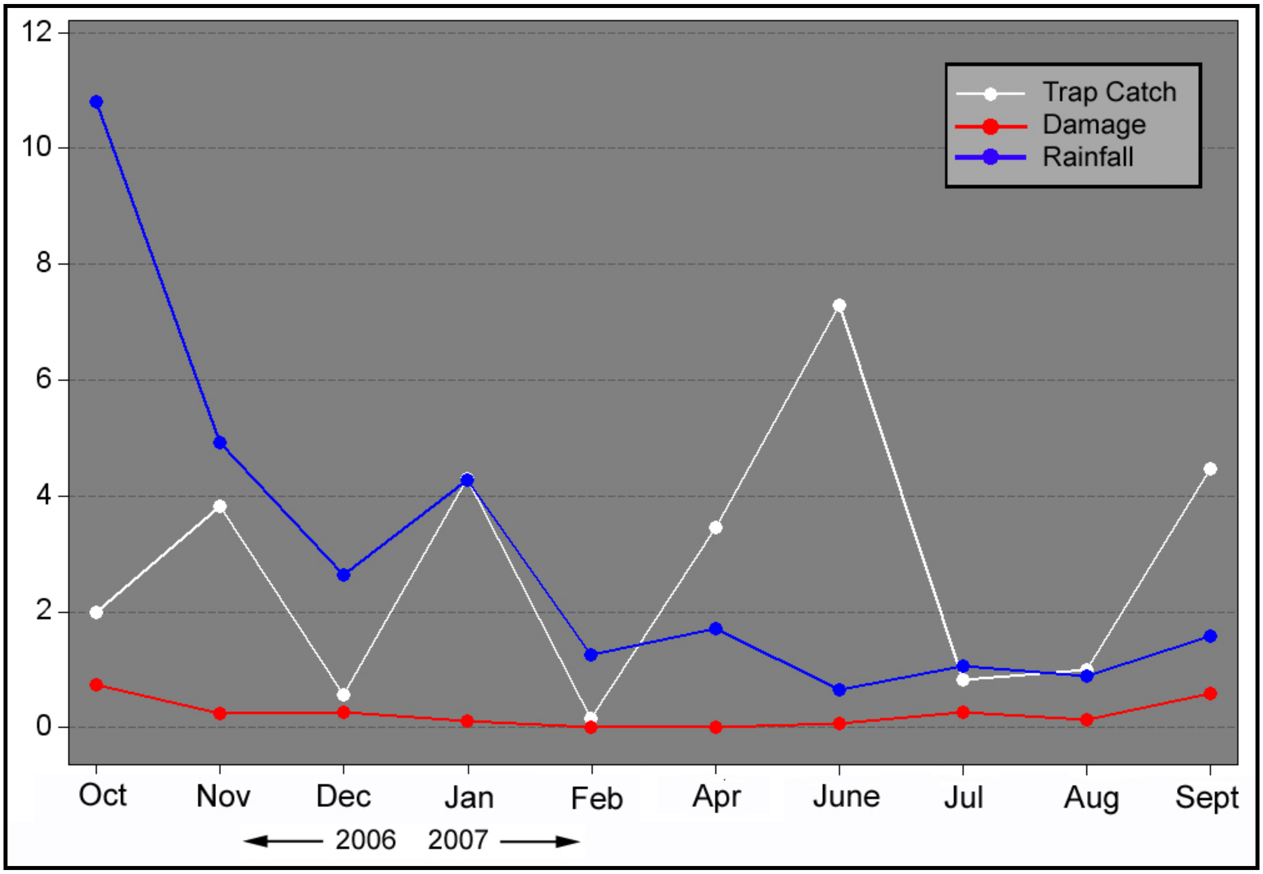

Figure 5.2.4 Damage and trap catch plotted against rainfall. Values are given as monthly averages and both sites were pooled. Y axis units follow for each variable: inches/month (rainfall); $\overline{X}$ BTB/trap (trap catch); *o/h/t* (damage).

Future research: NRS will continue monitoring *X. compactus* numbers in the field and at Nike using traps. These traps will only need to be checked once a month as they will be baited with a high release, long-lasting ethanol lure. Ten traps will be placed at each site and serve as sentinels, altering NRS to times of year when *X. compactus* is abundant. In addition, the ten largest trees at each site will be assessed once a month for damage. These data should better clarify the relationship between seasonal cures and *X. compactus* behavior as well as facilitate better prediction of *X. compactus* activity in the future.

Chapter 5.3 Molluscicide Field Test

Proposed action: Determine whether molluscicides might be used safely in a conservation setting. Work with cooperating agencies to get label permissions for use of molluscicides in forested areas.

Status: NRS carried out work supporting the development of an ecologically based area-wide control program for slugs in forested areas. Under an Experimental Use Permit (EUP) granted February 2007 and valid through February 2008, NRS carried out a field trial testing the safety and efficacy of the organic molluscicide, Sluggo® (Neudorff, Germany), in the KMU. Results summarized below were also presented as a poster at the 2007 Hawai‘i Conservation Conference (HCC). In fulfillment of the obligations stipulated in the EUP, NRS is currently preparing a document describing project conclusions for the Hawai‘i Department of Agriculture (HDOA) and the USFWS. This research is part of an effort to obtain Special Local Needs Labeling for Sluggo in Hawai‘i.

The purpose of the following study was to establish whether application of Sluggo in mixed native forest controlled slugs without adverse affects to common, native, non-endangered snails (Achatinellidae subfam. Tornatellidinae) hereafter referred to as “Tornatellids.”

Study: Safety and efficacy of Sluggo Deployment in Natural Areas

Methods: A grid of 12 plots, each 225m^2 and a minimum of 20m from its closest neighbor, was established in a gulch roughly 2ha in size within the KMU on February 2007. Pre-treatment monitoring confirmed both slugs and native Tornatellid snails were present at similar numbers in all plots and *Achatinella mustelina* absent. Six plots were randomly chosen to receive Sluggo treatments, those remaining served as controls. Sluggo was deployed a total of four times at two week intervals. At one week post-treatment and for two months after the final treatment (on April 11, 2007) slugs and Tornatellid snails were counted inside plots using the following methods.

1. a 20-minute timed search by the same person throughout the trial (McCoy 1999);
2. ten, 9 oz. pitfall cups baited with 6 oz. of beer (Cranshaw 1997);
3. six 0.5m^2, squares of moistened cardboard which serve as daytime refuge (Hawkins *et al.*1998).

Slug and snail counts from all three methods were summed prior to analysis. For the remainder of this document, “slug count” and “snail count” is used to refer to the sum of animals recorded using these three methods in combination.

Slug counts continued for an additional four months until June 12, 2007, when it became evident Sluggo was suppressing numbers in the treatment plots.

Analysis: Differences in slug response due to treatment over four months, and for snail response over two months, was undertaken using a general linear model (GLM).

Results and conclusions: Relative slug abundance over time in treated and untreated plots is shown in Figure 5.3a. Sluggo application significantly reduced slug numbers in treatment plots (P=0.007). GLM results show slug numbers were also significantly affected by time (P=0.001) and that there was no interaction between time and treatment (P=0.299). In other words, after the first Sluggo treatment, repeated applications did not appreciably improve suppression. Slug numbers remained significantly lower in treated plots two months after the final application on April 17. The failure of slugs to recover during this time was perhaps due to repeated applications.

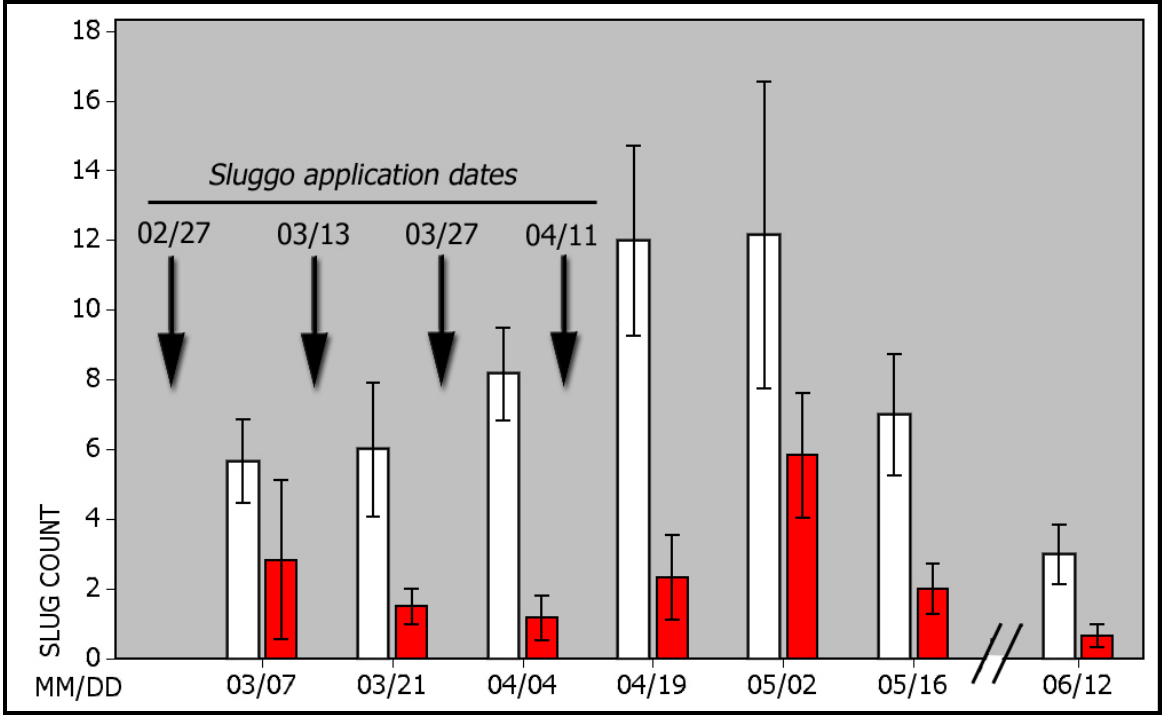

Figure 5.3.1 Mean number of slugs per 225m^2 plot over time in treated (red bars) vs. control (white bars) plots (N=6). Slug count refers to the sum of slugs counted using all three survey methods. Treatment significantly (P<0.05) reduced slug numbers. Bars are ± one SEM.

In contrast, snail numbers were not significantly affected by treatment (P=0.616) (Fig. 5.3.2) nor were they affected by any interaction between time and treatment (P=0.458). Though three methods were employed to asses Tornatellid abundance, snails were found during timed searches, presumably because they are arboreal. Thus, estimates of snail abundance are likely less accurate than those for slugs.

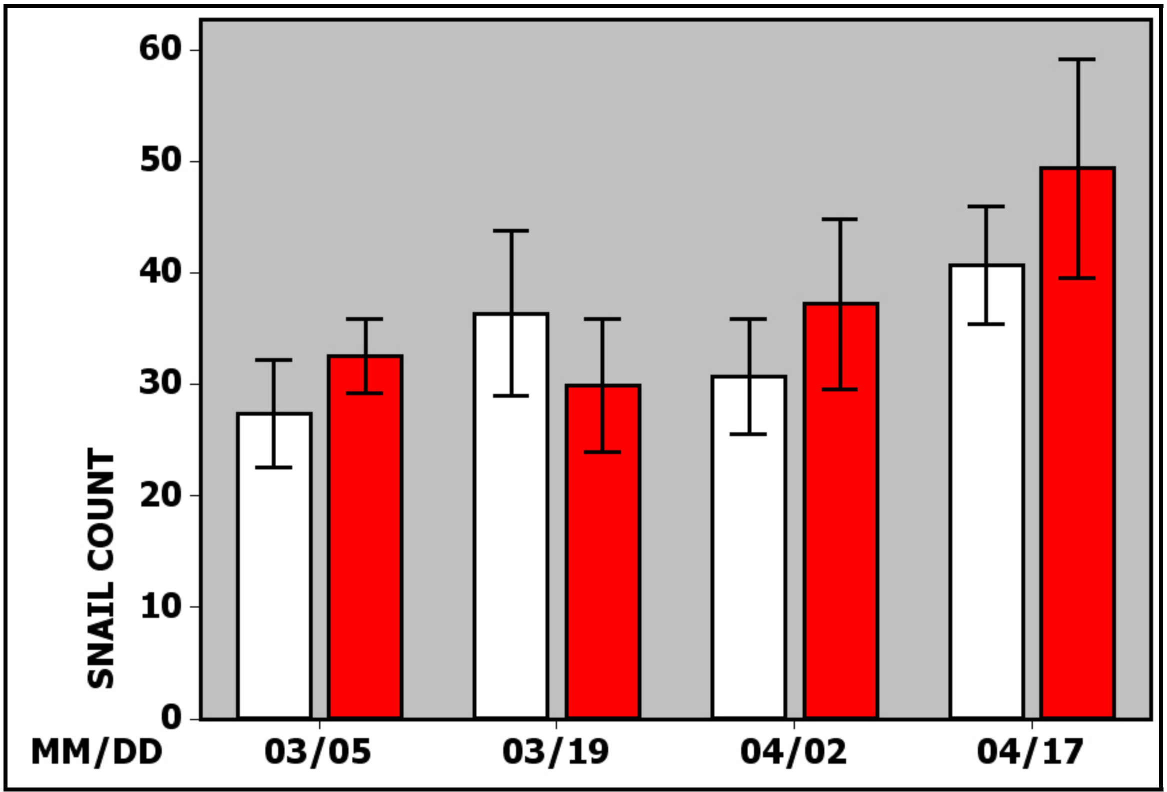

Figure 5.3.2 Mean number of native Tornatellid snails per 225m^2 plot over time in treated (red bars) vs. control (white bars) plots (N=6). Treatment did not significantly (P>0.05) affect snail numbers. Bars are ± one SEM.

As with the slugs, snail numbers were significantly influenced by time (P= 0.033) generally showing an upward trend between March and April. The significant effect of season on slug and snail numbers is unsurprising given that mollusks respond to changes in temperature and moisture (Nystrand and Granström 1997).

Though complete eradication of slugs was not achieved, Sluggo was responsible for, on average, a four-fold decrease in slug numbers in treatment plots. Slug numbers declined through two Sluggo applications but reached a plateau by the third and forth treatments. This suggests that repeated applications may be effective in preventing recovery over time, but do not play a role in the initial knockdown. Future tests will determine whether one or two treatments may be adequate in achieving the same reduction in slug numbers over time.

Future research: Of particular interest to NRS is whether Sluggo application can enhance seedling germination. This hypothesis will be tested in a six week trial beginning in December 2007 and ending before February 2008. Seeds from five species: *Cyanea superba, Cyrtandra dentata, Deiissea subcordata*, *Schiedea kaalae* and *Schiedea obovata* will be sown into a subplot 4m^2 located at the center of 12, 15 x 15 m plots. Germination and survival of seedlings will be recorded on a weekly basis with the expectation that a greater number will germinate and survive in those plots receiving treatment with Sluggo.

Chapter 5.4 *Drosophila* Surveys

Six species of Picture Wing flies (*Drosophila* spp.), endemic to O‘ahu were listed as endangered on 9 May 2006 in the Federal Register (USFWS 2006). The listed endangered Drosophila from O‘ahu are *D. aglaia, D. hemipeza, D. montgomeryi, D. obatai, D. substenoptera, D. tarphytrichia.* Army training lands contain habitat suitable for these flies. NRS contracted Dr. Steven Montgomery, prominent Hawaiian Entomologist and *Drosophila* expert, to conduct surveys. Appendix 5-1 is a survey summary prepared by Dr. Montgomery. Surveys began in December 2006 and will continue until NRS feel satisfied that all the prime habitats for the listed *Drosophila* have been adequately surveyed. A total of 20 field survey days were completed during this reporting period at 13 locations. Native *Drosophila* were observed at seven of the 13 locations. Figure 5.4.1 shows the survey locations. The numbered locations on the map are sites where native *Drosophila* were observed. Also shown on the map are sites where additional surveys are proposed.

Map removed,
available upon request

Figure 5.4.1 *Drosophila* Surveys in the Wai‘anae Mountains

Table 5.4.1 shows the results of the surveys by location as indicated in Figure 5.4.1. This is the first *Drosophila* survey work conducted on Army land on Oahu and therefore these data are important to show the current range of the endangered and non-endangered *Drosophila* taxa.

Table 5.4.1 *Drosophila* observed by location

Map Survey Number	Location	*Drosophila* observed
1	Mohiakea South Fork	*Drosophila gradata* *Drosophila oahuensis* *Drosohila haleakalae* *Drosophila inedita* *Drosophila crucigea*
2	‘Ōhikilolo	*Drosophila gradata* *Drosophila punalua* *Drosophila crucigea*
3	Central Wai‘eli, SB South	*Drosophila ambochila* *Drosophila punalua* *Drosophila crucigea*
4	Kahanahāiki	*Drosophila crucigea* *Drosophila hexachaetae* *Drosophila ambochila* *Drosophila inedita* *Drosophila punalua*
5	Ka‘ala	*Drosophila oahuensis*
6	Pu‘u Hapapa	*Drosophila ambochila* *Drosophila crucigea* *Drosophila distinguenda* *Drosophila punalua*
7	Pu‘u Kalena, SB West	*Drosophila montgomeryi** *Drosophila inflatus* *Drosophila crucigera* *Drosophila punalua*

*listed endangered

On field surveys, Dr. Montgomery sometimes collected *Drosophila* maggots to rear in lab conditions. This allowed him the opportunity to document taxa which were not observed in adult form. Some of the taxa identified in Table 5.4.1 were reared from maggots.

NRS apprenticed with Dr. Montgomery in order to build in-house capacity for conducting surveys and recognizing wing patterns of some native picture-wing flies. Dr. Montgomery provided our staff a “How-To-Fly” protocol that includes details of preparing bait and bait placement. This document is included as Appendix 5-1.

Map removed,
available upon request

Figure 5.4.2 *Drosophila* Surveys in the Koʻolau Mountains

Figure 5.4.3 Dr. Steven Montgomery surveying for *Drosophila* and baited sponges

The most significant find was made near Puʻu Kalena, in Schofield Barracks, West Range. One possible *Drosophila montgomeryi,* a listed endangered picture-wing, was observed on baits near patches of *Urera glabra*. It could not be confirmed as it flew off when approached and Dr. Montgomery was unable to capture it for photos and to make detailed notes. The only other native *Drosophila* that has a similar wing pattern is associated with *Pisonia* and there were no

Pisonia in the baiting area. *Drosophila montgomeryi* is associated with *U. glabra* and there were approximately 30 trees in the area. Another survey will be conducted at this location in the wet season. In addition, NRS will secure some field microscopes for closer examination and will take along a digital camera with a macro function.