Research Article

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Implementing sanitation practices against the hibiscus bud weevil *Anthonomus testaceosquamosus* (Coleoptera: Curculionidae)

https://doi.org/10.1515/flaent-2024-0023
Received April 3, 2024; accepted August 3, 2024;
published online November 7, 2024

Abstract: The hibiscus bud weevil, *Anthonomus testaceosquamosus* Linell (Coleoptera: Curculionidae), is an invasive pest of tropical hibiscus, *Hibiscus rosa-sinensis* L. (Malvaceae). While chemical and biological control alternatives have been identified, the viability of cultural control through sanitation (collecting and destroying dropped buds where larvae complete development) remains to be explored. The impact of adult hibiscus bud weevil infestation on flower bud abortion was studied in a greenhouse. Plants were infested with one mating pair of adult weevils, with non-infested plants as controls. Actively growing buds and dropped buds were counted weekly for four weeks. A subsequent shade house experiment evaluated the effect of sanitation on plant productivity. Groups of ten plants in a tunnel tent were infested with ten hibiscus bud weevil adult mating pairs per tent. Treatments included sanitation (weekly collection of dropped buds for eight weeks) and a no sanitation control. Five actively growing buds per tent were collected in search of eggs and larvae, while counts of actively growing buds and open flowers were recorded per plant. To identify efficient sanitation methods, the time required to remove fallen buds using three methods (manual, vacuum, and blower) was estimated. Hibiscus bud weevil infestation, using two adult weevils per plant, led to a 16-fold increase in bud abortion compared to non-infested plants. Sanitation reduced the proportion of dropped buds by 22 %, while no sanitation plants showed more actively growing buds, suggesting overcompensation in response to abortion. The vacuum method was the most efficient bud removal method tested. Sanitation can be an effective, practical, and complementary measure to reduce hibiscus bud weevil infestation in commercial nurseries.

Keywords: pest management; cultural control; invasive pest; flower bud abortion

Resumen: El picudo del botón del hibisco *Anthonomus testaceosquamosus* Linell (Coleoptera: Curculionidae) es una plaga invasiva del hibisco, *Hibiscus rosa-sinensis* L. Aunque existen alternativas de control químico y biológico, la sanitización como control cultural (destrucción de botones caídos donde la plaga completa su desarrollo), no ha sido estudiada. El impacto de la infestación por la plaga en el aborto de botones se estudió utilizando plantas infestadas con una pareja de adultos y plantas no infestadas como testigo en invernadero. Se contaron botones en desarrollo y caídos semanalmente durante cuatro semanas. El efecto de la sanitización en la productividad de las plantas se estudió usando grupos de 10 plantas en una tienda túnel, infestadas con 10 parejas de la plaga por tienda en casa de polisombra. Los tratamientos fueron sanitización (recolección de botones caídos durante ocho semanas) y sin sanitización. Se recolectaron cinco botones en desarrollo por tienda en busca de huevos y larvas, y se contaron botones en desarrollo y flores por planta. La evaluación de la eficiencia de tres métodos de sanitización (manual, aspirado y soplado) se estimó midiendo el tiempo de recolección de botones caídos. La infestación de la plaga resultó en un aumento de 16 veces en el aborto de botones en comparación con plantas no infestadas. La sanitización redujo los botones caídos en un 22 %, mientras que las plantas sin sanitización mostraron

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más botones en desarrollo, sugiriendo una respuesta compensatoria. El método de aspirado fue el más eficiente en la recolección. La sanitización demostró potencial como práctica complementaria a otras alternativas de manejo en la reducción de la infestación de la plaga.

Palabras clave: manejo de plagas; control cultural; plagas invasivas; aborto botón floral

1 Introduction

In the United States, nearly 70 % of the total number (approximately 10 million) of tropical hibiscus plants (*Hibiscus rosa-sinensis* L.; Malvaceae), hereafter hibiscus, sold in 2019 were produced in Florida (USDA-NASS 2020). As the number one U.S. county for floriculture, greenhouse, and nursery commodities (market value), Miami-Dade contributes significantly to hibiscus production in the state (USDA-NASS 2017). Therefore, when the hibiscus bud weevil, *Anthonomus testaceosquamosus* Linell (Coleoptera: Curculionidae), originally from northeastern Mexico and southern Texas, was detected on hibiscus in Miami-Dade county in 2017 (Skelly and Osborne 2018), there was legitimate cause for concern. Large hibiscus bud weevil infestations can lead to aesthetically poor hibiscus plants due to the damage resulting from hibiscus bud weevil feeding and oviposition activity (Bográn et al. 2003). Specifically, hibiscus buds drop from plants after hibiscus bud weevil females oviposit inside. Larval and pupal development occurs within these aborted buds, with hibiscus bud weevil adults emerging after approximately 16 days (egg to adult) at 27 °C (Revynthi et al. 2022).

Given that average temperatures exceed 25 °C for half of the year in Miami-Dade county, hibiscus nurseries in south Florida are forced to battle multiple hibiscus bud weevil generations each year (NOAA-NESDIS 2022). The significance of the threat posed by the hibiscus bud weevil to the South Florida hibiscus industry was well understood by the Florida Department of Agriculture and Consumer Services-Division of Plant Industry (FDACS-DPI), and the hibiscus bud weevil was designated as a regulated pest shortly after its discovery in the state (Skelly and Osborne 2018). To prevent the spread of the hibiscus bud weevil and to reduce its population, a compliance agreement was issued by FDACS-DPI to hibiscus growers found with the pest. The compliance agreement allows hibiscus growers to ship their products if they have taken action to guarantee that there is no indication of weevil presence.

Currently, the only management tool recommended by the compliance agreement is contact insecticide application. However, as an overreliance on pesticides can lead to adverse environmental effects and resistance issues, a variety of management solutions are necessary to manage pests sustainably (Barzman et al. 2015). While the development of tactics such as biological control, host plant resistance, and mass trapping can be important tools in integrated pest management (IPM) programs, a considerable amount of research, time, and effort must be invested before these management options are accessible to growers. Management options that do not require specialized knowledge or application technologies may prove useful.

In nursery and greenhouse production systems, sanitation practices (e.g., removal of weeds and plant debris) play a significant role in the maintenance of a clean production environment (Cloyd and Herrick 2021) and in the reduction of pesticide applications and associated costs (Kleczewski and Egel 2011). If not removed, pests developing within discarded or loose plant debris have the potential to reinfest growing plants (Cloyd and Herrick 2021). Hogendorp and Cloyd (2006) demonstrated the importance of removing or otherwise isolating (e.g., stored in containers with tight-sealing lids) greenhouse debris, as thousands of whiteflies, *Bemisia* spp. (Hemiptera: Aleyrodidae) and hundreds of western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), were found to emerge from greenhouse-collected plant debris placed in refuse containers over a 6-month period.

Sanitation practices are environmentally friendly, cost-effective, and easy to implement, as these activities can be performed during routine production procedures (Hogendorp and Cloyd 2006). Furthermore, as a key component of a systems-based pest management approach (i.e., a practice that focuses on pest prevention at each operational phase of a nursery system), sanitation practices can mitigate the concerns associated with regulated pests in nursery settings by providing growers with reduced pest movement through the crop cycles, and thereby improving their ability to respond and recover when pests are detected (Cochran et al. 2014).

Because hibiscus bud weevil development is completed within aborted hibiscus buds on the nursery ground, sanitation (e.g., removal and destruction of fallen hibiscus buds) can disrupt the hibiscus bud weevil life cycle and potentially be an effective management tactic for this pest. Previous recommendations for hibiscus bud weevil management have suggested that sanitation could help reduce hibiscus bud weevil numbers alongside crop rotation and well-timed pesticide applications in nursery settings (Bográn et al. 2003), but the effect of removing aborted buds remains to be tested. Therefore, we initially chose to determine the effect of the hibiscus bud weevil on infested plants by examining the number of fallen buds compared to non-infested plants. We hypothesized that infested plants would have a greater number of aborted flower buds than non-infested plants. Subsequently, we studied the effect of sanitation on plant infestation and productivity as measured by the number of fallen and actively growing buds, respectively. We hypothesized that the removal of infested fallen flower buds from hibiscus plants growing in semi-field conditions would significantly decrease hibiscus bud weevil abundance and significantly increase plant productivity when compared with hibiscus bud weevil-infested plants in which fallen flower buds were not removed.

Hibiscus production in South Florida is extensive, with nurseries maintaining monocultures of hibiscus plants in areas ranging from 2–4 ha. Hence, implementing sanitation practices is not always feasible because it is labor- and time-intensive. Therefore, we also chose to test the time efficiency of three different sanitation methods (manual, vacuum, and blower) for the collection and removal of fallen hibiscus buds from nursery settings. Understanding the magnitude of the effect of sanitation on hibiscus bud weevil population dynamics in hibiscus is important as more management tools are required for the development of a sustainable IPM program for this pest.

2 Materials and methods

2.1 Hibiscus plants

Between 180 and 200 hibiscus plants ('Painted Lady' variety) were used to maintain the hibiscus bud weevil colony. These plants were initially acquired from two local nurseries in Homestead, Florida, and grown outdoors at the Tropical Research and Education Center of the University of Florida in Homestead, Florida, where all experiments were conducted. Every three months, approximately 35 g of slow-release fertilizer (8–2–12: N–P–K; Diamond R Fertilizer, Fort Pierce, Florida, USA) was added to the plants, with overhead irrigation provided twice daily for 20 min.

2.2 Hibiscus bud weevil colony

The colony was maintained as described in Revynthi et al. (2022). Fresh hibiscus buds were provided to adults as food and oviposition substrate, in a ratio of one flower bud per adult weevil twice per week. Buds and weevils were kept inside 24 mesh cages 30.5 × 30.5 × 30.5 cm (BioQuip® Products, Rancho Dominguez, California, USA). The cages were evenly distributed inside two Percival I-36 LL incubators at 27 ± 1 °C, 12:12 h L:D, and 70 % relative humidity (RH), where environmental conditions were followed using a ThermoPro Tp50 (ThermoPro, Toronto, Ontario, Canada). Between 25 and 30 plastic food storage containers (20 × 19 × 7 cm, l × w × h) were used to house immature hibiscus bud weevil stages within hibiscus buds under the same environmental conditions as adults. The colony was supplemented weekly with hibiscus bud weevil adults from a local nursery, with an average of 46 adults per week over a year period. The colony supplied all adult weevils (approximately 1 week old) used in experiments.

2.3 Effect of hibiscus bud weevil infestation on dropped and actively growing hibiscus buds in greenhouse settings

To investigate the impact of hibiscus bud weevil infestation on the abortion of flower buds, two trials were conducted under greenhouse conditions (27 ± 2 °C; 70 % ± 10 % RH), where environmental conditions were followed using a ThermoPro Tp50 (ThermoPro, Toronto, Ontario, Canada), and using a completely randomized design. There were two treatments that consisted of releasing a pair of adult weevils (one female, one male) per plant (infested) compared to plants with no weevils (control). Bushy hibiscus potted plants (40–45 cm tall) of the variety 'Painted Lady' were contained individually in mesh cages (99 × 66 × 59.7 cm, h × l × w, 100 µm mesh diameter, BioQuip Products Inc., Compton, California, USA). The substrate used in the pots consisted of a mixture of Florida peat (40 %) and wood fines (60 %). Three potted (11.4 L) plants, with a minimum of 20 small-to-medium-sized flower buds (1.5–2.5 cm), were used per treatment and observed for four consecutive weeks. Observations consisted of recording the number of actively growing buds and the number of dropped buds per plant, respectively. The experiment was replicated twice, the first replicate from 11 November to 9 December 2020, and the second from 14 January to 4 February 2021.

2.4 Effect of sanitation on hibiscus bud weevil infestation and hibiscus plant productivity

Because hibiscus bud weevil development occurs within aborted hibiscus buds on the ground, we chose to assess the effect of the removal of fallen buds on hibiscus quality and hibiscus bud weevil population growth from groups of ten bushy hibiscus plants in a shade house under 50 % filtered light. The environmental conditions in Homestead during the time of the experiment (22 June to 8 August 2022) were obtained from the Florida Automated Weather Network, https://fawn.ifas.ufl.edu (27.7 ± 3.4 °C; 82.3 % ± 12 % RH). The plants' height and the type of substrate were as described previously. The experimental setup began by placing ten potted (11.4 L) 'Painted Lady' variety hibiscus plants approximately 0.3 m apart in the middle of a 4.3 × 2.4 × 1.6 m (l × w × h) tunnel tent (Quest Dixon 8 Person Tunnel Tent, Quest Outdoors, Louisville, Kentucky) (eight tents in total) in a section of a 60 × 40 m (l × w) shade house. Tents were considered replicates and allocated to one of the two treatments (sanitation and no sanitation) using a lottery method, with four replicates each, following a completely randomized design.

Prior to the start of the experiment, flowers and mature buds (>2.5 cm in length) were removed by hand from each hibiscus plant to establish a similar number of flower buds among plants. In each tent, one hibiscus bud weevil male and one female were manually released on each plant, with 20 adults per tent (ten males and ten females). Tents were arranged with east- and west-facing openings. Each hibiscus plant had a clear plastic plant saucer (30 cm diameter) placed underneath to retain water, and plants were watered three times per week. Irrigation was provided until each plastic plant saucer was full. Opaque water-resistant fabric was used to cover each tent from dusk to dawn and during rainfall events. While tents were covered, air exchange occurred via "No-See-Um" mesh windows in tent doors and along the width of each tent.

Once per week, five actively growing buds were collected from different plants (or fewer buds if less than five were available) within each tent; the plants from which actively growing buds were collected were alternated within a tent as often as possible. Under a stereoscope (Leica S9E, Wetzlar, Germany), actively growing bud dissections were performed to count the number of hibiscus bud weevil eggs and larvae within each bud. Dissections on all actively growing buds were conducted within 7 days of bud collection. Buds were stored at room temperature (23 °C; 60 % RH) prior to dissection. In addition to actively growing bud collections, weekly sampling of each tent included counting the number of actively growing buds per plant, open flowers per plant, and dropped buds per tent. All sampling data for a given week were collected during the same day prior to 12 PM. Eight weekly samples were collected throughout the duration of the experiment. In the sanitation tents, dropped buds were collected, placed in mesh sleeves (33 × 27 cm, l × w), and allowed to remain within tents while the immature hibiscus bud weevil stages within the dropped buds developed through adulthood. Mesh sleeves containing the dropped buds and emerging adult weevils from the sanitation treatment were kept inside the respective tents.

2.5 Time efficiency of sanitation collection methods

To identify methods that will optimize the implementation of sanitation under commercial settings, we tested the efficiency of three different sanitation methods (manual, vacuum, and blower). Observations were conducted within a rectangular enclosure of 5 × 2.6 × 1.2 m (l × w × h). Solid plastic panels were used to create each side and end boundary of the enclosure. Fifty 11.4-L pots (Home Depot, Homestead, Florida, USA) without plants but filled with substrate, as described earlier, were placed within the enclosure on the landscape fabric-covered shade house floor in five lines of 10 pots each (0.26 pots per ≈ 1 m^2) to simulate the plant spacing used in ornamental nurseries. Three stages of hibiscus bud decay (i.e., different times after collection from the plants) were established by placing freshly picked hibiscus buds (2–3 cm in length) in ventilated plastic boxes (20 × 19 × 7 cm, l × w × h) and allowing them to decay in Percival I-36 LL incubators (27 ± 1 °C, 12:12 h L:D, 60 % RH) for 2, 4, and 6–7 days. Each treatment replicate began by randomly spreading 75 decayed hibiscus buds (25 from each decay stage) throughout the enclosure by hand, and all buds that landed within pots were placed on the ground, simulating conditions in ornamental nurseries.

After buds were distributed, one of three different sanitation procedures was initiated: 1) manual collection: all buds were picked up by hand and placed in a plastic container (20 × 19 × 7 cm, l × w × h); 2) blower collection: starting from one end boundary, all buds from were blown with a Makita 36 V LXT® Brushless Blower (Makita, La Mirada, California, USA) to another end boundary and then placed by hand into a plastic container; or 3) vacuum collection: all buds were vacuumed via the vacuum attachment on the Makita Blower. All collection methods were carried out by moving in a zigzag manner from left to right (across the width of the enclosure), starting from one end boundary and moving across the length of the enclosure until the other end boundary was reached. The time required to collect all buds within a container or the vacuum was measured ("collection time" in seconds). Each treatment was replicated ten times. For blower collection replicates, the end boundary that the buds were blown towards featured an aluminum window screen instead of the solid plastic panel to allow the air (but not the buds) to pass through the boundary. This allowed all hibiscus buds within the enclosure to be picked up by hand after the blower aggregated them along the boundary screen. A total of five different individuals ("collectors") performed the bud collection and removal across all replicates. The treatments were replicated from 4–30 May 2023 using a completely randomized design.

2.6 Data analysis

In our experiments testing 1) the effects of hibiscus bud weevil infestation on bud flower abortion, 2) the effects of sanitation on hibiscus bud weevil infestation and plant productivity, and 3) the efficiency of sanitation methods, we used different statistical models to analyze the data. The selection of models was based on the data's characteristics, particularly the response variables' variance: mean ratio (Crawley 2013). The generalized linear mixed-effects model (GLMM) and linear mixed-effects model (LMM) were used to account for both fixed and random effects. Poisson distributions were used for response variables with a variance: mean ratio less than 3 unless convergence issues indicated the need to use negative binomial distributions. A negative binomial distribution of data was chosen for response variables with a variance: mean ratio greater than 3 to better handle overdispersion, which is very common in our case due to many zeroes. Model fit was evaluated using likelihood ratio tests, and in cases where models failed to converge, simpler models or the exclusion of certain data points were considered. The analyses included both fixed effects (i.e., treatment and week) and random effects (e.g., block, plant, tent, collector) to account for the data structure. Tukey-adjusted pairwise comparisons were performed to test differences among treatments and sampling weeks within each regression model. All analyses were conducted in R (v4.2.0) (R Core Team 2022). Regression analyses were completed using the *glmmTMB* (v1.1.3) package (Brooks et al. 2017), while post-hoc analyses used the *emmeans* (v1.7.3) (Lenth 2022) and *multcomp* (v1.4.18) (Hothorn et al. 2008) packages.

2.6.1 Effect of hibiscus bud weevil infestation in dropped and actively growing hibiscus buds in greenhouse settings

The number actively growing buds per plant was analyzed using a GLMM that included the categorial variables treatment and week, as well as their interaction as fixed effects; replicate (plant) nested within week, and the repetition of the experiments (blocks) were considered as random effects. Because the variance: mean ratio of the number of eggs per actively growing bud was <3 (2.68), a Poisson model was used to assess the effects of treatment and sampling week on the number of actively growing buds per plant. The same approach was taken for the number of dropped buds per plant, but as the inclusion of the interaction term prevented model convergence, the categorical fixed effects of treatment and weeks were individually included in the model. Because the variance: mean ratio of the number of dropped buds was >3 (6.29), a negative binomial model was used to assess the effects of treatment and sampling week on the number of dropped buds per plant.

2.6.2 Effect of sanitation on hibiscus bud weevil infestation and hibiscus plant productivity

The number of collected buds (actively growing per plant or dropped), eggs and larvae observed within actively growing bud dissections, and observed flowers per plant were separately analyzed via GLMM with a negative binomial error structure if response variables had a variance: mean ratio of >3 (actively growing buds per plant and dropped buds). For response variables in which the variance: mean ratio was <3 (flowers per plant, eggs observed within actively growing bud dissections), both Poisson and negative binomial models were constructed, and the difference in model fit was assessed via likelihood ratio test. Although the number of larvae observed within actively growing bud dissections had a mean: variance ratio of 1.6, a Poisson model was selected as the final model due to the convergence failure of the negative binomial model. For each analysis, model convergence was achieved by including only those sampling weeks in which at least one non-zero number was recorded among all observations. For this reason, models for the number of flowers per plant and the number of eggs and larvae observed within actively growing bud dissections did not include data from sampling week 2, sampling weeks 3, 6, 7, and 8, and sampling week 7, respectively.

As no flowers were observed during sampling week 2, data from that week were removed prior to modeling to allow for model convergence. Because the variance: mean ratio of the number of flowers per plant was <3 (2.0), both Poisson and negative binomial models were created to assess the effects of treatment and sampling week on the number of flowers per plant. As the negative binomial model provided a significantly better fit (likelihood ratio test: $X^2 = 18.1$, df = 1, $p < 0.001$) than the Poisson model, the former was chosen as the final model.

Each model included treatment (sanitation or no sanitation) and sampling week (1–8) as categorical fixed effects. An intercept that varied among replicates (tents) and among each hibiscus plant (nested within tents) was used for the random effect component for all models except for the number of eggs observed within actively growing bud dissections. The random effect component for this analysis included only a random intercept that varied among replicates to allow for model convergence. For each regression model, ANOVA was used to determine whether the mean number of collected buds (actively growing buds per plant or dropped buds) and flowers per plant and the mean number of eggs and larvae per actively growing bud differed between treatments for at least one sampling week. Tukey-adjusted pairwise comparisons among treatments and sampling weeks were conducted for each regression model.

As no eggs were observed within actively growing bud dissections during sampling weeks 3, 6, 7, and 8, data from these sampling weeks were removed to allow for model convergence. The random effect was reduced from what was used in all other analyses (a random intercept that varied among replicates and among each plant within replicates) to a random intercept that varied among replicates to allow for model convergence. Because the variance: mean ratio of the number of eggs per actively growing bud was <3 (1.3), both Poisson and negative binomial models were created to assess the effects of treatment and sampling week on the number of eggs per actively growing bud. As the negative binomial model failed to provide a significantly better fit (likelihood ratio test: X^2 = 0.83, df = 1, p = 0.360) than the Poisson model, the latter was chosen as the final model.

As no larvae were observed among actively growing buds during sampling week 7, data from that week were removed to allow for model convergence. Because the negative binomial model failed to converge, a Poisson model was used instead. As nearly all adult weevils from the sanitation replicates were collected in the first week of sampling, the analysis of the proportion of dropped buds per tent was done considering the first four weeks of sampling. The proportion of dropped buds was analyzed using a GLMM that included the categorical variables treatment and week, as well as their interaction, as fixed effects; replicate (tent) nested within week was designated as a random effect. The proportion of dropped buds was analyzed using a binomial distribution.

2.6.3 Efficiency of time in sanitation collection methods

Data were modeled using a LMM containing collection time as the response variable, collection method as the categorical explanatory variable, and collector as the random factor.

3 Results

3.1 Effect of hibiscus bud weevil infestation on dropped and actively growing hibiscus buds in greenhouse settings

The number of actively growing buds per plant was influenced by the interaction between treatment and the week of observation (χ^2 = 8.22; df = 3; P = 0.041) (Figure 1A). A higher number of actively growing buds per plant was observed in the second and third weeks in the control and the second week in the infested plants. However, the number of actively growing buds was not significantly affected by treatment alone (χ^2 = 0.27; df = 1; P = 0.598) but varied with observation time (χ^2 = 12.38; df = 3; P = 0.006). Infested plants exhibited 15 to 17 times more dropped buds per plant than control plants that naturally aborted a few

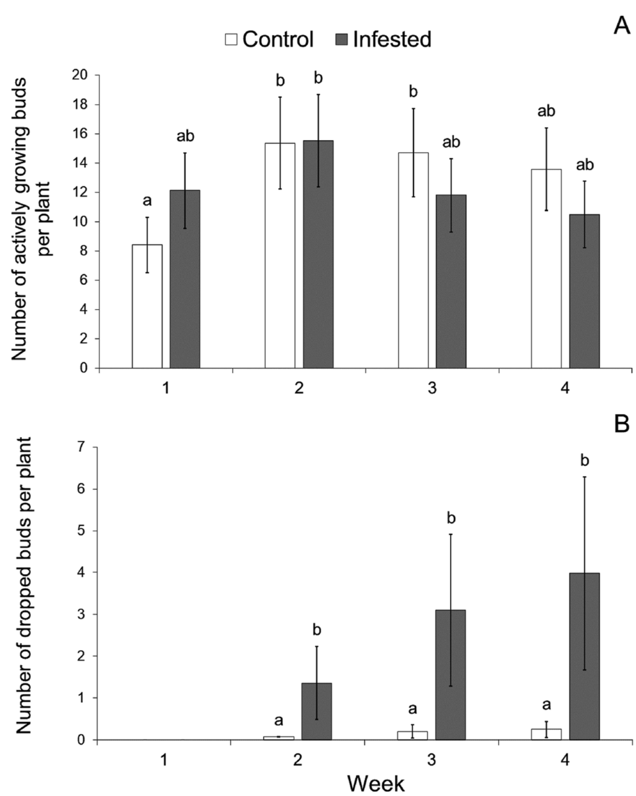

Figure 1: Actively growing buds (A) and dropped buds (B) per hibiscus plant (estimated marginal means ± SEM; generalized linear mixed-effects model), across 4 weeks of growth after the infestation of two or zero hibiscus bud weevil adults per plant (infested and control, respectively) in greenhouse trials (n = 3, experiment replicated twice). In the number of actively growing buds, significant differences across treatments and weeks are illustrated with lowercase letters, while lowercase letters separate means among treatments across weeks in the number of dropped buds (Tukey adjustment, α = 0.05).

flower buds (χ^2 = 19.49; df = 1; P < 0.001). However, no effects were observed due to the sampling week (χ^2 = 2.96; df = 3; P = 0.396) (Figure 1B).

3.2 Effect of sanitation on hibiscus bud weevil infestation and hibiscus plant productivity

Across weeks, significantly more actively growing buds per plant were observed in the no sanitation treatment in comparison to the sanitation treatment (χ^2 = 20.55; df = 1; P < 0.001). Additionally, more actively growing buds were observed during the first week of sampling than in any other sampling week (χ^2 = 374.78; df = 7; P < 0.001) (Figure 2A). Furthermore, significantly more flowers per plant were observed in the no sanitation treatment in comparison to the sanitation treatment across weeks (χ^2 = 9.10; df = 1; P = 0.002). More open flowers per plant were observed during the first week of sampling than in any other sampling week (χ^2 = 41.98; df = 6; P < 0.001) (Figure 2B). The no sanitation treatment had significantly more dropped buds per plant in comparison to the sanitation treatment across sampling weeks (χ^2 = 11.15; df = 1; P < 0.001); while more dropped buds were observed during the first week of sampling than in any other sampling week (χ^2 = 326.11; df = 7; P < 0.001) (Figure 2C).

The number of eggs per actively growing bud did not vary significantly either with the treatments (χ^2 = 1.52; df = 1; P = 0.217) or with the sampling weeks (χ^2 = 1.97; df = 3; P = 0.578). However, in each sampling week in which eggs were observed in actively growing buds (sampling weeks 1, 2, 4, and 5), more eggs were observed in replicates with no sanitation than in those in which sanitation procedures were performed (Figure 3A). Although there were no significant differences between sanitation and no sanitation treatments on the number of larvae per actively growing bud (χ^2 = 0.86; df = 1; P = 0.350), in each sampling week in which larvae were observed in actively growing buds (all sampling weeks except sampling week 7), more larvae were observed in replicates with no sanitation than in those in which sanitation procedures were performed. Across treatments, significantly more larvae per actively growing bud were observed during the first week of sampling than in any other sampling week (χ^2 = 80.62; df = 6; P < 0.001) (Figure 3B).

Seventy percent of all female (20/29) and 90 % of all male (28/31) weevils emerging from dropped buds collected from sanitation replicates were from the first week of sampling. At least one weevil was collected in each sampling event across all sanitation replicates except for sampling weeks 5 and 8. During the first 4 weeks, a significantly higher proportion of dropped buds was observed in the no sanitation treatment compared to those tents receiving sanitation (χ^2 = 26.39; df = 1; P < 0.001), with an estimated marginal mean 22 % larger (0.41 ± 0.05 SEM dropped buds per plant) than that observed when dropped buds were weekly collected (0.32 ± 0.05 SEM dropped buds per plant). No effects were observed due to sampling week (χ^2 = 3.08; df = 3; P = 0.379), and no interaction effects between treatment and sampling week were detected (χ^2 = 5.65; df = 3; P = 0.129).

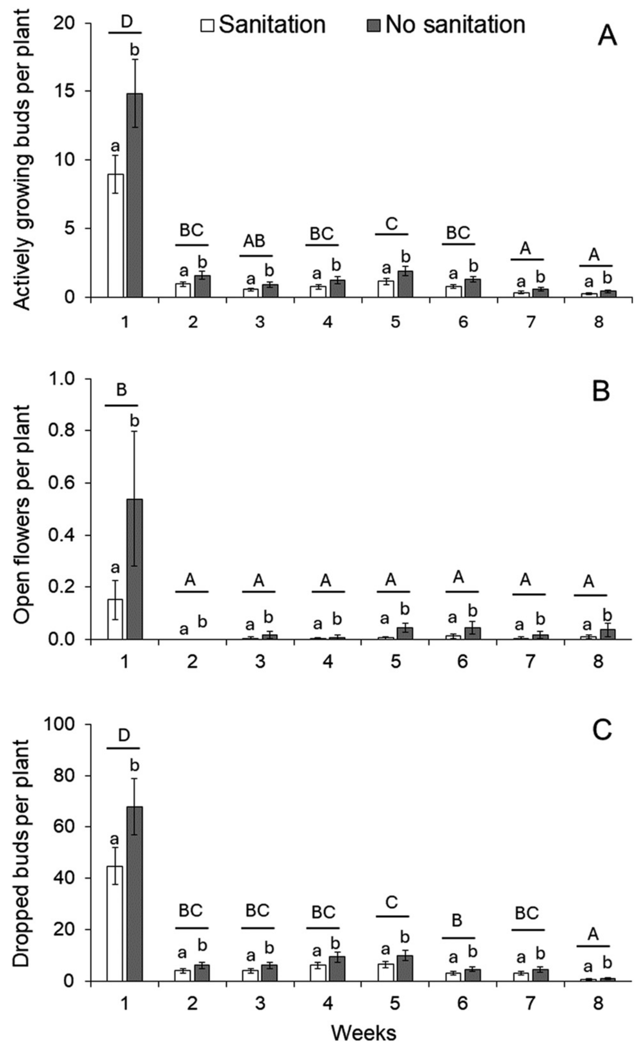

Figure 2: Actively growing buds (A), open flowers (B), and dropped buds (C) per hibiscus plant (estimated marginal means ± SEM; generalized linear mixed-effects model), across 8 weeks of growth after the infestation of 20 hibiscus bud weevil adults (ten male, ten female) in tunnel tents containing ten plants. Sanitation tents were subject to weekly sanitation via collecting all fallen buds, while sanitation was not performed in no sanitation tents (n = 4). Within weeks, statistically different treatments are separated with lowercase letters, while statistical differences across weeks are separated with uppercase letters (Tukey adjustment, α = 0.05).

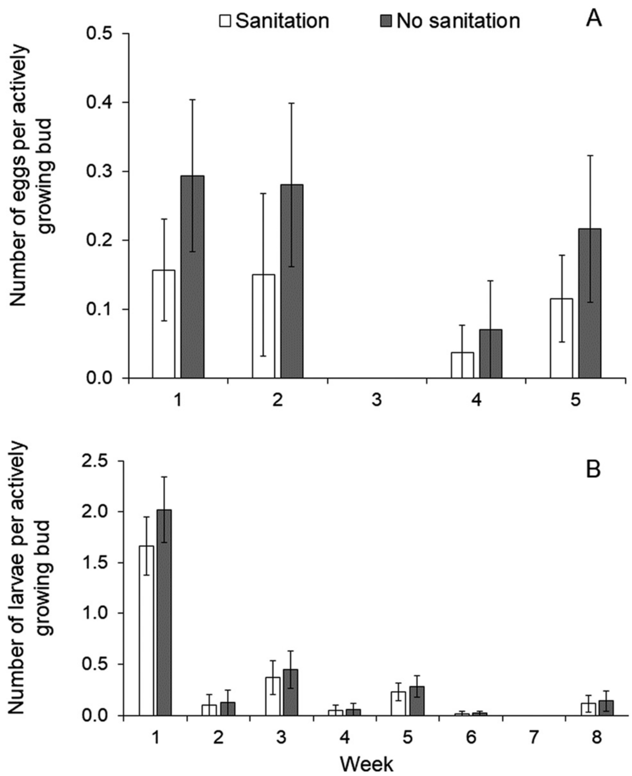

Figure 3: Number of eggs (A) and larvae (B) per actively growing bud on hibiscus (estimated marginal means ± SEM; generalized linear mixed-effects model), recorded over 8 weeks following the infestation of 20 hibiscus bud weevil adults (ten males, ten females) in tunnel tents containing ten plants. In sanitation tents, weekly sanitation was conducted by collecting all fallen buds, while no sanitation was performed in the no-sanitation tents (n = 4).

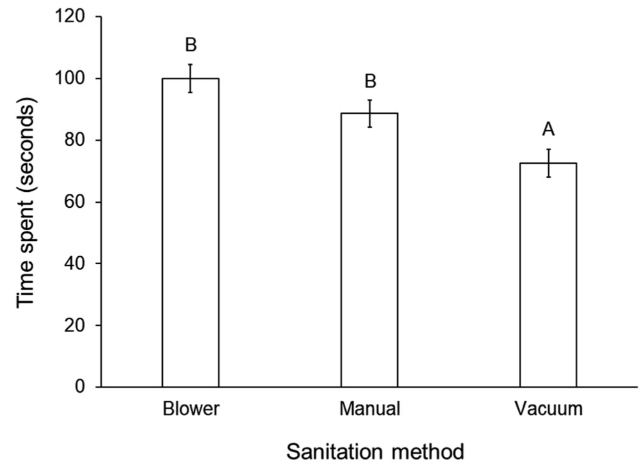

Figure 4: Time spent (s) to collect a total of 75 hibiscus flower buds using three different collection methods (blower, manual, and vacuum) (estimated marginal means ± SEM; linear mixed-effects model). Experiments were conducted within an approximately 13 m^2 area containing 50 11.4-L plant pots featuring the plant spacing (0.26 pots per ≈ 1 m^2) used in commercial nursery settings. Statistically different treatments are separated with uppercase letters (Tukey adjustment, α = 0.05).

3.3 Efficiency of sanitation collection methods

The vacuum collection method was significantly more efficient than either the manual collection method (approximately 20 % more time was required) or blower collection method (approximately 30 % more time was required) at gathering 75 decayed hibiscus buds from the enclosure (χ^2 = 30.19; df = 2; $P < 0.001$), with no differences between the latter two (Figure 4).

4 Discussion

Previous reports presented anecdotical information on the effects of hibiscus bud weevil infestation on plant productivity (Bográn et al. 2003; Revynthi et al. 2021). Per our observations, after females lay their eggs in the flower buds, they subsequently move to the petiole. There, they pierce the tissue several times, weakening it and potentially making it prone to falling. We propose that this behavior increases female-inclusive fitness via reduced competition, as the induction of bud drop ensures that no other female will lay eggs within the dropped bud. Oviposition exclusion behaviors have previously been reported for the *Anthonomus* genus, as pepper weevil *Anthonomus eugenii* Cano (Coleoptera: Curculionidae) females plug oviposition sites using frass in combination with a pheromone to repel additional oviposition from conspecific females (Addesso et al. 2007). In our study, we revealed the effects of hibiscus bud weevil adult infestation on tropical hibiscus flower bud abortion. Notably, plants infested with hibiscus bud weevil exhibited a significant surge in dropped buds, approximately 16 times greater than in the control group, underscoring the need to address this regulated pest. Consequently, it also emphasizes the need for sustained efforts in implementing a comprehensive IPM program to effectively manage the long-term impact of infestations.

Cultural control, defined as the biological or physical alteration of the cultivated field environment during its establishment or growth (Chang et al. 2017), usually presents a cost-effective option that aligns with sustainable alternatives. Such actions potentially serve as a substitute for, or a complement to, chemical insecticides, which are the predominant control method applied in ornamental production. Additionally, these cultural control practices can potentially prevent the development of conditions that reinforce the building of population cycles (All 1999; Norris et al. 2003).

Contrary to our hypothesis, plants in the no sanitation group showed a higher number of actively growing buds and flowers per plant than those in the sanitation treatment in shade house experiments. This phenomenon was similarly observed in the first week of the greenhouse experiment that tested the effects of infestation; however, in the latter scenario, the increase in actively growing buds was not statistically significant. This unexpected increase in plant reproductive organ growth in response to the lack of sanitation, and possibly associated with an increase in pest infestation, may represent the first reported case of overcompensation – a plant's enhanced growth following damage – in tropical hibiscus. The concept of overcompensation was first identified by Paige and Whitman (1987), and later studies (Paige 2018) have provided further evidence of its adaptive value in plants growing under different environmental stressors. Overcompensation may manifest not just in tissue regeneration, but also in the plant's production of chemical defenses (van der Meijden et al. 1988; Miles-Mesa et al. 2017). Although our research did not initially aim to investigate this physiological trait in 'Painted Lady' tropical hibiscus, our findings suggest that these plants may exhibit a form of host plant tolerance to hibiscus bud weevil infestation. This trait could be advantageous in commercial production settings, as it could constitute a first line of defense (e.g., plant tolerance) in the face of hibiscus bud weevil infestations. However, it is also important to consider that within the range of induced plant defenses, there is also a spectrum of plant manipulation by insects and mites (e.g., gall formation, manipulation of host plant development, and nutritional quality) (Favery et al. 2020). These processes involve the manipulation of plant physiology and development for the benefit of the herbivores. Therefore, at this stage, we cannot rule out the possibility that hibiscus bud weevil females could be inducing higher flower bud production to increase their own fitness. Furthermore, our observations warrant additional research into the specific types of plant responses employed by this hibiscus cultivar in the face of this pest.

In the shade house experiment, a higher number of dropped buds per plant were found in the no sanitation treatment (Figure 2C). This phenomenon is evidently linked to feeding and oviposition activity. Additionally, it was associated with a greater number of actively growing buds observed in the same treatment. Therefore, estimating the proportion of dropped buds provides a way to account for this linkage and helps in contrasting the level of damage between treatments. A higher proportion of dropped buds in the no sanitation treatment aligns with the tendency of higher reproductive activity of the hibiscus bud weevil adults on the plants. This tendency is reflected in a higher number of eggs and larvae per actively growing bud, although this trend was not statistically significant (Figure 3). It is worth noting that during the initial two weeks of the shade house experiment, an infestation of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) was detected across treatment replicates. Even though this infestation was promptly controlled after releases of the natural enemies *Proprioseiopsis ovatus* (Garman) and *Neoseiolus longispinosus* Evans (Acari: Phytoseiidae), it could have resulted in the sudden reduction in plant productivity (i.e., flower buds and flowers) after the first week.

We also demonstrated that the vacuum was the most efficient of all tested collection methods in terms of the time spent on the operation. Additionally, it is important to note that using this system offers additional benefits. Specifically, the vacuum used in these trials shreds the buds it collects, which facilitates the disposal of these final crop residues as surviving larvae and pupae are eliminated in the process. The latter provides a more cost-effective scenario in virtue of the increasing costs of labor in the industry. The identification of a fast and efficient sanitation tool will make the implementation of sanitation easier and more acceptable to the ornamental industry.

In general, sanitation is well accepted in ornamental production and considered a cornerstone strategy for IPM for significant fungal diseases. Removing potential inoculum through sanitation is a key element in limiting the spread of these problems (Benson 2007; Bika et al. 2021). The accumulation of crop residues in the field also creates favorable conditions for insect pests. The benefits of sanitation have been extensively discussed not only in greenhouse environments (Cloyd and Herrick 2022), but also in pest management in open field scenarios. For instance, the larvae of the false wireworms *Pterohelaeus* spp. (Coleoptera: Tenebrionidae) that feed on plant residues can also target seedlings of various crop plants such as maize, barley, sunflower, soybean, and tomato. Therefore, proper management of crop residues will decrease the chance of infestation on germinating seedlings (Wilson-Rummenie and Radford 2007). In orchards, the management of fallen nuts plays an important role in controlling the navel orangeworm *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae). Removing the overwintering larvae has proven effective in reducing infestations in the subsequent season (Wilson et al. 2020).

In southern Florida, hibiscus bud weevil populations are usually low between August and January, coinciding with a low availability of hibiscus plants in the nurseries (Revynthi et al. 2021). Pest populations start growing from February until July, with a population peak between March and June during the shipment period. If preventive measures such as sanitation are exercised beginning in February targeting initial pest population growth, other pest management alternatives (e.g., chemical, biocontrol, repellents) may be more efficient at sustaining low pest population numbers during spring. Considering the hibiscus bud weevil life cycle, which at 27 °C can be completed within 16 days (Revynthi et al. 2022), sanitation can be an IPM strategy that reduces hibiscus bud weevil populations in a nursery if implemented twice a month.

While sanitation is an environmentally friendly and effective method, it should not be expected to regulate hibiscus bud weevil on its own. This is because some dropped buds might be unreachable by collection methods, including manual collection. Therefore, complementary alternatives ought to be considered. The application of entomopathogenic nematodes like *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae), which can target fallen buds hidden under the substrate, or those missed at ground level, would be advantageous (Vargas et al. 2024). Currently, additional options for managing hibiscus bud weevil have been proposed, including the use of systemic and contact insecticides (Vargas et al. 2023; Greene et al. 2023). Moreover, the potential application of entomopathogenic fungi and bacteria warrants further study. To effectively integrate a broader range of options into a hibiscus bud weevil IPM program, additional research is needed to enhance monitoring techniques and test the effectiveness of traps baited with lures (Ataide et al. 2024). To foster the implementation of preventive practices that ideally focus on sustainable methods, targeting the initial signs of pest colonization in early spring is vital if crop losses are to be prevented during the shipping season in early summer.

Acknowledgments: We gratefully acknowledge Juleysy Rodriguez, Maria Alejandra Canon, Isamar Reyes, and Owen McManes for technical support. Our appreciation also goes to the Florida Nursery and Landscape Association, the Miami-Dade County Agricultural Manager's Office, Railroad Nursery, and Nature's Way Farms.
Research ethics: Not applicable.
Informed consent: Not applicable.
Author contributions: Conceptualization: A.D.G., G.V. and A.M.R.; methodology: A.D.G., G.V., Y.V.H., L.L., and A.M.R.; writing-original draft preparation: A.D.G. and G.V.; writing-review and editing: A.D.G., G.V., Y.V.H., L.L. and A.M.R.; visualization and data analysis: A.D.G. and G.V.; supervision and funding acquisition: A.M.R. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Use of Large Language Models, AI and Machine Learning Tools: None declared.
Conflict of interests: The authors state no conflict of interest.
Research funding: This research was funded by the National Horticulture Foundation (Award ID: AGR DTD 01-17-2020) and the United States Department of Agriculture/Florida Department of Agriculture and Consumer Services, Specialty Crop Block Grant Program 21SCBPFL1022 (Contract #28486). Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the United States Department of Agriculture.
Data availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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