Final Project Report

Detection and Management Strategies for the Control of *Prosapia bicincta* (Twolined Spittlebug) in Hawaii

PO No. C20556

For the Period of
October 1, 2021 – March 31, 2023

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Report

This report covers the full project period from October 1, 2021, through March 31, 2023. The work describe herein was completed through multiple funding sources including this HDOA contract (No. 68126: October 1, 2019 – June 30, 2022, $673,989), a USDA-ARS grant (Agreement 58-2040-9-010: November 12, 2020 – June 30, 2023; $160,312) and this HISC grant (PO No. C20556: October 1, 2021 – March 31, 2023, $184,788).

The objectives of the work under these various contracts were 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 Twolined spittlebug (TLSB). Additional objectives specific to HISC PO C20556, that were not addressed since the project was not fully funded, included investigation: 1) into methods of reseeding damaged lands; 2) silicon fertilization trials; 3) remote sensing technology to detect TLSB infestations, movement vectors, and damage levels. The work and deliverables of each of these objectives are discussed in the following sections. The attached appendix includes example outreach materials, screen shots of the Twolined Spittlebug Management Tool smartphone application, and a detailed report on the 2020-2021 host plant resistance trails.

Twolined 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 November of 2017 revealed that the pest had rapidly expanded its range and as of September 2021 infested over 278 sq. miles or about 178,369 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.

Personnel

The funding provided through the HISC grant (C20556) in part provided support for two TLSB research technicians, two graduate students and partial support (0.5 FTE) for a Range Research Technician. In addition, the funding provided for opportunities to consult with and engage a spittlebug expert in research and development of project outputs.

Outreach and Education

Outreach and education activities included attendance and information booth/displays at industry and trade conferences (Hawaii Island Landscape Association, Hawaii Cattlemen's Council), holding workshops, and professional meeting presentations. Over the project period we have disseminated information on TLSB to various stakeholders through eight oral and poster presentations, three meetings and site tours, five video recordings and one podcast, three rack cards, a website and one extension publication. Two other extension publications are currently in draft. Finally, we developed and released (November 2022) the Twolined Spittlebug Identification and Management Tool smartphone application for both apple and android phones. The app allows users to identify, photograph, geolocate and report TLSB sightings that will then be mapped by the TLSB team via an administrative website application. The smartphone app greatly increases the team's ability to accurately map and track TLSB population outbreaks. Additionally, the app helps landowners locate, track, and quantify TLSB populations, and based on the population density of TLSB nymphs determine the degree and timing of potential pasture damage. This will facilitate adoption of integrated pest management strategies recommended within the app and linked to data on nymph densities. Below is list of the outreach and educational activities accomplished over the full period of this project.

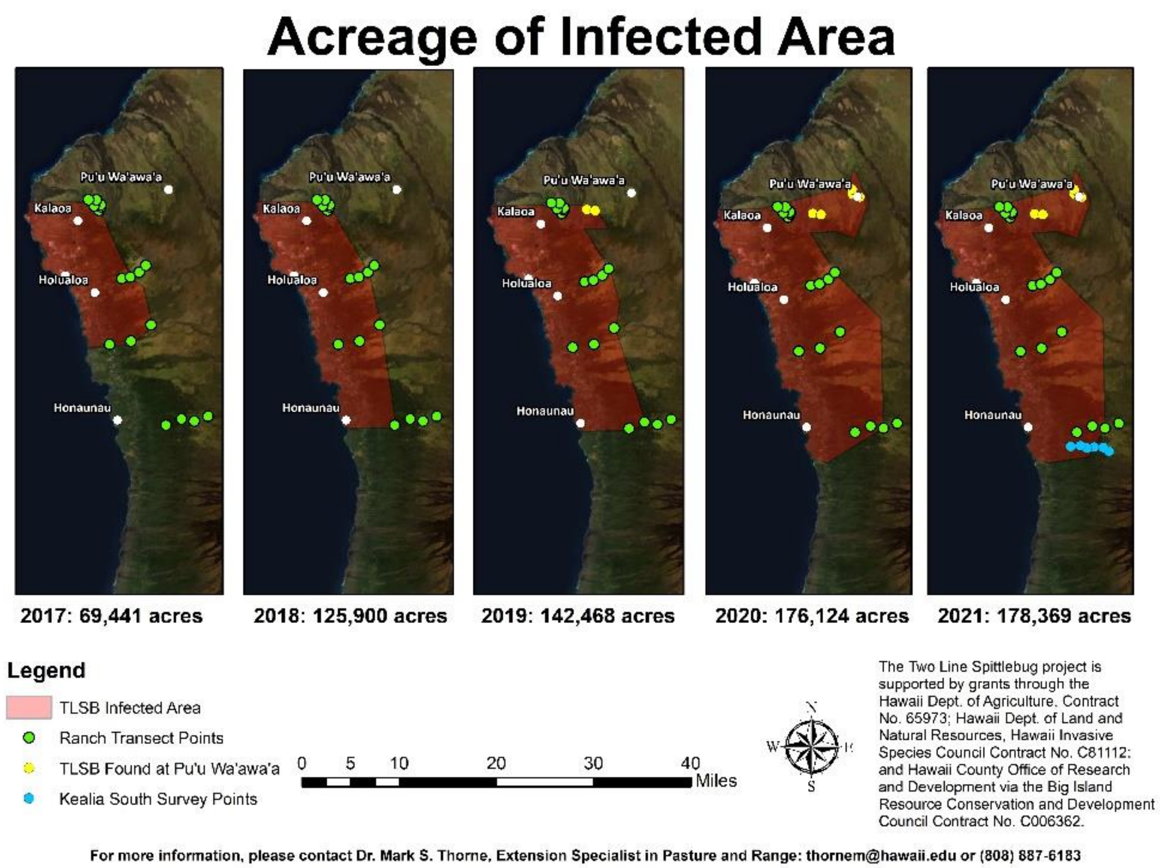


Figure 1. Map showing increase in the range of Twolined Spittlebug infestation from 2017-2021. Green dots are monthly survey sites. Yellow and blue dots represent scouted TLSB populations.

Outreach and education activities for the project period include:

- Poster Presentation – Thorne, M.S., S. Wilson, M. Wright, D. Peck, and M. Oshiro. 2022. Plant community composition changes following Twolined Spittlebug (*Prosapia* *bicincta*) infestations in Hawaii. Society for Range Management Annual Meeting, Albuquerque, NM, February 6-11, 2022.

- Oral Presentation – Thorne, M.S. 2022. Twolined Spittlebug in Hawaii – Research Update. Hawaii Invasive Species Awareness Month, Hawaii Invasive Species Council, February 16, 2022.
- Oral Presentation – Thorne, M.S. 2022. Twolined Spittlebug: A pasture pest in paradise. Invited Lecture, School of Natural Resources and the Environment, University of Arizona, March 16, 2022.
- Poster Presentation - Thorne, M.S., S. Wilson, M. Wright, D. Peck, and M. Oshiro. 2022. Plant community composition changes following Twolined Spittlebug (*Prosapia bicincta*) infestations in Hawaii. Association of Natural Resource Extension Professionals, Kalamazoo, Michigan, May 31 – June 3, 2022
- Oral Presentation – Thorne, M.S., 2022. Twolined Spittlebug (*Prosapia bicincta*): A pasture pest in paradise. California-Pacific Section, Society for Range Management, Kamuela, Hawaii, October 6, 2022.
- Tour of Kona Experiment Station Greenhouse and discussion on TLSB in Hawaii with state legislators, November 10, 2022
- Oral presentation – Wilson, S., M.S. Thorne, M. Wright, and D. Peck. 2022. Establishment, pest status, and management of the twolined spittlebug, *Prosapia bicincta*, in Hawaii, Entomological Society of America Joint Annual Meeting, November 14, 2022
- Booth and poster display on TLSB, Hawaii Cattlemen's Council Annual meeting, November 18-19, 2022.
- Poster Presentation – Thorne, M.S., M. Wright, S. Wilson, D. Peck, and M. Oshiro. 2023. Development of a decision support tool to assist rangeland managers with control of Twolined Spittlebug in Hawaii. Society for Range Management Annual Meeting, Boise, Idaho, February 11-17, 2023.
- Meeting, tour, and discussion on TLSB in Hawaii with state legislators at the Kona Research Station (II), February 24, 2023.
- Oral presentation – Wilson, S., M.S. Thorne, M. Wright, and D. Peck. 2023 Establishment, pest status, and management of the twolined spittlebug, *Prosapia bicincta*, in Kona, CTAHR Showcase & Research Symposium, March 27, 2023.
- In cooperation with BIISC, updated Twolined Spittlebug Alert! Rack card (Appendix A)
- In cooperation with BIISC, developed Twolined Spittlebug Identification Rack Card (Appendix B)
- In cooperation with BIISC, developed Twolined Spittlebug Smartphone Application information card (Appendix C)
- In Collaboration with USDA-NRCS developed information videos on Twolined Spittlebug
    - Twolined Spittlebug: A New Threat to Hawaii's Agriculture video; https://youtu.be/m16mfcQM0QQ
    - Twolined Spittlebug – Raise Awareness video: https://vimeo.com/510897324
- Updated Twolined Spittlebug Page on Hawaii Rangelands Website: https://rangelandsgateway.org/twolined-spittlebug

- Developed and released (November 2022) the Twolined Spittlebug Management Tool smartphone/computer application (See Appendix D)
- Extension Publication: Twolined Spittelbug Identification Key (March 2022).
- Hawaii Cattlemen's Council TLSB committee – ongoing participation and consultation to committee formed in response to threat TLSB poses to the livestock industry. Committee is concerned with reporting information to HCC membership and development of legislative measures to support industry.

Field Surveys – Detection and Surveillance
Monthly field surveys of transects established in 2017 revealed several trends in Twoline Spittlebug population dynamics (Figure 2). Generally, TLSB populations decline in October or November of each year and remain low until diapause breaks in the spring, usually late March to early April, depending on elevation. Typically, TLSB nymph populations rapidly increase into April and peak in May and June, followed by a slight and brief decline. A second TLSB nymph population density peak occurs each year around August to September, again, depending on elevation. Then the pest again enters diapause late October or early November.

Low elevation locations typically see diapause break sooner than the higher elevation sites (Figure 2). Presumably this is due to warmer temperatures in the lower elevation sites. Higher elevation TLSB nymph populations tend to have slightly contracted generational periods relative to lower elevation populations since they break diapause, often, a few weeks later than at lower elevations. The start of diapause seems to be variable across years, sites, and elevations. It is likely that resource quality (grass), temperature, day length, and growing season precipitation combine to influence when adult TLSB females begin laying diapause eggs.

Hot, dry conditions do not seem to be favorable to TLSB nymph development and survival. For example, our Hualalai site low elevation (2,000 ft.) transects HUL-T6 and HUL-T5 are located in a geographically summer dry zone, typically receiving, on average, less than 11 inches of rain between June and October. TLSB activity for these two transects was typically low (Figure 2), with the exception of 2020 which was an exceptionally wet summer receiving nearly 29 inches between the March and September spittlebug season. In 2020 TLSB nymph populations at HUL-6 and to a lesser degree HUL-5, were higher than in any other year since the observations began (Figure 2). Precipitation across the years between 2018 – 2021 within most of the TLSB infested zone (above 2,000 ft. elevation) was above average during the summer months, perhaps supporting higher population densities of the pest than would have been detected otherwise. Indeed, drought conditions that started in mid-October 2021 persisted into the TLSB season of 2022 and as of June, TLSB population densities across all sites were much reduced relative to past years (Figure 2).

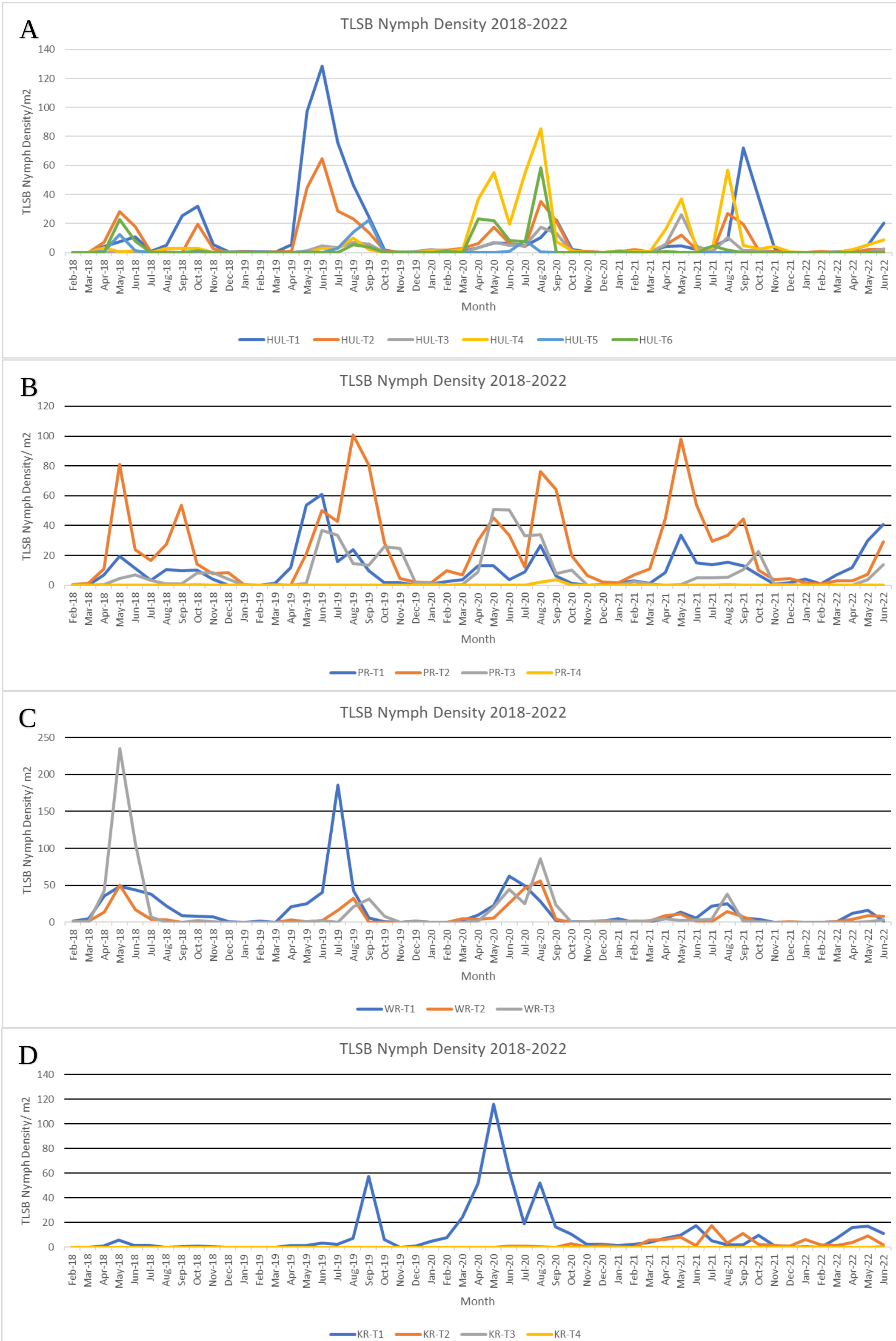


Figure 2. Monthly survey data for 17 transects located at Hualalai (A), Palani (B), Wall (C), and Kealia (D).

Integrated Pest Management Protocols

This project supported the development of the following IPM protocols for TLSB for ranchers and homeowners to include:

Producers/land managers needing to make decisions about implementing IPM protocols will:

1. Collect data on nymph density (counts of nymphs per unit area) to determine potential for resource damage
2. Collect data on TLSB adult activity via sweep net counts to assess effects of IPM protocols implemented
3. Use Monitoring protocols during and post treatment on nymph and adult activity

Using data collected on instar dimensions (length and width; Table 1) we derived three age classes to assist in the classification of nymph population density distributions that allow observers to project the approximate days to expected adult outbreaks (Table 2).

Table 1. Twolined Spittlebug Nymph instar stage, proposed age class distribution and expected days to adulthood by instar and age class, dimensions by age class based on TLSB nymph body length data analyses 12/08/20 (data sheet available on TLSB Google Drive).

Instar Stage	Age Class	Approximate Expected Days to Adult	Expected size/dimensions (mm)		Range in Length (+/- SD)
			Width	Length	
1	Age Class 1	50 days	0.13	0.43	0
2	Age Class 1	40 days	0.61	1.80	2.05-1.55
3	Age Class 2	30 days	0.97	2.77	3.46-2.07
4	Age Class 2	20 days	1.43	4.57	5.19-3.95
5	Age Class 3	10 days	2.06	7.63	8.98-6.28

Table 2. Summary Twolined Spittlebug Age Classes (1, 2, or 3) with dimensions and expected days to adulthood.

Age Class	Age class dimensions (mm)		Approximate Days Expected to Adult
	Width	Length	
1	< 0.6 mm	< 2.1 mm	More than 35 days
2	1.0 -1.4 mm	2.1-5.2 mm	Between 15 and 35 days
3	>1.5 mm	> 5.3 mm	Within 15 days

Note: Approximate Days Expected to Adult assumes an average 50 days from egg hatch to adult and an even development rate of 10 days between instar stage and selected to be the half-way point between classes in days.

Based on these age class distributions and the approximate threshold of significant damage for Kikuyu grass pasture at more than 60 nymphs/m^2 we derived three potential damage levels

(Table 3) that relate to a series of recommended IPM strategies (Recommended Management Actions, see below).

Table 3. Age Class distribution and Expected Days to Adult by Nymph Density estimates in relation to Potential Future Damage. Yellow (damage level 1) light to moderate forage loss; Orange (damage level 2) moderate to heavy forage loss; Red (damage level 3) heavy to catastrophic forage loss.

Age Class	Nymph Density/Potential Future Damage			Expected Days to Adult
	< 10/ m2	11-59/m2	> 60/m2	
1	1	2	3	More than 35
2	1	2	3	15-35
3	2	3	3	Within 15

Management Actions by "Potential Future Damage Level":

Damage Level 1:

1. Intensive grazing –
    a. Objective: reduce suitability of pasture conditions for nymph and adult habitat.
    b. Grazing should reduce forage biomass by more than 65% within one week and be followed by at least 40 days, but no more than 50 days of rest. (Estimated cost $10/acre)
2. Monitoring of TLSB nymph and adult activity –
    a. Objective: determine effectiveness of intensive grazing and continued need
    b. repeat grazing bouts as needed every 40-50 days as precipitation/forage production allows until detection of nymph/adult activity is zero.

Damage Level 2 (step 1 is essential, step 2 weighed against economic/environmental costs):

1. Intensive grazing
    a. Objective: reduce suitability of pasture conditions for nymph and adult habitat.
    b. Grazing should reduce forage biomass by more than 65% within one week and be followed by at least 40 days, but no more than 50 days of rest.
    c. Estimated cost $10/acre
2. Strategic pesticide application (one or the other product, but not both)
    a. Contact pesticide (for example Carbaryl)
        i. Objective: control adults
        ii. Estimated Cost $40/acre/application)
    b. Systemic Pesticide (lambda-cyhalothirin/Chlorantraniliprole)
        i. Objective: Control nymphs and adults
        ii. Must have Restricted Use Pesticide Permit
        iii. Estimated Cost $45/acre/application
    c. Pesticide applications must be applied post grazing and animal withdrawal periods considered; follow all product label requirements.
3. Monitoring of TLSB nymph and adult activity –

   a. Objective: determine effectiveness of intensive grazing and strategic pesticide applications
   b. repeat grazing bouts as needed every 40-50 days as precipitation/forage production allows until detection of nymph/adult activity is zero.
   c. Repeat pesticide applications according to label instructions

<u>Damage Level 3:</u>

1. Intensive grazing
   a. Objective: reduce suitability of pasture conditions for nymph and adult habitat.
   b. Grazing should reduce forage biomass by more than 65% within one week and be followed by at least 40 days, but no more than 50 days of rest.
   c. Estimated cost $10/acre
2. Strategic pesticide application (one or the other product, but not both)
   a. Contact pesticide (for example Carbaryl)
      i. Objective: control adults
      ii. Estimated Cost $40/acre/application)
   b. Systemic Pesticide (lambda-cyhalothirin/Chlorantraniliprole)
      i. Objective: Control nymphs and adults
      ii. Must have Restricted Use Pesticide Permit
      iii. Estimated Cost $45/acre/application
   c. Monitor and repeat applications according to product label
   d. Pesticide applications must be applied post grazing and animal withdrawal periods considered; follow all product label requirements.
3. Monitoring of TLSB nymph and adult activity –
   a. Objective: determine effectiveness of intensive grazing and strategic pesticide applications
   b. repeat grazing bouts as needed every 40-50 days as precipitation/forage production allows until detection of nymph/adult activity is zero.
   c. Repeat pesticide applications according to label instructions
4. Herbicide applications
   a. Objective: reduce weed cover followed by repeat applications as needed
   b. Estimated cost $50/acre/application.
5. Seed damaged patches/pastures
   a. Objective: Reestablish pasture forage productivity and ecological condition
   b. Reseed with a grass/legume combination suitable for the elevation/soil conditions of the pasture (See UH- Cooperative Extension for recommendations) following initial intensive grazing activity.
   c. Estimated cost $30/acre.
   d. Rest from grazing during establishment phase (3-6 months).
   e. Once new pasture is established, manage grazing, monitor TLSB activity.

These IPM protocols have been compiled and incorporated into the ***Twolined Spittlebug Management Tool*** smart phone application to be released to the public soon (See appendix D). Additionally, these protocols will be published in an extension publication currently in draft.

Biological Control Agent Exploration

We are working, locally, on potential biological control agents. Though we have not made any new progress on the indigenous entomopathogenic 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.

Greenhouse pesticide screening trials were initiated last fall at the end of the TLSB season. To determine whether *Beauveria bassiana* (formulated as Botanigard®) is potentially effective against twolined spittle, trials were conducted in Petri dishes (dipped adult TLSB, Botanigard compared with controls), and in caged trials (adults). Two dose rates of Botanigard® were applied: the label rate (1x), and a 4x dose. An untreated control was included in the trials, and a labelled pesticide, Sevin® was included as a standard treatment in the cage trials. Four replicates of five adult TLSB were used for each treatment. Morality was assessed daily for seven days following treatment. Data were analyzed using Kaplan-Meir curves in Prism (GraphPad) to determine probability of survival of TLSB in each treatment, median survival, and to compare the survival curves with logrank tests.

The insects treated in Petri dishes died quickly after treatment in the 4x Botanigard treatment, and died at a slightly slower rate in the 1x treatment, confirming their susceptibility to *B. bassiana*. The caged trials produced median survival of 7 days for the controls; 5 days for Botanigard 1x; 4 days for Botanigard 4x; and 1 day for Sevin. The survival curves are shown in Figure 3. Isolates were collected and grown on PDA plates in the lab to confirm that *B. bassiana* was the causal agent in TLSB that died and had signs of mycosis.

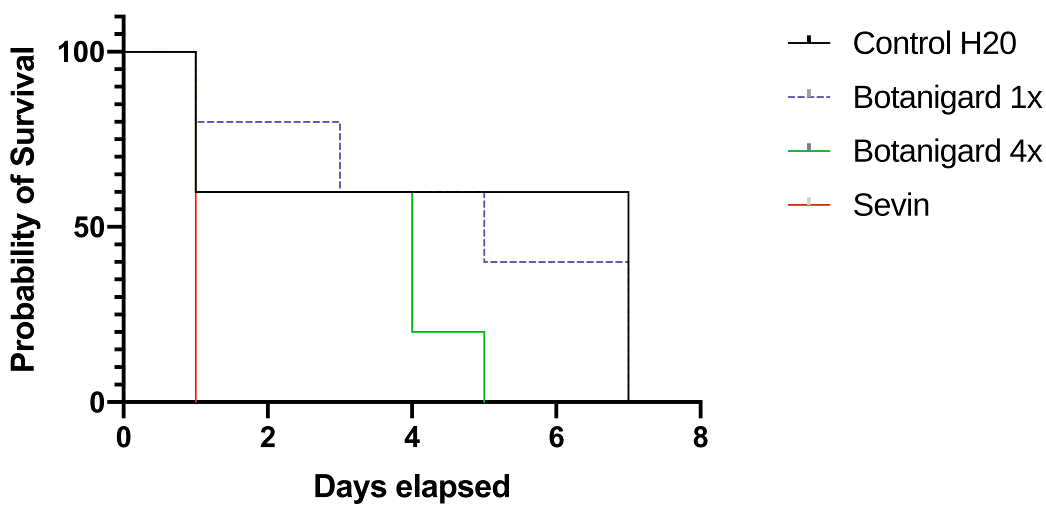


Figure 3. Kaplan-Meir survival curves for TLSB exposed to 3 pesticide treatments and a control. The curves were significantly different (logrank test, $P < 0.05$).

These results suggest that while TLSB are susceptible to Botanigard, treatment at the label rate produced slow mortality, not different from the control. The 4x Botanigard treatment resulted in death of all treated TLSB by the fifth day following treatment. These early trials suggest that while TLSB adults are susceptible to the fungal strain in BotaniGuard, spraying at the recommended rate (4X) may not be economically efficient at the field scale. Additional trials are needed to further assess the effectiveness of BotaniGuard on TLSB adults. These would include controlled experiments in the greenhouse and field-scale trials.

Exploration of potential biological agents in the home range of TLSB were put on hold because of the COVID-19 crisis.

Twolined 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 were carried out over the 2021 TLSB season. These included host-plant specificity or preference trials and adult density threshold (economic injury level) trials. The results of this work allow the development of treatment thresholds for use in the field, and guide work on selection of resistant or tolerant pasture grass varieties.

Using established protocols we conducted trials on 9 varieties of grasses, including two experimental varieties developed in Brazil to be spittlebug resistant (Cayana and Sabia), one variety developed in Columbia for spittlebug resistance (Cayman), along with Mulato II, a *Brachiaria* spp. hybrid developed for spittlebug resistance, Gatton panic, Bahia Tifton 9, Marandu (*Brachiaria brizantha*), and three other naturalized grasses commonly found in Hawaii pasture systems (Kikuyu, fountain grass and Yorkshire fog). A detailed report of the 2020-2021 trails is provided in Appendix E. Results of the 2022 trials are still being analyzed and are not currently available.

We established nine field research units across three cooperating ranches to quantify pasture species susceptibility and resistance under field conditions. Establishment began in February and was not completed until August of 2021. Establishment was more difficult than anticipated and several blocks had to be reseeded. Consequently, the trials and monitoring of TLSB activity on the plots did not start until the 2022 TLSB season.

On each ranch, three units were completed along an elevational gradient that spanned different forage zones and soil types. Each unit contained 32 plots measuring 2 meters x 2 meters in size and arranged in a randomized split-plot design with one half of each research unit excluded from grazing through fencing and the other half exposed to grazing (Figure 4 and 5). This arrangement will allow testing of the eight varieties of grasses (4 replications per variety per research unit) for TLSB infestation rates, tolerance/resistance, and forage quality and productivity, across different elevations and under grazed and ungrazed conditions (Table 4).

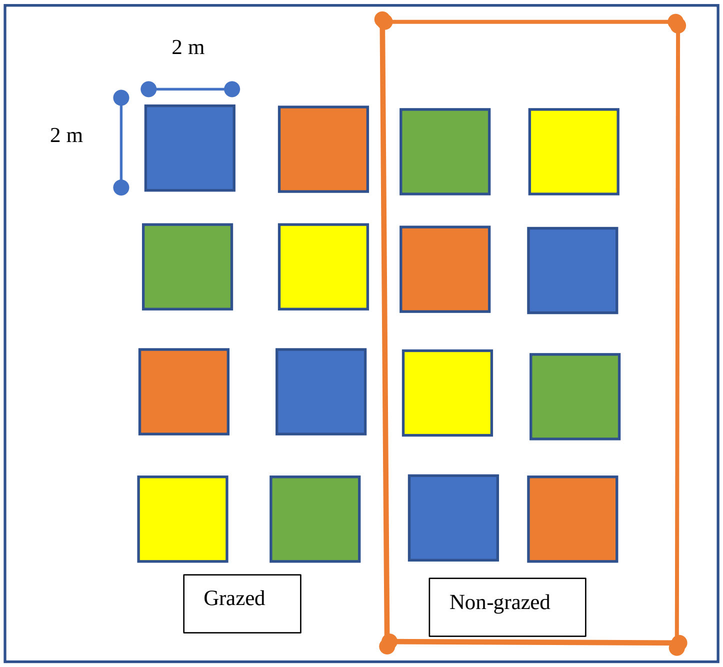


Figure 4. Example layout of research unit showing randomized split-plot design of different grass species (color coded) plots exposed to grazing or not (enclosed in fencing depicted in orange). Note, only 16 plots shown.

Table 4. Grass species/varieties, listed by common name, scientific name, our code designation, and a description. These eight forage grasses will be tested for TLSB infestation rates, resistance, forage quality and productivity over the summer 2022 TLSB season in nine different research units spanning different ecological zones on three different cooperating ranches. Establishment of the research units began in February 2021 and was completed in August 2021.

Grass	Scientific Name	Code	Description
Sabia	Brachiaria Hybrid v Sabia	BrHyvS	Barenbrug developed hybrid
Cayana	Brachiaria Hybrid v Cayana	BrHyvC1	Barenbrug developed hybrid
Bahia T9	Paspalum Notatum v Tifton 9	PanoT9	USDA developed variety of Pensecola Bahia
Gatton Panic	Panicum maximum v Gatton	Pama	
Kikuyu	Pennisetum clandistinum	Pecl	
Marandu	Braciaria brizantha v Marandu	BrbrM	Base species of Brachiaria for Hybrids with spittlebug resistance
Mulatto II	Brachiaria Hybrid v Mulatto II	BrHyvM	CIAT developed Brachiaria hybrid with spittlebug resistance
Cayman	Brachiaria Hybrid v Cayman	BrHyvC2	CIAT developed Brachiaria hybrid with spittlebug resistance

Figure 5. Completed research unit at PR-1 (left) and research unit at WR-2 (right) being seeded.

Future Research and Extension Needs:

Surveys of established transects on the four ranch properties should continue monthly in order to continue to monitor TLSB activity; elucidate biology and ecology of TLSB in Hawaii; allow for collection of samples of nymphs and adults for study and propagation for greenhouse trials on grass selections and pesticides and inform the refinement of existing IMP protocols as well as the development of new strategies. Spittlebug activity needs to be evaluated on each of the nine field research units each month during the peak TLSB season for the next 2-3 seasons. Likewise, forage samples from the plots need to be collected over the next 2-3 seasons to evaluate quality and productivity across different ecological zones and elevations in the face of TLSB pressure.

We believe that an important next step is to use the existing TLSB population data collected between 2017 – 2022 to develop models, using remote sensing, and GIS technology to detect movement vectors for the pest, niche partitioning, and predict potential large-scale outbreaks and damage to pastures, all of which would enhance biosecurity efforts.

Appendix

Appendix A

ALERT!

NEW PEST ATTACKING PASTURE GRASSES

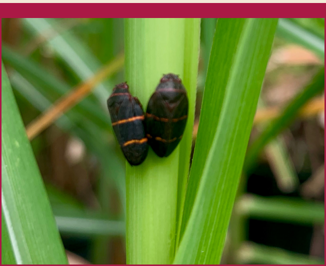
TWOLINED SPITTLEBUG

(Prosapia bicincta)

First detected in Kealakekua in 2016, this new invasive pest has been quickly spreading across the west side of Hawai'i island, causing the loss of thousands of acres of kikuyu and pangola grass pastures. Pastures do not recover after infestation, but instead are replaced by invasive plants like fireweed, pamakani, and blackberry. The TLSB is a serious threat to the ranching industry in Hawaii, where most of our livestock depend on the grasses attacked by the insect.

- **Adults can jump into vehicles so keep doors and windows closed**
- **Do not move plant and soil material from grassy areas on the West side**
- **Always clean your gear and vehicle after visiting an infested area**

Scan to watch TLSB documentary



Peak activity is from April to July so be on high alert for active adults!

Nymphs suck plant nutrients from grass to form this protective spittle mass

Appendix A

TWOLINED SPITTLEBUG

(Prosapia bicincta)

TLSB has been reported in residential areas, attacking lawns, sod, orchards, and other landscapes. Unlike other spittlebugs in Hawai'i, the TLSB nymph spittle mass is found at or even below the soil level, making them difficult to detect. Adults kill the grass by sucking nutrients from the plant. Adult TLSB actively jump and fly around, and can become trapped in vehicles and gear. Please use care and inspect vehicles and gear for TLSB when leaving pastures and natural areas that are infested to avoid transporting them to other areas around the island.

Early TLSB damage in a kikuyu grass pasture

What can you do?

- **BE AWARE** when traveling in & out of infested areas
- **BE ALERT** & watch for signs of damage to sod/pastures
- **REPORT** sightings of the twolined spittle bug

If you encounter TLSB or grass damage:

Take a picture and send to biisc@hawaii.edu or send us a message on Facebook or Instagram.

: @UHBIISC

: @bigislandinvasivespecies

For more information, visit www.biisc.org/tlsb

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Appendix B

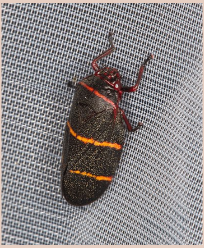
ARE YOU SURE IT IS twolined spittlebug?

An identification guide for spittlebugs in Hawai'i

The following key will help you identify the Twolined Spittlebug (Prosapia bicincta; TLSB) and distinguish it from two other species of spittlebugs that exist in Hawaii. These other two species have been in Hawai'i for some time and do not pose an economic or environmental threat like TLSB.

ADULTS:

Twolined Spittlebug
(Prosapia bicincta)



- ~10 mm in length
- Red eyes
- Reddish-black legs
- Two orange stripes across the back

Meadow Spittlebug
(Philaenus spumarius)

- ~6 mm in length
- Starts out green and then turns brown or grey

Sunflower Spittlebug
(Clastoptera xanthocephala)

- ~3 mm in length
- Red eyes
- Color ranges from black to light brown

0 mm
5 mm
10 mm

NYMPHS:

Twolined Spittlebug
(Prosapia bicincta)

- ~0.4 mm to 8 mm
- Red eyes
- Brownish head, yellow/cream-colored abdomen with two faint pink spots

Meadow Spittlebug
(Philaenus spumarius)

- ~0.3 mm to 6 mm
- Red eyes
- Soft, elongated body
- Change from orange to yellow to green as they grow

Sunflower Spittlebug
(Clastoptera xanthocephala)

- ~0.1 mm to 3 mm
- Very small
- Green to pale yellow

ARE YOU SURE IT IS twolined spittlebug?

see other side for photos of spittle masses

Appendix B

ARE YOU SURE IT IS twolined spittlebug?

An identification guide for spittlebugs in Hawai‘i

The nymphs of all three species live and mature in a spittle mass they produce that protects them from environmental extremes and various predators. Make note of where on the plant the spittle masses are located.

SPITTLE MASSES:

Twolined Spittlebug
(Prosapia bicincta)

- Located primarily on the roots and base of grass plants

Meadow Spittlebug
(Philaenus spumarius)

- Can be found on the leaves of grasses, forbs, and small shrubs

Sunflower Spittlebug
(Clastoptera xanthocephala)

- Found primarily on the leaves and stems of forbs, shrubs like Pamakani

If you encounter TLSB:

Take multiple photos and report the location to **643pest.org**

: @UHBIISC

: @bigislandinvasivespecies

For more information, visit tlsbhawaii.com

Funding provided by the Hawaii Dept. of Agriculture under contract No. 65973 awarded to UH-Mānoa.

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Appendix C

DO YOU HAVE TWOLINED SPITTLEBUG?

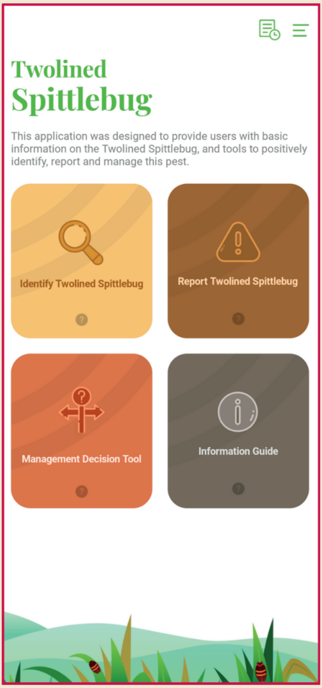
Download the smartphone app today

Developed by the University of Hawai'i- CTAHR, the 'Twolined Spittlebug Tool' is a great resource for any rancher, land manager, or resident who wants to learn more about how to identify, report, and manage twolined spittlebug (TLSB) infestations. The app is available on both Apple and Android devices.

Get the app today and join the effort to manage this destructive pest!

Apple iOS:

Android:



Appendix C

Learn

Learn about the different spittlebugs present in Hawai'i, basic biology of TLSB, and its impacts on pasture grasses.

Identify

Correctly identify TLSB and distinguish it from other spittlebug species. If it is TLSB, report the sighting in the app.

Report

It's important to track the spread of TLSB. If you've spotted this pest, submit a report!

Manage

The app helps ranchers and land managers determine what IPM strategies they need to employ for their situation.

Learn more about the app:

For more information, visit tlsbhawaii.com

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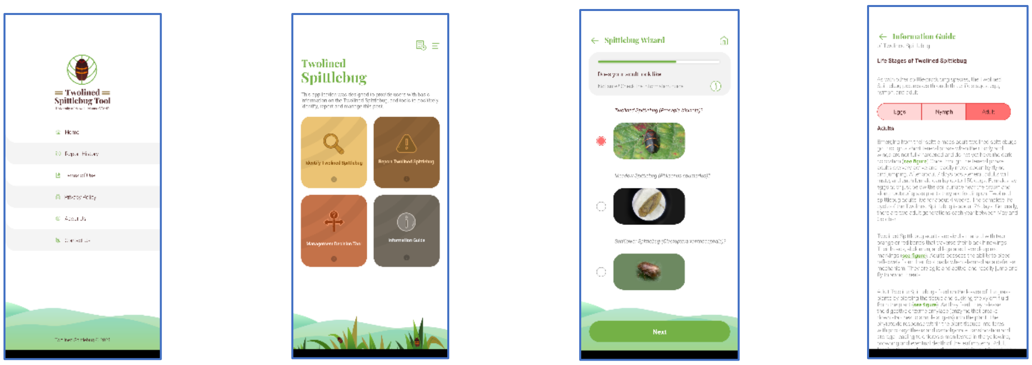
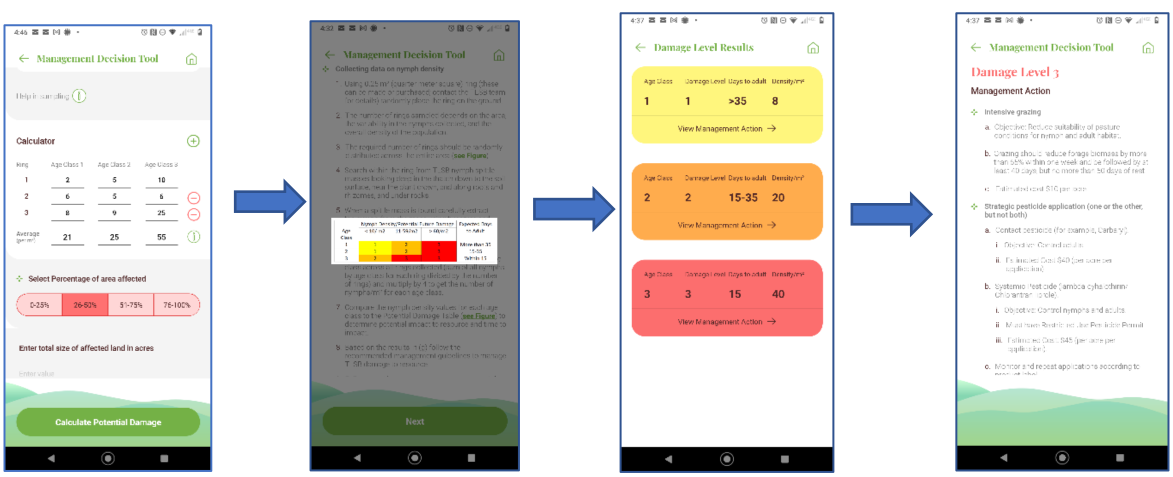
Appendix D

Twolined Spittlebug Management Tool
Smartphone Application Screenshots

The Twolined Spittlebug Management Tool smartphone application has been developed to provide users with basic information on the pest; and tools to identify and report the presence of Twolined Spittlebug; quantify nymph density and adult activity; and based on potential damage levels, determine appropriate IPM strategies for managing and recovering from an infestation. The tool will be released to the public fall of 2022. The images below are screenshots of the tool.



Startup and Home screen for the Twolined Spittlebug Management Tool smartphone application. Home screen provides access to four tools to identify, report, manage, and learn about TLSB.



In the management tool of the application, users are provided protocols to estimate Twolined Spittlebug nymph densities by age (size class) distribution. This data is then used to determine the potential impact and timing of the forthcoming adult population. This allows the user to preemptively select the appropriate IPM protocol and employ it at the appropriate time.

Appendix E

Data summary report:
Host plant resistance, tolerance, and susceptibility to adult *Prosapia bicincta* feeding
Shannon Wilson

Background

It is crucial to prevent the further spread of *Prosapia bicincta* in Hawaii and implement effective and efficacious management strategies in order for the cattle industry in the state to persist. However, despite the severe economic impacts inflicted by this pest, an effective integrated pest management program does not exist because of the paucity of information on the basic biology and ecology and its high diversity of habitat associations and hosts. Thus, it is vital to conduct further research on this pest complex and the development and implementation of control strategies.

Since pastures in most regions are low value crops that occupy vast areas, past control efforts against spittlebugs in have typically been directed toward the development of low-cost methods that ranchers can easily implement over thousands of hectares. Host plant resistance is widely accepted as the most effective method to combat spittlebugs since it is low cost, sustainable, environmentally sound, has a cumulative effect, can be easily incorporated into ranches, and is compatible with other tactics. Species and varieties of grasses that have shown resistance to spittlebugs do exist, but the level of resistance is dependent on the spittlebug species and require specific soil fertility, or association with legumes for nitrogen fixation. The use of sustainable, environmentally friendly management tactics like host plant resistance may be essential for Hawaii Island, because the rangelands are typically comprised of thousands of hectares of kikuyugrass pastures surrounded by and intertwined with native forests at various elevations. Thus, management must also take into account these nearby delicate native ecosystems. Since 70% of Hawaii's cattle are raised on sod-forming grasses and for many generations' ranches have mainly consisted of kikuyu and pangola pastures, deep rooted, conventional practices will need to be adjusted with the introduction of spittlebug resistant grasses. The unfortunate reality is that due to the susceptibility to *P. bicincta* feeding, existing kikuyu-pangolagrass pastures across Hawaii Island are severely threatened. Thus, the future of livestock production in these pasture systems will be dependent on the incorporation of more tolerant or resistant species of grasses. Additionally, host plant resistance is more compatible with Hawaii's unique rangelands since certain management strategies employed in other regions such as controlled burning on fields of Bermudagrass in Georgia, may be too risky for the multitude of native species in these areas that are vital to these ecosystems. Furthermore, chemical control, which is widely used against pests in high-value crops, is typically either too costly or unfeasible to apply over thousands of hectares of pastures. Additionally, insecticides may be ineffective against the extremely mobile adults and unable to penetrate through viscous spittle masses that protect the nymphs under thick thatches of grass. Due to these factors that make host plant resistance a more promising, sustainable, and long-term management strategy for Hawaii Island, research has been conducted by the University of Hawaii College of Tropical Agriculture and Human Resources Cooperative

Extension Service evaluating the resistance, tolerance, and susceptibility of different forage grass species and varieties to *P. bicincta* feeding in the hopes of providing ranchers with better forage options to replace kikuyu. Additionally, kikuyu response to different infestation levels of adult spittlebugs was assessed to determine the threshold at which the adults cause phytotoxemia and kill the grass, which is an imperative measure for estimating the economic impact. The results of these grass trials may be extremely significant for preserving Hawaii Island's rangelands.

Research Objectives

1) Evaluate host plant susceptibility by categorizing damage inflicted by adult *P. bicincta* feeding and comparing the variation of disruption adults cause between different grass species/varieties
2) Assess kikuyu response to different levels of adult infestation to determine significant threshold for survivability to an attack

Methodology

Insect collection and infestation

1. *Prosapia bicincta* adults were field collected using sweep nets (Bioquip) and aspirated (Bioquip) into a container to be transported to the greenhouse
2. All adults were then placed in a cage (Bioquip) with a pot of kikuyu grass and left for 24 hours to acclimate in the greenhouse
3. After 24 hours live adults were aspirated out through the sleeve of the cage
4. Each cage of experimental grass units was infested with adults while the cages of control units remained uninfested
5. 24 hours following infestation, dead adults in the cages were replaced by live ones
6. Insect survival was assessed on days 1, 4, 8, and 12

Host plant resistance trials

- Test species:
    - 2020: Kikuyu, Limpo Big Alta, Limpo #508607, Marandu, Orchard, Signal
    - 2021: Bahia T9, Cayana, Cayman, Fountain, Kikuyu, Marandu, Mulatto II, Sabia, Yorkshire Fog

1. Plant units:
    a. 2020: Cuttings of 2-4 sprigs (2 for larger/taller grasses and 3-4 for smaller/shorter grasses) of similar biomass containing crown and roots from 6 different grass species (Kikuyu, Marandu, Signal, Limpo Big Alta, Orchard, Limpo #508607) were taken from mother plants
    b. 2021: Six seeds per species were planted into 4" square pots (McConkey Co.)
2. The roots of the cuttings in 2020 were dusted with Root Boost rooting hormone (Garden Tech) and then transplanted into 4" square pots

3. Cuttings & seeds were planted using a 1:1 mixture of expanded perlite (Wilking mining & trucking inc.) for aeration and moisture control, and a soil growing mix with mycorrhizae (Sunshine Aggregate Plus)
    a. The amount of growing medium used for each pot was about 120 g
4. Six replications were carried out for each grass species with each rep containing 3 experimental units (infested) and 3 control units (uninfested) per grass species
5. Within each rep, plants were grown and treated consistently under the same conditions, however across different reps there was variability due to circumstances based on space and watering availability in the greenhouse and aphid infestations that necessitated insecticide applications
6. Pre-experiment growing conditions:
    a. Watered two times per day for 2 minutes
    b. ½ tsp of nutricote total 13-11-11 type 100 fertilizer (Arysta LifeScience) was added to each pot after 1 week of growing for cuttings and upon planting for seedlings
    c. 2020: after two weeks of growing plants were trimmed to about 12" in order to further standardize the units across species and subsequently trimmed every 2 weeks until used for testing
    d. 2021: seedlings took around 6 weeks to grow and were then trimmed to about 12" and subsequently trimmed until used for testing
7. After growing for a minimum of 4-6 weeks the pots were ready to be infested and were placed into insect cages (BugDorm) in a complete randomized block design and fitted with drip irrigation to maintain soil moisture
    a. Watered two times per day for 1 minute
8. Photos and observations of plants were taken on Day 0 before being infested
9. Prior visible damage from water/heat stress and aphids was noted
10. After acclimating in stock cage for 24 hours adults were aspirated and each experimental unit was infested
    a. 2020: Six *Prosapia bicincta* adults per experimental unit
    b. 2021: Increased infestation to 12 adults since seeds produced more plant biomass than when taken from cuttings
11. 24 hours after infestation dead adults were replaced
12. Photos were taken and observations and adult mortality were recorded on days 1, 4, 8, and 12
13. Plants were evaluated for adult damage 12 days after infestation
14. Damage was scored on a 1-6 visual scale based on CIAT resistance breeding program scale (see below) to estimate tolerance (1-2.25= resistant, 2.26-3.5= tolerant, >3.5=susceptible):

Appendix E

Figure 1. CIAT visual damage scale to assess tolerance to spittlebug herbivory

1.0	1.1 – 2.25	2.26 – 3.5	3.55 – 4.75	4.76 – 6.0

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

1.0	No visible damage
1.1 - 2.25	1-25% of foliar area affected -few leaves on lower position of plant curling, crinkling, and slight yellowing
2.26 - 3.5	26-50% foliar area affected -crinkling and curling, yellowing, bronzing, and browning leaves in the middle and lower positions
3.55 - 4.75	51-75% foliar area affected -crinkling and curling all over, yellowing, bronzing and browning, and drying of leaves
4.76 - 6.0	76-100% foliar area affected and dry -extreme curling yellowing, bronzing and browning, and drying of leaves and defoliation and stunted growth

Appendix E

Table 2. Damage rating per percent damage

% Damage	Damage Rating	% Damage	Damage Rating	% Damage	Damage Rating
0	**1**	36	2.80	72	4.60
1	1.05	37	2.85	73	4.65
2	1.10	38	2.90	74	4.70
3	1.15	39	2.95	**75**	**4.75**
4	1.20	40	3.0	76	4.80
5	1.25	41	3.05	77	4.85
6	1.30	42	3.10	78	4.90
7	1.35	43	3.15	79	4.95
8	1.40	44	3.20	80	5.0
9	1.45	45	3.25	81	5.05
10	1.50	46	3.30	82	5.10
11	1.55	47	3.35	83	5.15
12	1.60	48	3.40	84	5.20
13	1.65	49	3.45	85	5.25
14	1.70	**50**	**3.5**	86	5.30
15	1.75	51	3.55	87	5.35
16	1.80	52	3.60	88	5.40
17	1.85	53	3.65	89	5.45
18	1.90	54	3.70	90	5.50
19	1.95	55	3.75	91	5.55
20	2.0	56	3.80	92	5.60
21	2.05	57	3.85	93	5.65
22	2.10	58	3.90	94	5.70
23	2.15	59	3.95	95	5.75
24	2.20	60	4.0	96	5.80
25	**2.25**	61	4.05	97	5.85
26	2.30	62	4.10	98	5.90
27	2.35	63	4.15	99	5.95
28	2.40	64	4.20	**100**	**6.0**
29	2.45	65	4.25		
30	2.50	66	4.30		
31	2.55	67	4.35		
32	2.60	68	4.40		
33	2.65	69	4.45		
34	2.70	70	4.5		
35	2.75	71	4.55		

15. On day 12 the above ground biomass fresh weights were obtained by cutting each grass at soil level and weighing on a scale (Ohaus) right away
16. The root portion was gently rinsed and separated from the soil material and then rung out and patted with paper towels and left to air dry for 60 minutes
17. After air drying the root mass the fresh weight was obtained
18. For each plant the biomass and root mass portions were placed in separate paper bags and labeled
19. The plant materials air dried for 1-4 days, depending on availability of oven and scale, and were then placed in an oven to dry at 65 °F for a minimum of 48 hours
20. After at least 48 hours in the oven the dry weights were recorded and stored for later analyses

<u>Adult impact on kikuyu</u>

1. Well established mother plants of Kikuyu in gallon pots were quartered up
2. Each quarter of similar estimated biomass and root mass was transplanted into a gallon size pot using a 1:1 mixture of expanded perlite for aeration and moisture control, and a soil growing mix with mycorrhizae
3. The pots were placed on tables containing irrigation with emitters for watering and were set to water the grasses 2 times per day for 3 minutes
4. After 2 weeks of growing, the grasses were trimmed to about 12 inches to further standardize the units and 1 tsp of nutricote total 13-11-11 type 100 fertilizer was added to each pot
5. Field collected adults were aspirated from the stock cage and used to infest the cages containing the experimental grass pots
    a. Treatments: 0 (control), 5, 10, or 15 adults per pot
    b. Replicated 4 times in complete randomized block design with 10 pots per rep used for each infestation level
6. Adults were allowed to feed for 12 days and then the plants were immediately scored for damage on the 1-6 visual scale above
7. Following the damage rating, the above ground biomass fresh weights were obtained by cutting each grass at soil level and weighed right away
8. The root portion was gently rinsed and separated from the soil material and then gently rung out and patted with paper towels and left to air dry for 90 minutes and the fresh weight was obtained
9. For each plant the biomass and root mass portions were placed in separate brown paper bags and labeled for later analyses
10. Bags dried in an oven at 65 °F for at least 48 hours and then dry weights were obtained

Results

2020 Grass Trials

Appendix F

Figure 2a. Uninfested (control) vs infested (experimental) Kikuyu on day 0 and day 12

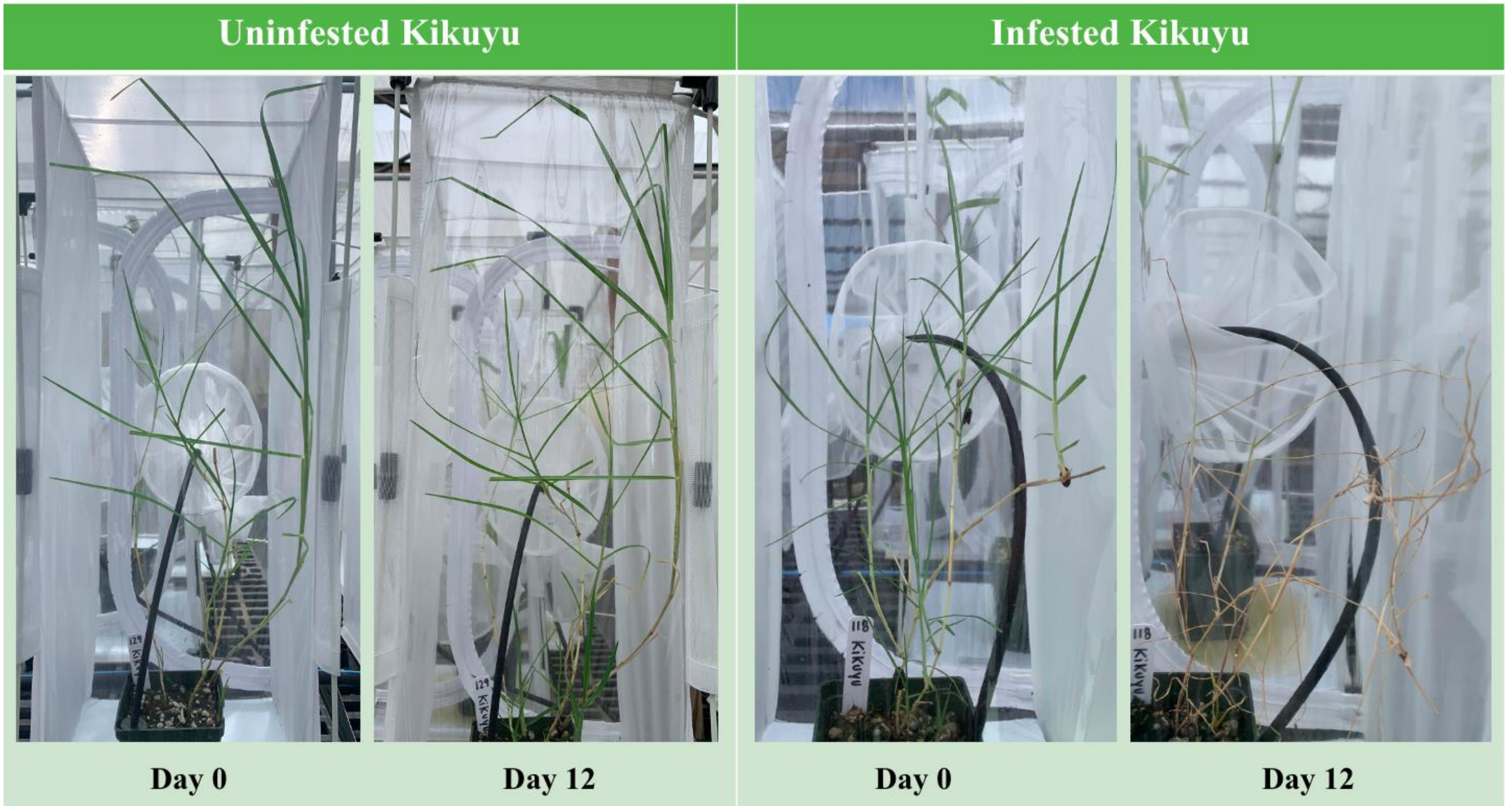


Figure 2b. Uninfested (control) vs infested (experimental) Marandu on day 0 and day 12

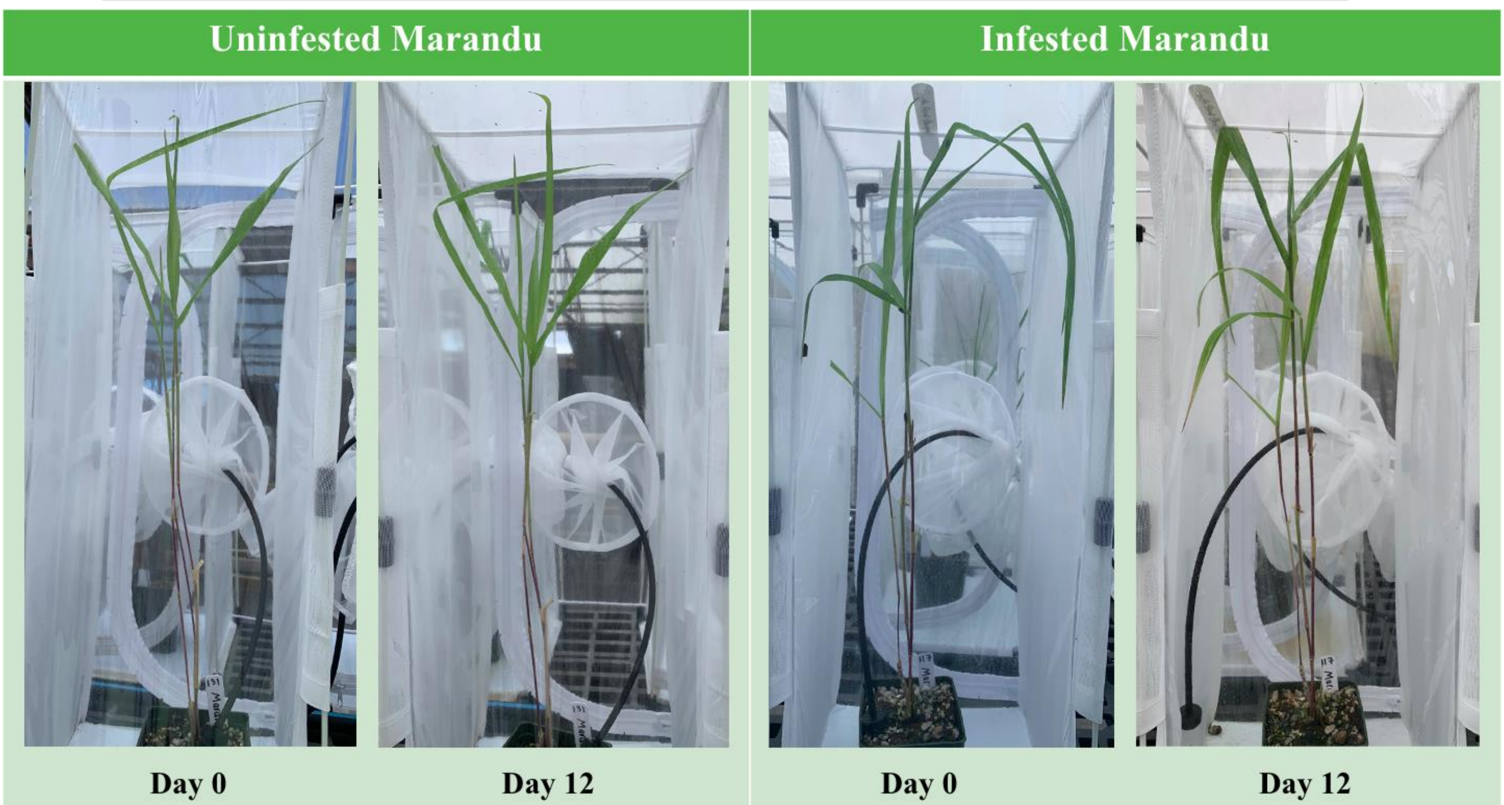


Figure 3: Average damage ratings of 6 grass species to adult spittlebug feeding

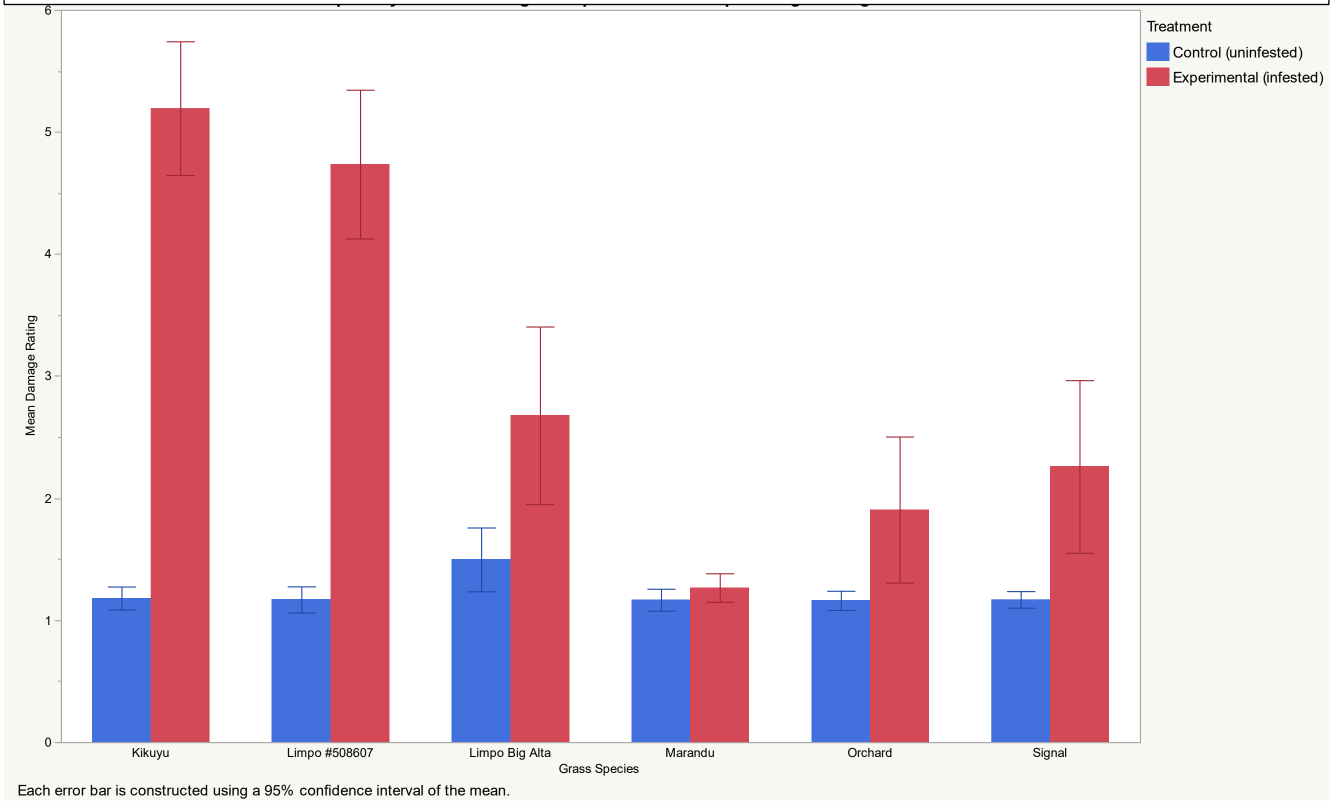


Figure 4: Resistance, tolerance, & susceptibility of 6 grass species to adult spittlebug feeding

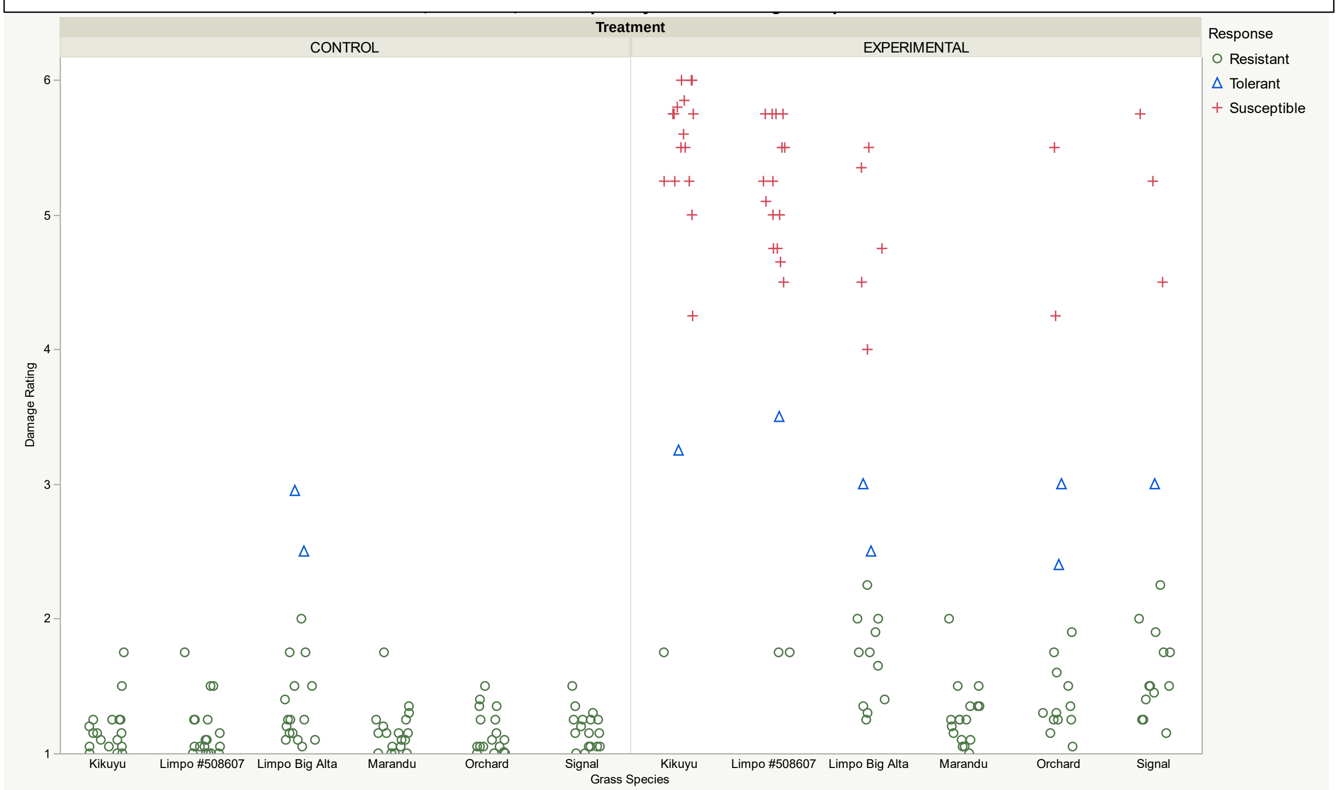


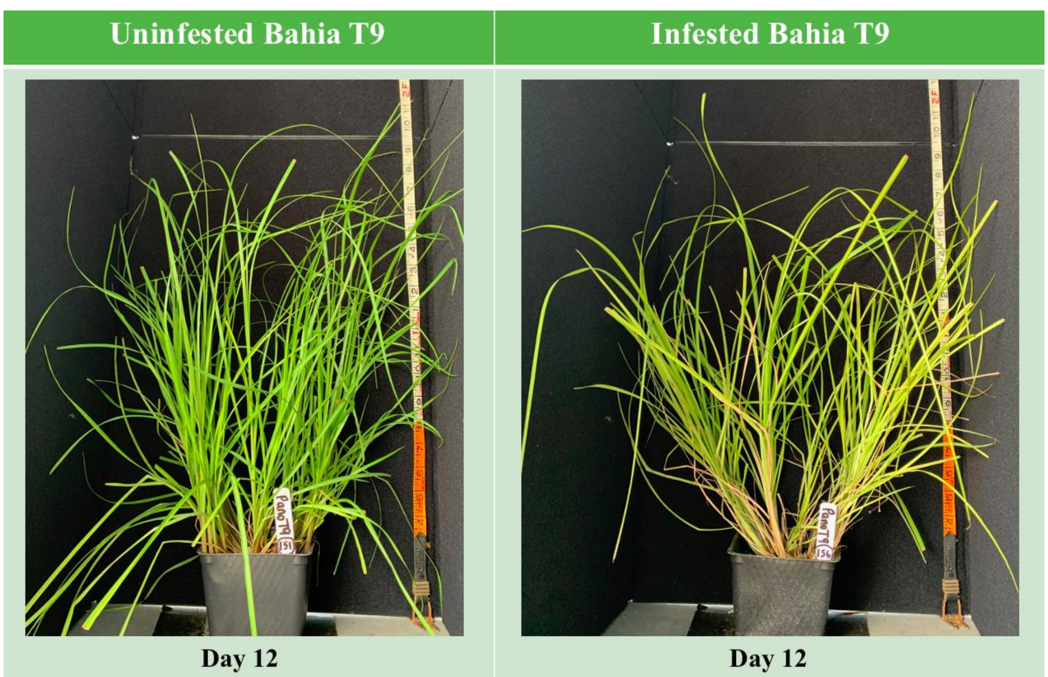
Table 3. Descriptive statistics 2020 trials

		Plant damage rating								
Grass species	**Treatment**	**N**	**Mean**	**Std Dev**	**Min**	**Max**	**Var**	**Std Err**	**CV**	**Median**
Kikuyu	CONTROL	18	1.18055556	0.18953417	1	1.75	0.0359232	0.04467363	16.0546593	1.15
	EXPERIMENTAL	18	5.19444444	1.10092059	1.75	6	1.21202614	0.25948947	21.1941932	5.55
Limpo #508607	CONTROL	18	1.16944444	0.21498214	1	1.75	0.04621732	0.05067178	18.3832708	1.075
	EXPERIMENTAL	18	4.73611111	1.22484159	1.75	5.75	1.50023693	0.28869793	25.861758	5.05
Limpo Big Alta	CONTROL	18	1.49722222	0.52537724	1.05	2.95	0.27602124	0.1238326	35.0901309	1.25
	EXPERIMENTAL	18	2.67777778	1.46370021	1.25	5.5	2.1424183	0.34499745	54.6610036	2
Marandu	CONTROL	18	1.16666667	0.18068497	1	1.75	0.03264706	0.04258786	15.4872832	1.15
	EXPERIMENTAL	18	1.26666667	0.23452079	1	2	0.055	0.05527708	18.5147991	1.25
Orchard	CONTROL	18	1.16166667	0.15804876	1	1.5	0.02497941	0.03725245	13.6053455	1.1
	EXPERIMENTAL	18	1.90555556	1.20377945	1.05	5.5	1.44908497	0.28373354	63.1720995	1.325
Signal	CONTROL	18	1.16944444	0.13627275	1	1.5	0.01857026	0.03211979	11.6527764	1.15
	EXPERIMENTAL	18	2.25833333	1.42212041	1.15	5.75	2.02242647	0.335197	62.9721216	1.625

2021 Grass Trials

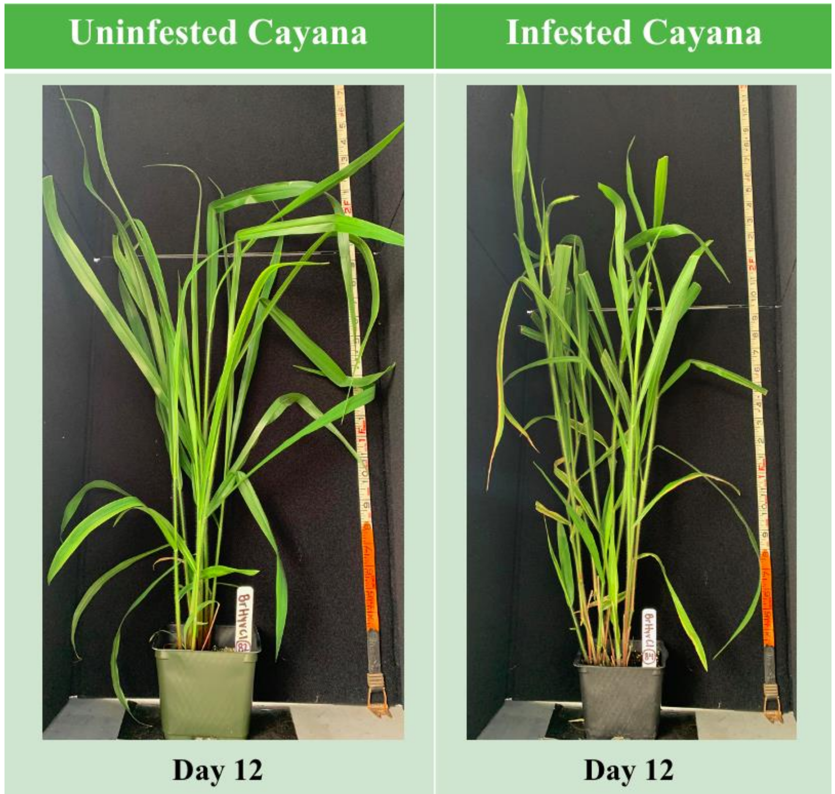
Figure 5a-i. Uninfested (control) vs infested (experimental) for each species on day 12

5a.

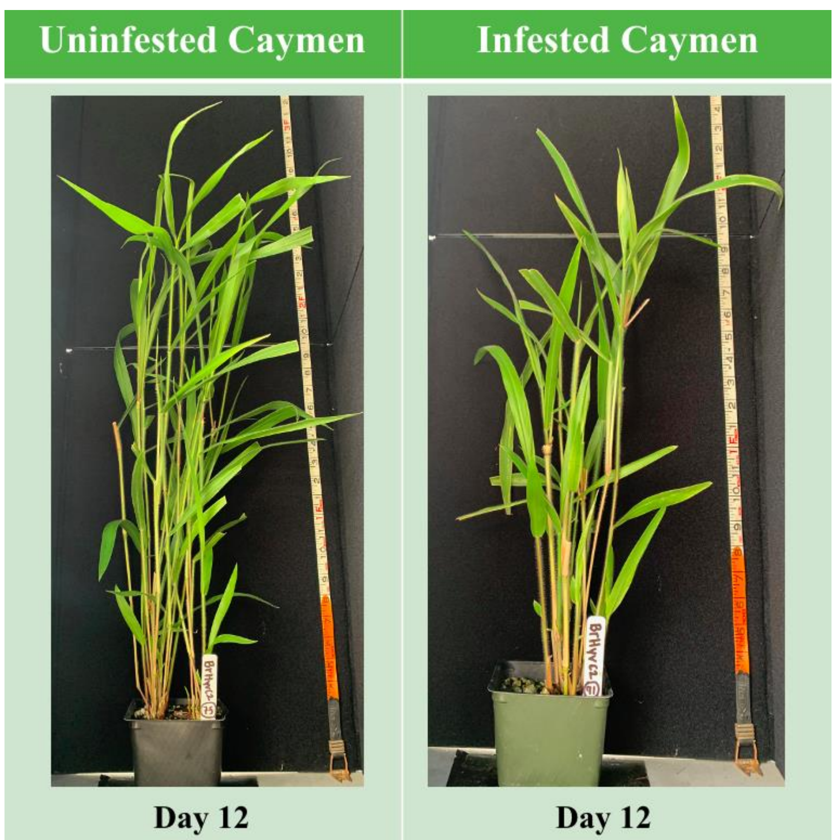


Appendix E

5b.

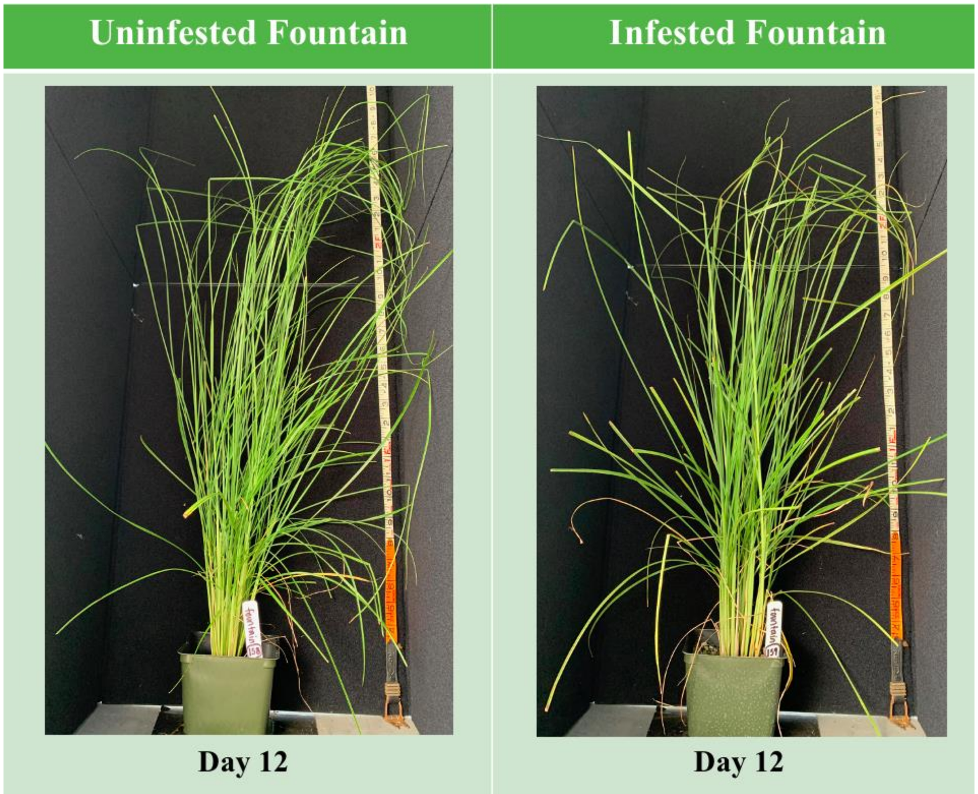


5c.

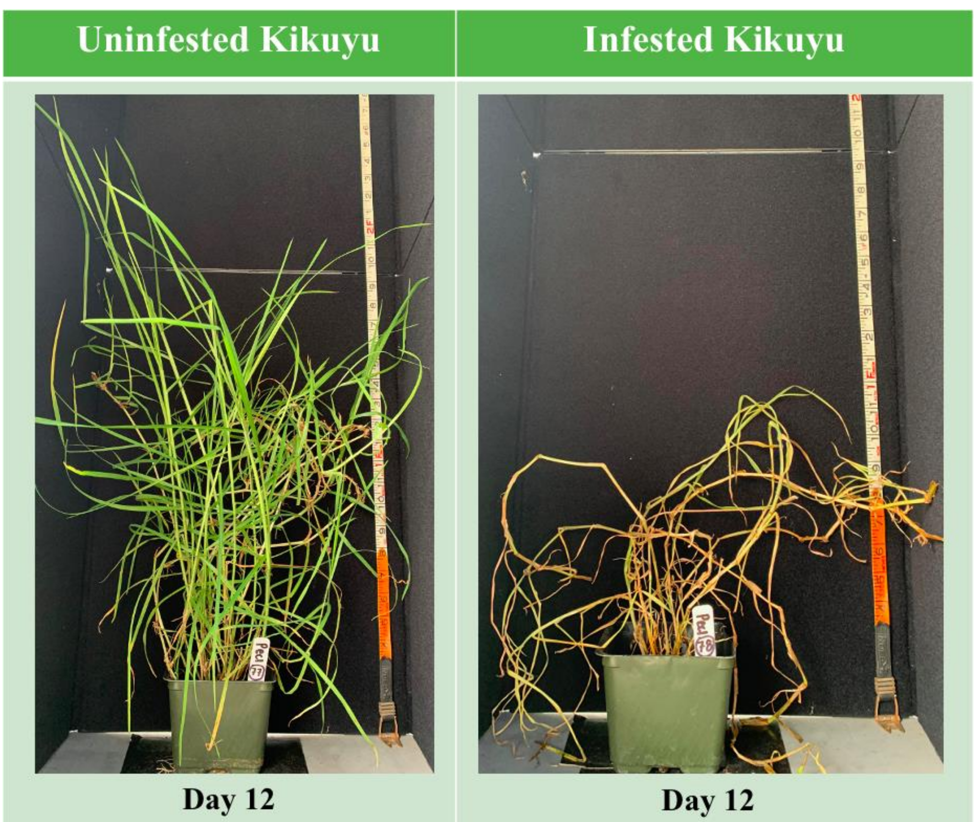


Appendix E

5d.



5e.



Appendix E

5f.

Uninfested Marandu	Infested Marandu

Day 12	Day 12

5g.

Uninfested Mulatto II	Infested Mulatto II

Day 12	Day 12

Appendix E

5h.

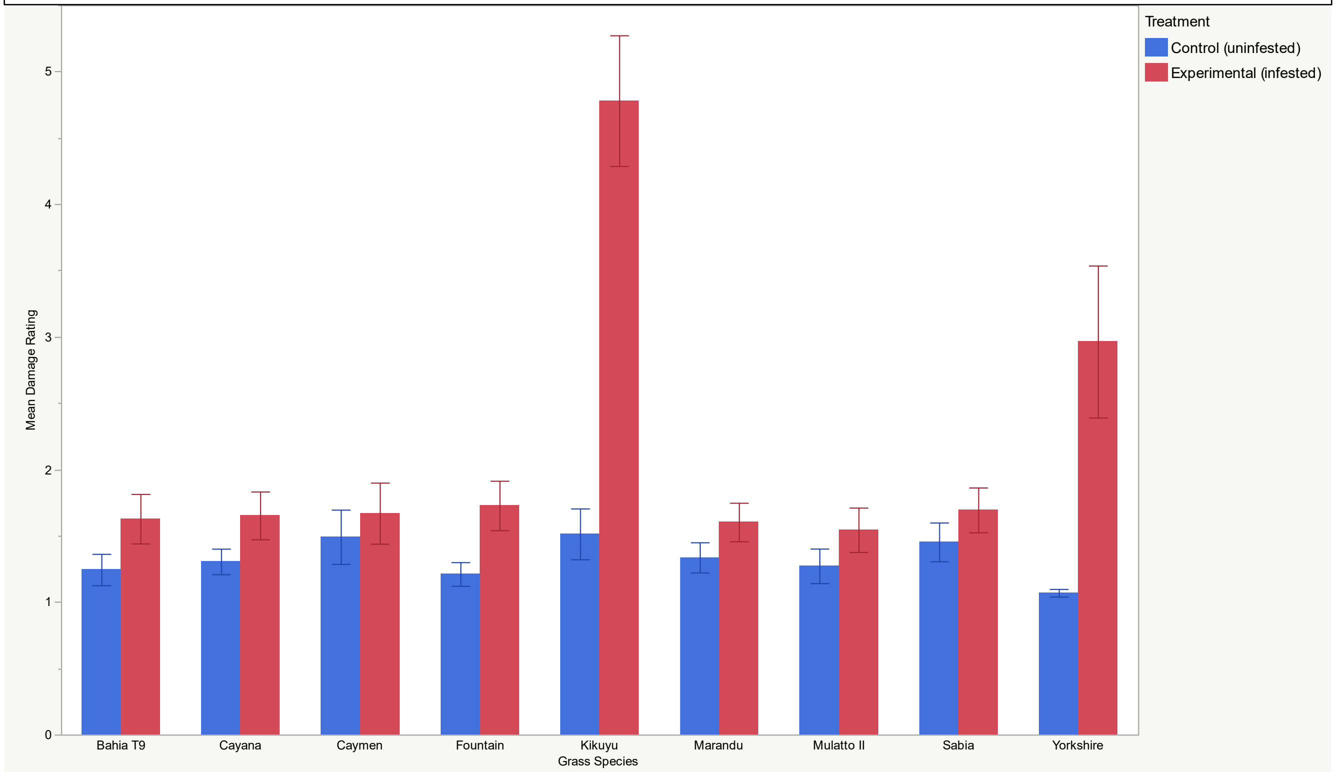
Uninfested Sabia	Infested Sabia
	
Day 12	Day 12

5i.

Uninfested Yorkshire Fog	Infested Yorkshire Fog	
	Tolerant	Susceptible
		
Day 12	Day 12	

Appendix E

Figure 6: Average damage ratings of 9 grass species to adult spittlebug feeding



Each error bar is constructed using a 95% confidence interval of the mean.

Figure 7: Resistance, tolerance, & susceptibility of 9 grass species to adult spittlebug feeding

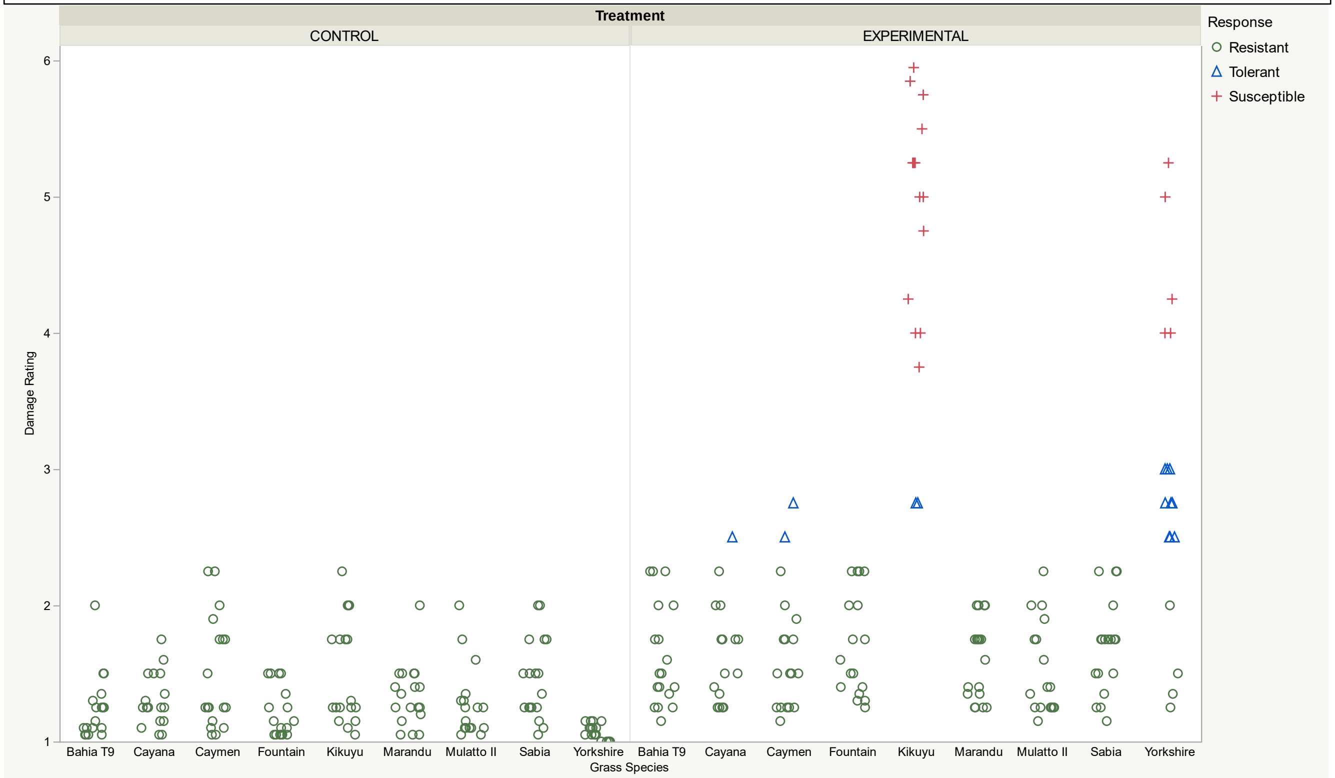


Table 4. Descriptive statistics 2021 trials

		Plant damage rating								
Grass species	**Treatment**	**N**	**Mean**	**Std Dev**	**Min**	**Max**	**Var**	**Std Err**	**CV**	**Median**
Bahia T9	CONTROL	18	1.24444444	0.23756664	1.05	2	0.05643791	0.05599499	19.0901764	1.2
	EXPERIMENTAL	18	1.62777778	0.37503812	1.15	2.25	0.14065359	0.08839733	23.0398848	1.5
Cayana	CONTROL	18	1.30555556	0.19394427	1.05	1.75	0.03761438	0.0457131	14.8553056	1.25
	EXPERIMENTAL	18	1.65277778	0.3627802	1.25	2.5	0.13160948	0.08550811	21.9497267	1.625
Caymen	CONTROL	18	1.49166667	0.41168592	1.05	2.25	0.16948529	0.0970353	27.5990559	1.25
	EXPERIMENTAL	18	1.66944444	0.46277262	1.15	2.75	0.2141585	0.10907655	27.7201569	1.5
Fountain	CONTROL	18	1.21111111	0.17950549	1.05	1.5	0.03222222	0.04230985	14.8215545	1.15
	EXPERIMENTAL	18	1.72777778	0.3746458	1.25	2.25	0.14035948	0.08830486	21.6836799	1.675
Kikuyu	CONTROL	18	1.51388889	0.38493909	1.05	2.25	0.1481781	0.09073101	25.4271694	1.275
	EXPERIMENTAL	18	4.78055556	0.99100942	2.75	5.95	0.98209967	0.23358316	20.7300053	5.125
Marandu	CONTROL	18	1.33611111	0.22868264	1.05	2	0.05229575	0.05390102	17.1155409	1.3
	EXPERIMENTAL	18	1.60277778	0.29178569	1.25	2	0.08513889	0.06877455	18.2049997	1.675
Mulatto II	CONTROL	18	1.27222222	0.26190604	1.05	2	0.06859477	0.06173184	20.5865006	1.2
	EXPERIMENTAL	18	1.54444444	0.33645596	1.15	2.25	0.11320261	0.07930343	21.7849184	1.4
Sabia	CONTROL	18	1.45277778	0.29379476	1.05	2	0.08631536	0.06924809	20.2229661	1.425
	EXPERIMENTAL	18	1.69444444	0.33993463	1.15	2.25	0.11555556	0.08012336	20.0617161	1.75
Yorkshire	CONTROL	18	1.06944444	0.05723761	1	1.15	0.00327614	0.01349103	5.35208804	1.05
	EXPERIMENTAL	18	2.96388889	1.15233849	1.25	5.25	1.32788399	0.27160879	38.8792741	2.75

Appendix E

2020 Kikuyu threshold trials

Figure 8: Kikuyu response to varying levels of adult infestation

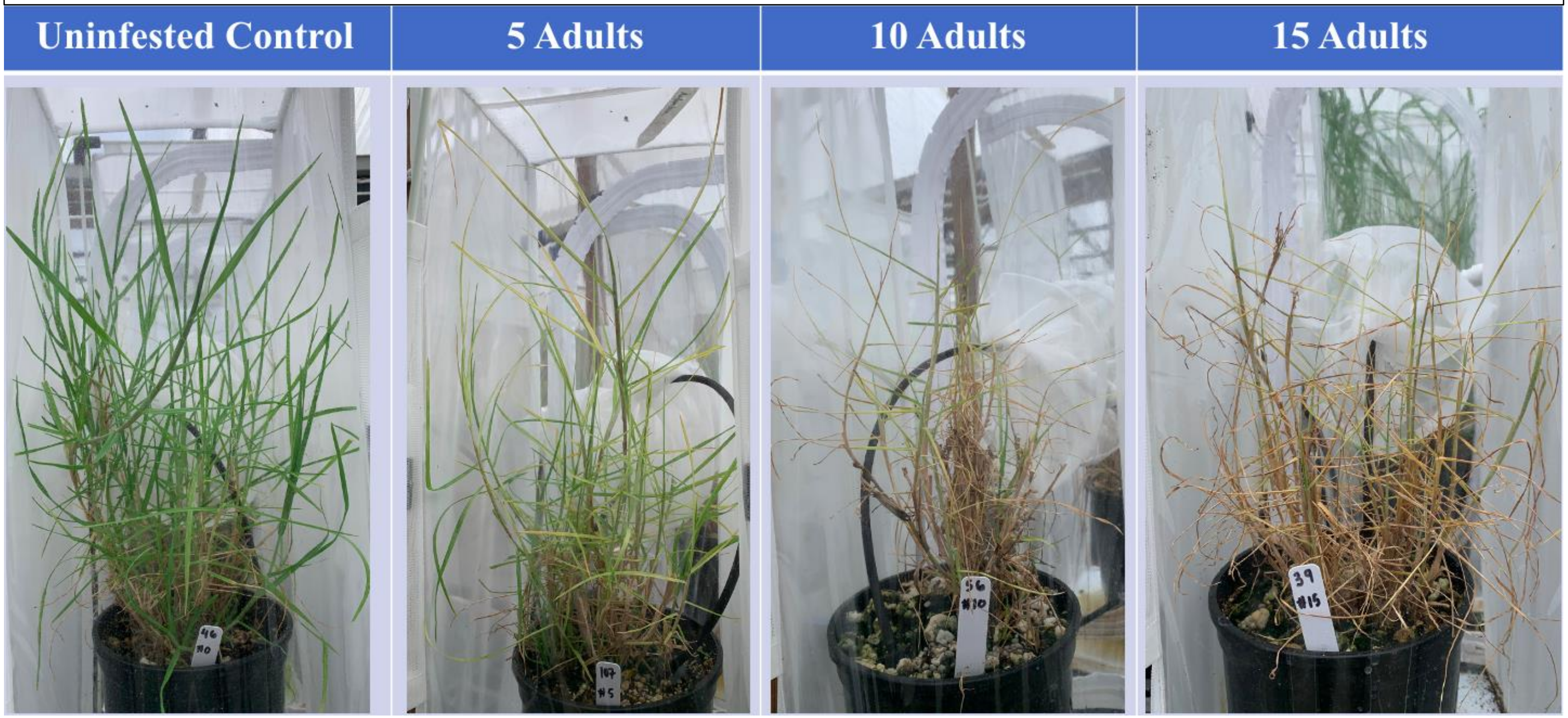
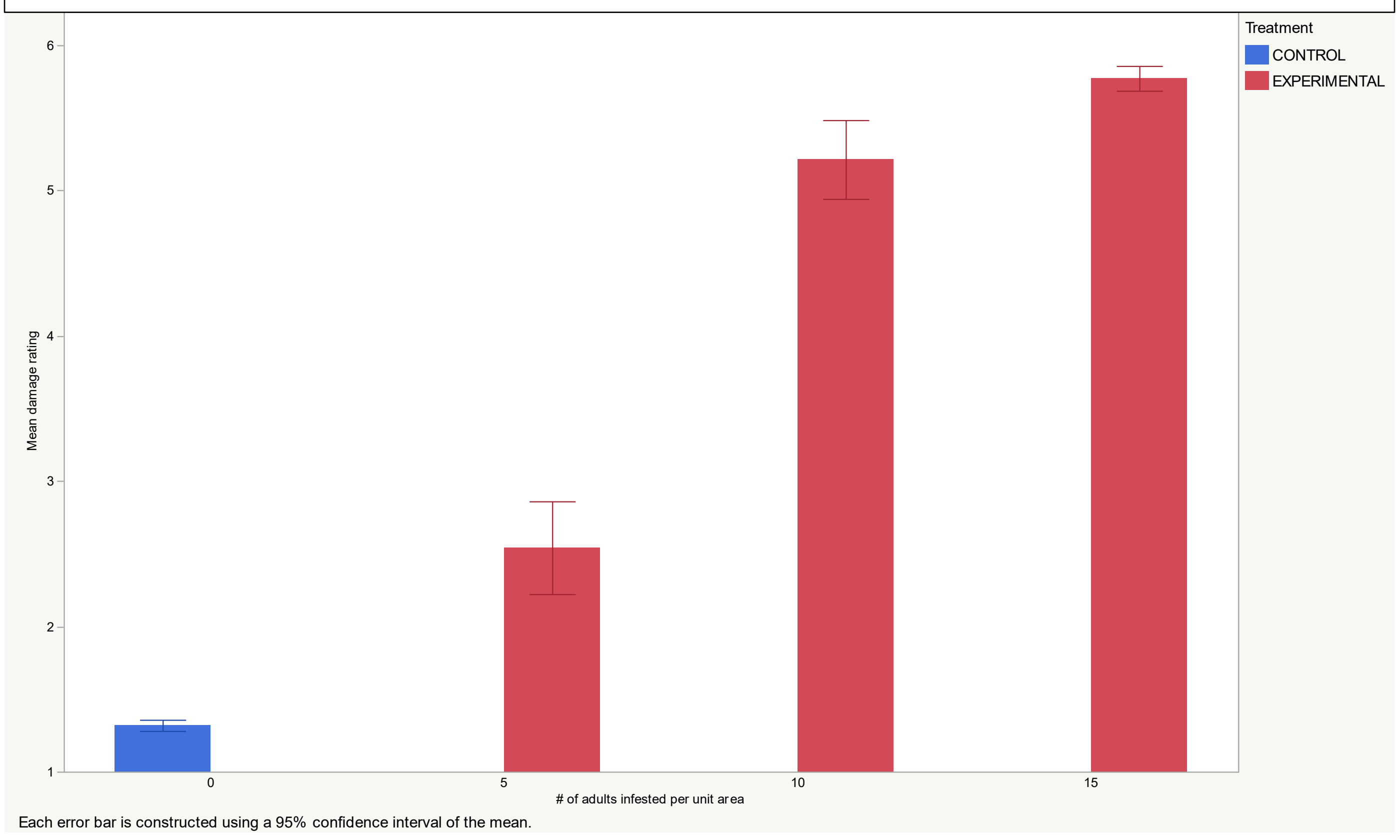


Figure 9: Threshold for kikuyu survivability to an attack per square foot



Appendix E

Table 5. Descriptive statistics kikuyu threshold trials

		Plant damage rating								
# Adults	**Treatment**	**N**	**Mean**	**Std Dev**	**Min**	**Max**	**Var**	**Std Err**	**CV**	**Median**
0	CONTROL	36	1.31805556	0.11411321	1.15	1.75	0.01302183	0.01901887	8.65769372	1.275
5	EXPERIMENTAL	36	2.54027778	0.94330872	1.25	5	0.88983135	0.15721812	37.1340777	2.5
10	EXPERIMENTAL	36	5.20972222	0.80203486	3	5.95	0.64325992	0.13367248	15.394964	5.55
15	EXPERIMENTAL	36	5.76805556	0.25217703	4.75	6	0.06359325	0.0420295	4.37195909	5.825

Discussion & Conclusion

The results of six replications carried out in 2020 evaluating the resistance, tolerance, and susceptibility of six different grass species (Orchard, Kikuyu, Signal, Limpo Big Alta, Marandu, and Limpo #508607) to adult *P. bicincta* feeding are revealed in Figures 3 and 4. 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 in Figure 4 as the average damage ratings seen in Figure 3 for the controls of Orchard, Kikuyu, Signal, Limpo Big Alta, Marandu, and Limpo #508607 were 1.16±0.16, 1.18±0.19, 1.17±0.14, 1.50±0.53, 1.17±0.18, and 1.17±0.21 respectively. Thus, the uninfested control plants help support the validity of the study. Furthermore, the infested experimental plants for Orchard, Kikuyu, Signal, Limpo Big Alta, Marandu, and Limpo #508607 had average damage ratings of 1.91±1.20, 5.19±1.10, 2.26±1.42, 2.68±1.50, 1.27±0.23, and 4.74±1.22 respectively. Consequently, these results suggest Marandu had the highest resistance to adult *P. bicincta* feeding, followed by Orchard. There was little variance between the experimental data points and statistical average for Marandu, therefore its values for plant damage were relatively consistent. Conversely, Orchard had much more variance compared to Marandu, thus its data points for plant damage were more spread out over a range of values. Signal and Limpo Big Alta fell in the tolerant category, but both species had the highest amount of variance overall. Lastly, Limpo #508607 and Kikuyu proved to be very susceptible, with Kikuyu having the highest average damage rating, suggesting it is the most susceptible of the 6 species tested. Photo comparisons of the uninfested control plants and infested experimental plants of the most (Kikuyu) and least (Marandu) susceptible species on days 0 and 12 are illustrated in Figures 2a and 2b.

The experimental design and efforts put into these research trials produced meaningful results and prompted additional trials on more grass varieties in 2021. Thus, six more replications were conducted using nine different species (Bahia T9, Cayana, Caymen, Fountain, Kikuyu, Marandu, Mulatto II, Sabia, and Yorkshire Fog). In Figure 6, the average damage ratings for the control plants of Bahia T9, Cayana, Caymen, Fountain, Kikuyu, Marandu, Mulatto II, Sabia, and Yorkshire Fog were 1.25±0.24, 1.31±0.19, 1.49±0.41, 1.21±0.18, 1.51±0.38, 1.34±0.23, 1.27±0.26, 1.45±0.29, and 1.07±0.06 respectively. Hence, the average damage ratings for the control plants of each species were all within the resistance category in Figure 7 and further support the validity of this study. Additionally, the average damage ratings for the experimental plants of Bahia T9, Cayana, Caymen, Fountain, Kikuyu, Marandu, Mulatto II, Sabia, and Yorkshire Fog were 1.63±0.38, 1.65±0.36, 1.67±0.46, 1.73±0.37, 4.78±0.99, 1.60±0.29, 1.54±0.34, 1.69±0.34, and 2.96±1.15 respectively. Compared to the previous year, the average damage rating for the experimental Kikuyu plants was slightly lower, but still proved to be highly susceptible, while the average for Marandu was slightly higher, yet still fell in the resistant category. Variation in these averages may be due to the difference in biomass and insect infestation levels between the two years since the plant units in 2020 had less biomass because they were taken as cuttings, while in 2021 they were planted from seed which resulted in a greater biomass and necessitated a higher infestation level. Moreover, the averages of Bahia T9, Cayana, Caymen, Fountain, Mulatto II, and Sabia were in the resistant category. The only species in the tolerant category was Yorkshire Fog, but it had the highest amount of variation of all the species due to significant differences in plant damage among the experimental units which is illustrated in Figure 5i. The results of this research seems promising and the diversity of grass species that proved to be more tolerant to adult *P. bicincta* feeding hold the potential to replace highly susceptible Kikuyu pastures on Hawaii rangelands. Host plant resistance will be a vital control strategy in an integrated pest management program. Thus, the next step will be to see if the results observed in the greenhouse will translate to the field in a multitude of climate conditions, habitats, and elevations

Figure 9 shows the results of four 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±0.11, 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±0.94, 5.21±0.80, and 5.76±0.25 respectively. Therefore, the Kikuyu plants were tolerant to treatment with 5 adults, but highly susceptible to treatment with 10 or more adults. These results suggest Kikuyu plants can tolerate at least 5 adults per square foot, but the threshold for Kikuyu survivability to an attack was 10 or more adults per square foot. The results of this research have important implications and could be applied to the field to estimate an economic threshold for pastures and recommend at what density of infestation control should be implemented. Estimating economic damage is important for providing economic incentives for support of research on pest management strategies that could be applied to *P. bicincta* on Hawaii rangelands. This could help illustrate the major threats to the cattle industry in the state and how that will impact food and job security and the ecosystem services provided by pastures.