Semi-annual Project Report

Prosapia bicincta (Two-lined Spittlebug) Detection and Control in Hawaii
Contract No. 68126

For the Period of
April 1, 2020 – September 30, 2020

Submitted by:
Mark S. Thorne, Ph.D.
Extension Specialist in Range and Livestock Management
University of Hawaii-Manoa Cooperative Extension
Kamuela Extension Office
67-5189 Kamamalu Road,
Kamuela, HI 96743

And

Mark G. Wright, Ph.D.
Professor and Specialist in Entomology
Plant & Environmental Projection Sciences
University of Hawaii-Manoa
3050 Maile Way Rm. 310
Honolulu, HI 96822

Progress Report

This report covers the period from April 1, 2010 through September 30, 2020. The objectives of the work under this contract are to 1) provide rancher outreach and education; 2) conduct surveys to detect and monitor TLSB populations; 3) develop Integrated Pest Management (IPM) protocols (Pesticides, Grazing management, Forage replacement); 4) conduct biological control agent exploration; and 5) research the biology and ecology of Two-lined spittlebug (TLSB). The work and deliverables of each of these objectives are discussed in the following sections. The attached appendix includes pictures of TLSB damaged pastures and example data output from the project.

Two-lined spittlebug was first detected in Kailua-Kona, on the Big Island of Hawaii in September of 2016 where it had caused damage to nearly 2,000 acres of pastureland. Monthly pasture surveys that began in in November of 2017 have revealed that the pest has rapidly expanded its range and as of August of 2020 infests over 275 sq. miles or about 176,124 acres (Figure 1). In highly infested areas, the TLSB has resulted in nearly 100% die back of key pasture grasses including Kikuyu (*Pennisetum clandestinum*) and pangola (*Digitaria eriantha*) grasses. The loss of these important livestock forages provides entry for the establishment of many undesirable, and often invasive plants including Pamakani (*Eupatorium adenophorum*), wild blackberry (*Rubus* spp.), fireweed (*Senecio madagascariensis*), Hilo grass (*Paspalum conjugatum*), several other minor grasses of low forage quality, and other weeds. The weeds tend to replace the dead grasses permanently, reducing the quality and usability of the pasture for livestock production. The rate of spread of this pest combined with its devastating impacts on Hawaii's rangelands threatens the economic sustainability of the Hawaii livestock industry.

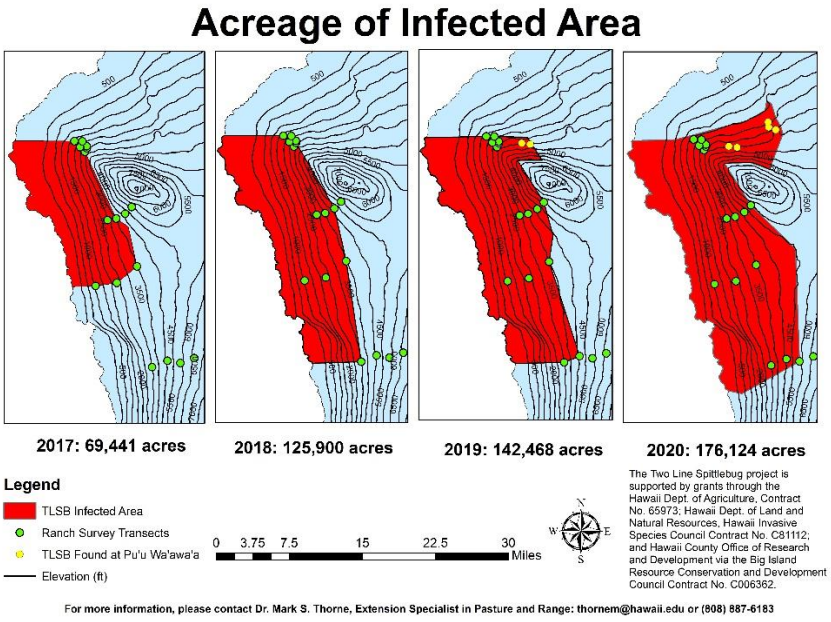


Figure 1. Map showing increase in the range of Twoline Spittlebug infestation from 2017-2020. Green dots are monthly survey sites. Yellow dots represent scouted TLSB populatons.

Personnel
The current contract provided support for two TLSB research technicians, two graduate students and a partial support for a third Range Research Technician.

Outreach and Education
Outreach and education activities for this reporting period include:

- Provided a presentation on TLSB in Hawaii at University of Hawaii/CTAHR Invasive Pest Working Group Meeting
- Provided field training on identification and management of TLSB for landowners and DoFaW personnel at PuuWaaWaa Natural Area
- Provided a recorded Video presentation on TLSB in Hawaii at the UH/CTAHR Livestock Extension Field Day Program (Available at: Livestock Extension Virtual Field Day 2020 | Global Rangelands and on YouTube at: https://youtu.be/Opw52jWrnYg).

Field Surveys – Detection and Surveillance
Monthly field surveys of established transects continue. Two-line spittlebug activity between April and September was high being the prime season for spittlebug activity. The pest typically breaks diapause in April with increased activity through May and June. Adult populations peak, typically, in late June and late August, or early September. Monthly reports on the survey data from each location are available.

Integrated Pest Management Protocols
We continue to work on developing and refining IPM protocols for TLSB for ranchers and homeowners. These will be compiled and published via extension publications and other media later this year. An identification guide for the spittle bug species present in Hawaii is being prepared.

Biological Control Agent Exploration
We are working, locally, on potential biological control agents. Though we have not made any new progress on the indigenous insect-pathogenic nematode, it remains an interesting line of research. A pathogenic fungus was observed to cause mortality of adult *P. bicincta*, and samples were collected for submission to the USDA insect pathogen lab in Ithaca, NY. We initiated exploratory trials on BotaniGuard as potential control agent for adult TLSB. In these trials Adult TLSB were either dunked or sprayed with either BotaniGuard solution at or 4X the recommended concentration. Results were mixed. Mycosis was observed most prominently in the dunked versus sprayed adults and in the 4x rate than in the recommended rate. These early trials suggest that while TLSB adults might be susceptible to the fungal strain in BotaniGuard, spraying at the recommended rate may not be economically efficient at the field scale. Additional trials are planned for this spring to further assess the effectiveness of BotaniGuard on TLSB adults. Exploration of potential biological agents in the home range of TLSB have been put on hold because of the COVID-19 crisis.

Twoline Spittlebug Biology and Ecology

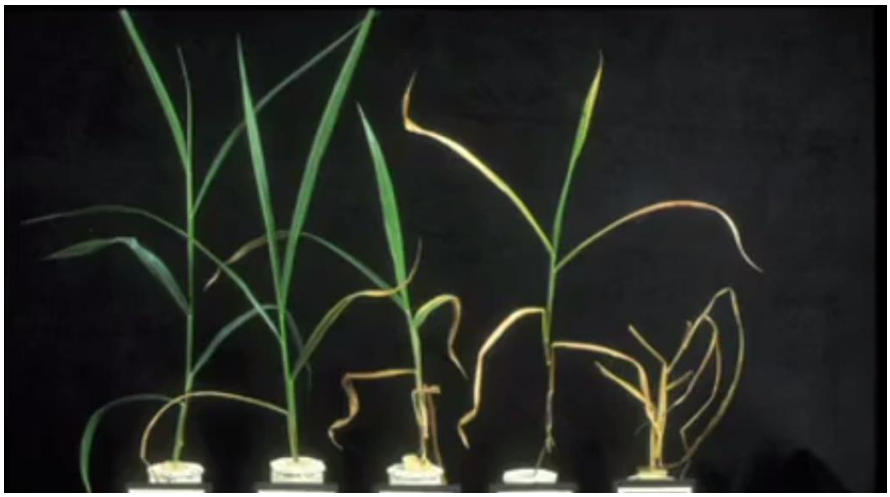
In addition to the data collected during the monthly surveys that revel important aspects of the biology and ecology of TLSB, several controlled experiments are underway. These include host-plant specificity trials and adult density threshold (economic injury level) trials.

A summary of the results of these trials are presented here:

Objectives and methodology

1) Evaluate host plant resistance, tolerance, and susceptibility across different forage grass species and categorize damage inflicted by adults and calculate percent water loss of plant biomass and roots before vs. after infestation
    a. Thus far, 6 replications of this study have been carried out on 6 different grass species. Experimental plant units were infested with 6 adults each for 12 days. Photos, observations, and adult mortality were recorded on days 0, 1, 4, 8, and 12 and then evaluated for adult damage on day 12 using a 1-6 visual scale (see figure 2 and table 1) with specific scores for each percent of damage (see table 2) to estimate tolerance (1-2.25= resistant, 2.26-2.3= tolerant, >3.5=susceptible).

2) Assess plant response of kikuyu to different levels of adult infestation to determine threshold at which phytotoxemia is caused in order to estimate economic impact per unit area
    a. Presently, 4 replications of this study have been carried out on kikuyu grass using 4 different treatment levels of adult infestation (0, 5, 10, and 15 adults). Plants were infested for 12 days and photos, observations, and adult mortality were recorded on days 0, 1, 4, 8, and 12. Adult damage was assessed on day 12 using a 1-6 visual scale with specific scores for each percent of damage to estimate tolerance (1-2.25= resistant, 2.26-2.3= tolerant, >3.5=susceptible).



1.0	2.25	3.5	4.75	6.0

Figure 2. Visual damage scale.

Table 1. Damage descriptions of 1-6 visual scale to estimate tolerance (1-2.25= resistant, 2.26-3.5= tolerant, >3.5=susceptible)

1	No visible damage
2.25	25% of foliar area affected -few leaves on lower position of plant curling, crinkling, and slight yellowing
3.5	50% foliar area affected -crinkling and curling, yellowing, bronzing, and browning leaves in the middle and lower positions
4.75	75% foliar area affected -crinkling and curling all over, yellowing, bronzing and browning, and drying of leaves
6.0	All foliar area affected and dry -extreme curling yellowing, bronzing and browning, and drying of leaves and defoliation and stunted growth

The results of 6 replications evaluating the resistance, tolerance, and susceptibility of 6 different grass species (orchard, kikuyu, signal, limpo big alta, marandu, and limpo #508607) to adult TLSB feeding are revealed in figure 3. Using the damage rating scale (1-2.25= resistant, 2.26-3.5= tolerant, >3.5=susceptible) it is evident that the control plants for each species fell well within the resistance category as the average damage ratings for the controls of orchard, kikuyu, signal, limpo big alta, marandu, and limpo #508607 were 1.16, 1.18, 1.17, 1.55, 1.17, and 1.17 respectively. Thus, the controls prove the validity of the study. Furthermore, the experimental plants for orchard, kikuyu, signal, limpo big alta, marandu, and limpo #508607 had average damage ratings of 1.91, 5.08, 2.26, 2.68, 1.27, and 4.74 respectively. Thus, these results suggest marandu had the highest resistance to adult TLSB feeding, followed by orchard. Additionally, signal and limpo big alta fell in the tolerant category. Lastly, limpo #508607 and kikuyu proved to be susceptible, with kikuyu being very highly susceptible. Therefore, the experimental design and efforts put into these research trials have produced meaningful results. Thus, additional future replications using more grass varieties should be carried out.

Figure 4 shows the results of 4 replications assessing kikuyu response to different levels of adult infestation. The 0 adult infestation level was considered the control and had an average damage rating of 1.32, which falls well below the maximum rating to be considered resistant, thus revealing the legitimacy of the study. The average damage ratings for adult infestation levels of 5, 10, and 15 were 2.54, 5.21, and 5.76 respectively. Therefore, the kikuyu plants were tolerant to treatment with 5 adults, but susceptible to treatment with 10 or more adults. These results suggest the kikuyu plants could tolerate at least 5 adults per unit area, but the threshold for phytotoxemia caused was at least 10 or more adults per unit area. Thus, additional research that

investigates the threshold between 5-10 adults will be necessary to determine at what infestation level the plant response is no longer tolerant.

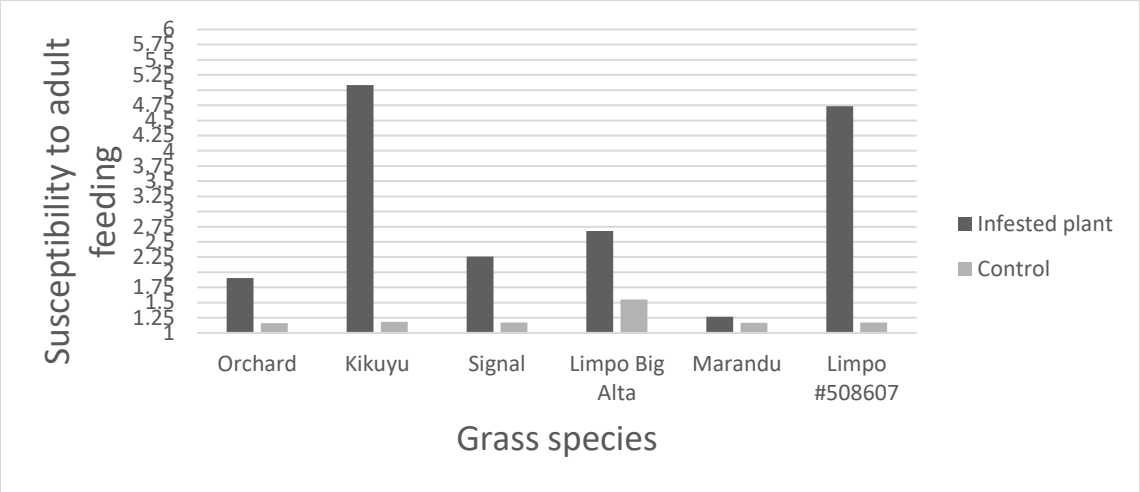


Figure 3. Resistance, tolerance, and susceptibility of different grass species to TLSB adult feeding.

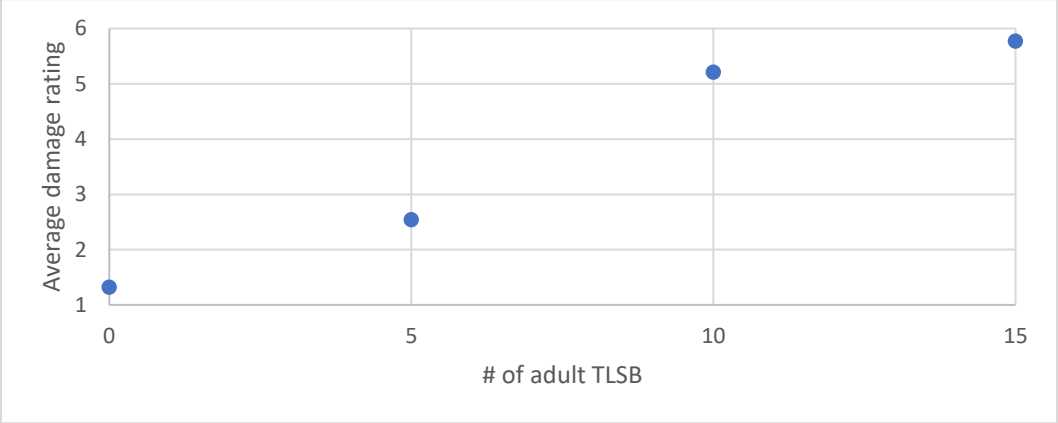


Figure 4. Kikuyu grass response to different levels of adult infestation.

Future Plans:
Surveys of established transects on the four ranch properties will continue monthly to monitor TLSB activity, establish biology and ecology of TLSB in Hawaii, collect samples of nymphs and adults for study, and propagation for greenhouse trials on grass selections and pesticides, and inform the development of integrated pest management strategies.

In addition to the monthly monitoring of the established transects, every two weeks on a select ranch, collection surveys will be conducted to gain insight into the population dynamics of the

TLSB across seasons along an elevational gradient. Additional surveys of affected ranchlands within the Kona area will be conducted over the summer on several ranches to quantify the extent and range of damages by TLSB.

We are planning to present a TLSB training webinar series in the spring that will bring together the expertise of several people and the TLSB team to engage and inform Hawaii Livestock Producers on our current knowledge and management protocols for TLSB.

At least two extension publications will be drafted for publication this spring or summer; one will focus on correctly identifying TLSB in the field and the other will provide management protocols for ranchers.

A Mobile Application developer has been selected and work has begun on the Twoline Spittlebug Mobile Application that will 1) allow users to report, picture, and geolocate TLSB activity; 2) provide for field identification and estimate level of infestation of TLSB; 3) provided a decision support tool for management decisions based on infestation thresholds; and 4) provide general information on the biology and ecology of TLSB. We expect to have a working mobile application by this spring.