Research Article

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Phenology of *Nipaecoccus viridis* (Hemiptera: Pseudococcidae) in Florida citrus groves

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Abstract: Hibiscus mealybug, *Nipaecoccus viridis* (Newstead) (Hemiptera: Pseudococcidae), is an invasive pest of citrus in Florida. It causes deformation and premature abortion of developing fruit. To date, hibiscus mealybug has been reported causing damage in commercial citrus groves in central and southern regions of Florida. As a recent invader, there is no information available on the phenology of this invasive pest on citrus in Florida. We sampled hibiscus mealybug population densities in six commercial citrus groves in central Florida throughout 2021 using absolute and relative sampling techniques. Results from absolute sampling showed that hibiscus mealybug completes multiple generations per year, with three of them being clearly defined. The first (March) and second (June) generations pose the greatest threat to citrus production in Florida. Results from relative sampling showed that a corrugated cardboard band trap is an effective tool for detecting and quantifying the population density of the pest in citrus in Florida. Our results also showed that the seasonal abundance of hibiscus mealybug was not influenced by new vegetative growth of citrus trees, nor by environmental factors measured at Florida Automated Weather Network stations close to the sampling sites. This description of the seasonal phenology of hibiscus mealybug will help improve the timing and effectiveness of management efforts for controlling this invasive pest in citrus groves in Florida.

Keywords: invasive; lebbeck mealybug; commercial citrus; sampling; seasonal biology; timing for management action

Resumen: La cochinilla del hibisco, *Nipaecoccus viridis* (Newstead) (Hemiptera: Pseudococcidae), es una plaga invasora de los cítricos en Florida. Provoca deformación y aborto prematuro del fruto en desarrollo. Hasta la fecha, se ha informado que la cochinilla del hibisco ha causado daños en plantaciones comerciales de cítricos en las regiones central y sur de Florida. Debido a que es un invasor reciente, no existe información sobre la fenología de esta plaga invasora en los cítricos en Florida. En 2021, tomamos muestras de las densidades de población de cochinilla del hibisco en seis plantaciones comerciales de cítricos en el centro de Florida utilizando técnicas de muestreo absoluto y relativo. Los resultados del muestreo absoluto demuestran que la cochinilla del hibisco completa varias generaciones por año, tres de las cuales están claramente definidas. La primera (marzo) y segunda (junio) generación presentan la amenaza más relevante para la producción de cítricos en Florida. Los resultados del muestreo relativo indicaron que una trampa con banda de cartón corrugado es una herramienta efectiva para detectar y cuantificar la densidad poblacional de la plaga en cítricos en Florida. Nuestros resultados también mostraron que la abundancia estacional de la cochinilla del hibisco no fue influenciada por el nuevo crecimiento vegetativo de los árboles de cítricos, ni por factores ambientales medidos en las estaciones de la Red Meteorológica Automatizada de Florida (FAWN) cercanas a los sitios de muestreo. Esta descripción de la fenología estacional de la cochinilla del hibisco ayudará a mejorar el momento y la eficacia de los esfuerzos de manejo para controlar esta plaga invasora en los huertos de cítricos de Florida.

Palabras clave: plaga invasora; cochinilla de lebbeck; cítricos comerciales; muestreo; biología estacional; calendario para la acción de gestión

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1 Introduction

The hibiscus mealybug, also known as the lebbeck mealybug, *Nipaecoccus viridis* (Newstead) (Hemiptera: Pseudococcidae), is a pest of concern in citrus growing regions worldwide (García-Morales et al. 2016; Sharaf and Meyerdirk 1987).

Hibiscus mealybug is minute and usually exhibits cryptic feeding behaviour, which makes its detection challenging during phytosanitary and quarantine inspections. Consequently, hibiscus mealybug can easily be introduced into new geographical areas through international trade of plant materials and agricultural products (Hulme et al. 2008; Pieterse et al. 2010). Hibiscus mealybug is native to Asia (Evans and Dooley 2013), but due to its high invasive success, populations have been documented attacking numerous plant species in 67 countries in Asia, Africa, Australia, South America, the Caribbean, the Pacific Islands, and North America (CABI 2021; García-Morales et al. 2016; Thomas and Leppla 2008). Hibiscus mealybug has previously been noted as a potential invasive pest in Florida (Evans and Dooley 2013). Due to the ease of spread from one field to another on workers' clothing and farm equipment, and the favorable climatic conditions for establishment of tropical and sub-tropical invasive species in Florida (Diepenbrock and Ahmed 2020; Middleton and Diepenbrock 2022a; Thomas and Leppla 2008; USDA-HZ 2020), the pest has become widespread in commercial groves in southern and central Florida (Olabiyi et al. 2023a).

The hibiscus mealybug is highly polyphagous and its potential host range in Florida includes several economically important food crops and ornamental plants (Deeter and Ahmed 2023; Diepenbrock and Ahmed 2020; García-Morales et al. 2016; Olabiyi et al. 2023a). Hibiscus mealybugs are phloem feeders and attack all above ground parts of citrus trees. They feed on sap of citrus trees using their piercing-sucking mouthparts, resulting in reduced vigor and stunted growth of citrus trees (Diepenbrock and Ahmed 2020). Dieback occurs from feeding activity of mealybugs at terminal regions of infested citrus branches (Meyerdirk et al. 1988). High population densities of hibiscus mealybug can cause defoliation, abortion of developing fruit (Hattingh et al. 1998), and the death of young citrus trees (Diepenbrock and Ahmed 2020). The economic impact of hibiscus mealybug on Florida citrus production is yet to be estimated. Nevertheless, up to 50 % fruit drop has been reported in some citrus groves in southern Africa (Hattingh et al. 1998).

Mealybug infestation is primarily managed with the application of insecticides in citrus groves (Franco et al. 2004). However, hibiscus mealybugs exhibit characteristics that limit the effectiveness of foliar applied insecticides. Adults and late instar females have thick protective wax covering on their bodies that reduces direct contact with droplets of insecticide sprays. Mature females lay their eggs in tightly woven and protective waxy ovisacs that reduce penetration of insecticide spray. Likewise, pupal males are protected from penetration of insecticide spray droplets by their filamentous cocoon. Early immature instars (both sexes) and adult males, on the other hand, lack this thick waxy covering, and are consequently highly susceptible to insecticide application (Middleton and Diepenbrock 2022b). Hibiscus mealybugs are usually found feeding in protected locations like cracks and crevices in plant stems, leaf axils, and beneath the fruit calyx, where they are less likely to encounter droplets from insecticide spray (Diepenbrock and Ahmed 2020). Therefore, the seasonal population biology of this mealybug must be described to determine optimal timing for management actions against the early instars in citrus groves.

Monitoring protocols have been developed to detect several mealybug species using both direct and indirect sampling techniques (Beltrà et al. 2013; Millar et al. 2002; Walton et al. 2004). Direct (absolute) sampling involves visual examination of specific plant parts to count all living mealybugs. Like most mealybug species, hibiscus mealybug has clumped spatial distribution (Nestel et al. 1995) and are usually hidden in cryptic locations on citrus trees (Diepenbrock and Ahmed 2020; Olabiyi et al. 2023a). Therefore, direct sampling can be time-consuming and labor intensive (Geiger and Daane 2001; Walton et al. 2004). Moreover, there is a possibility of under-sampling if the mealybug infestation is not found in the sample unit. Indirect (relative) sampling techniques are usually less labor intensive and involve the use of various trap designs such as corrugated cardboard bands that are wrapped around trunks and branches of trees to monitor mealybug population densities (Beltrà et al. 2013; Goolsby et al. 2002; Martínez-Blay et al. 2018), and pheromone-baited sticky traps to monitor, and potentially remove, alate adult male mealybugs (Hall et al. 2008; Sun et al. 2002; Walton et al. 2004). Sticky traps can be baited with either synthetic sex pheromones, when available, or live virgin female mealybugs (Meyerdirk et al. 2001; Serrano et al. 2001). Although the sex pheromone of female hibiscus mealybug has been identified (Levi-Zada et al. 2019), we used live virgin female mealybugs in this study because effective synthetic lures are not yet available.

In the Mediterranean basin, hibiscus mealybug develops and reproduces continuously throughout the year, through multiple and overlapping generations, with decelerating developmental time in winter (Peri and Kapranas 2012). Due to the ideal year-round climatic conditions that favours the establishment of hibiscus mealybug in Florida (USDA-HZ 2020), and the multivoltine nature of this pest (Sharaf and Meyerdirk 1987), we expect multiple population peaks (generations) throughout the year. Documenting the seasonal trends of hibiscus mealybug populations in Florida citrus groves should facilitate the design of successful management programs to control the pest. For example, understanding seasonal population trends will inform selection of insecticide(s) with appropriate mode(s) of action and facilitate accurate timing of management actions to coincide with occurrence of susceptible life stages.

Hibiscus mealybug was first documented on citrus trees in commercial groves in Highlands county in June 2019 (Diepenbrock and Ahmed 2020). Because of its ability to disperse, it has now become widespread in commercial citrus groves among 22 counties in central and southern regions of Florida (Deeter and Ahmed 2023). As a recent invader, there is no information on the seasonal population trends of hibiscus mealybug populations in Florida citrus groves. Therefore, the main objectives of this study were to: (1) describe the trends in the seasonal abundance of hibiscus mealybug in commercial citrus groves in Florida by an absolute sampling method (visual examination of plant material), (2) compare the obtained results with results from relative sampling procedures (corrugated cardboard band traps and sticky traps) to potentially identify simpler monitoring methods for evaluating hibiscus mealybug populations, and (3) describe the relationship between the seasonal abundance of hibiscus mealybug and new vegetative growth of citrus in Florida.

2 Materials and methods

2.1 Mealybug

Virgin female mealybugs used in this study were obtained from a colony maintained on potted volkamer lemon, *Citrus volkameriana* V.Ten. & Pasq. (Rutaceae), in an indoor insectary (25 ± 5 °C, 70 ± 10 % relative humidity (RH), and a photoperiod of 16:8 h L:D) at the Citrus Research and Education Center (CREC), Lake Alfred, Florida since 2019. The colony originated from field-collected individuals from Highlands county, Florida (27.34°N, 81.34°W). As there is a history of misidentification of hibiscus mealybug for other species such as the cottony cushion scale, *Icerya purchasi* Maskell (Hemiptera: Monophlebidae) (Diepenbrock and Ahmed 2020), the mealybugs were visually identified in the field using a guide published by Olabiyi et al. (2023a) and identity of field-collected mealybugs was confirmed in the laboratory using morphological characteristics (Gullan 2000; Miller et al. 2014) and mealybug identification keys presented in Wakgari and Giliomee (2005).

2.2 Survey sites

Six commercial citrus groves located in three counties in central Florida were used for this study: Hardee (27.4934°N, 81.7959°W), DeSoto (27.2159°N, 81.8584°W), and Polk (28.0445°N, 81.6279°W) (Supplementary Fig. 1). All citrus groves sampled were planted with *Citrus sinensis* (Osbeck) 'Valencia' (Rutaceae). The citrus groves were sampled biweekly from February 2021 through January 2022 (Martínez-Blay et al. 2018). Biweekly averages of meteorological parameters including temperature (measured at 2 m above ground level), windspeed (measured at 10 m above ground level), relative humidity, and total rainfall from the six commercial citrus groves were obtained from the Florida Automated Weather Network stations located at Ona, Arcadia, and Lake Alfred, throughout the sampling period.

2.3 Absolute sampling

In each of the six commercial citrus groves, five citrus trees that presented visual evidence of hibiscus mealybug infestation (fruit damage and sooty mold) in the previous growing season, were randomly selected, and marked. An absolute sample was taken biweekly by collecting 20 cm long twigs, chosen at random, from the canopy of each marked tree. Absolute samples were individually bagged and stored in a freezer until examination in the laboratory. Samples were examined under a Leica MZ6 stereomicroscope (Leica Microsystems Inc., Wetzlar, Germany) to identify developmental stages of mealybugs captured. Mealybugs collected in each absolute sample were separated into the following developmental groups: first instars (crawlers), immatures (2nd–3rd instar females and 2nd instar–pupal stage males), and adult females. The numbers of mealybugs in each developmental group were recorded. Although adult males were not among the target developmental stages for sampling in this part of the experiment, we looked for adult males while examining absolute samples to help us time our deployment of baited sticky traps for monitoring adult male populations in the groves.

2.4 Relative sampling

2.4.1 Corrugated cardboard band traps

Corrugated cardboard band traps (CCBTs) were used to sample mealybugs in the marked citrus trees at the six sites (Beltrà et al. 2013; Martínez-Blay et al. 2018). One CCBT, approximately 20 cm wide, was secured around the trunk of each marked tree at about 20 cm up from the ground (Supplementary Fig. 2a). Four CCBTs, approximately 5 cm wide, were wrapped around four main branches of the trees, one in each cardinal direction, using garden ties (Oligei Twist

Tie, China) (Supplementary Fig. 2b) (Martínez-Blay et al. 2018). Sampling was done biweekly. On each sampling date, CCBTs were individually bagged, transported to the laboratory, and examined under a Leica MZ6 stereomicroscope to determine the developmental stages and the number of each stage captured as with absolute sampling.

2.4.2 Baited sticky traps

We exploited the attraction of winged adult males to sex pheromones produced by live virgin females in designing our virgin female baited sticky trap (VFBST) (Levi-Zada et al. 2019). The VFBST design was adapted from previous investigations on other mealybug species (Meyerdirk et al. 2001; Serrano et al. 2001). Each VFBST was baited with 30–50 third instar live virgin female hibiscus mealybugs, on 3.5 cm diameter *C. volkameriana* leaf discs on agar gel in sterile 3.5 × 1.0 cm Petri dishes (ThermoScientific NUNC IVF dishes, Thermo Fisher Scientific, Roskilde, Denmark) sealed with Parafilm® M (Bemis Company Inc., Neenah, Wisconsin) (Supplementary Fig. 3a). The virgin female baits were suspended in 7 × 10 cm fine mesh bags (Boshen store online at Amazon) over 20.0 × 18.5 cm white sticky panels (Trécé Inc., West Adair, Oklahoma) in white Scentry LP delta traps (Scentry Biologicals Inc., Montana) (Supplementary Fig. 3b). We initiated deployment of VFBSTs in May 2021 after confirming the presence of adult males from our absolute samples in April 2021. Five baited sticky traps (VFBST), and five un-baited but otherwise identical, sticky traps (blank controls) were deployed randomly in the canopy of the same five marked trees used for assessing CCBTs at each site. The traps were deployed at approximately 1.5 m above the ground and approximately 15 m apart. Sampling was conducted biweekly and sticky cards and baits were replaced every 7–14 days depending on site access. Sticky cards were transported to the laboratory and examined for adult males using a Leica MZ6 stereomicroscope.

2.5 Citrus flush phenology

Flush shoot density was monitored at the six commercial citrus groves (*Citrus sinensis*) to investigate the relationship between vegetative flush phenology and abundance of hibiscus mealybug. In citrus, flush are young, newly expanded leaves that occur soon after budbreak. Citrus flush expands as the leaves mature and are no longer considered flush once they begin to harden (Hall and Albrigo 2007). Newly emerged flush shoots were quantified by placing a PVC cube (23 × 23 × 23 cm) into a randomly selected area of the outer tree canopy and recording the total number of flush present within the frame of the cube. Each tree was sampled from each of the four cardinal directions, 1–2 m aboveground (Hall and Albrigo 2007). Flush shoot density was recorded biweekly at each study location from February 2021 through January 2022. We sampled the abundance of feather flush, the youngest stage of flush, because it is a vegetative growth stage that offers optimal conditions for oviposition and development of many important citrus pests, including *Diaphorina citri* Kuwayma (Hemiptera: Psyllidae), resulting in positive correlation between flush density and some insect population fluctuations (Tsai and Liu 2000).

2.6 Statistical analysis

Data from each sampling method were analysed separately. Data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. As data were not normally distributed, a zero-inflated generalized linear mixed model with negative binomial distribution was fit for both sampling methods, with the developmental stage and sampling month as fixed effects, and experimental sites as a random effect using the glmmTMB package in R (Brooks et al. 2017). To estimate the effect of sampling month on abundance of hibiscus mealybug on citrus trees at the sample sites, the glmmTMB models fit for both data sets were subjected to analysis of variance using the "Anova" function in the car package in R (Fox and Weisberg 2019). Means of hibiscus mealybug abundance were separated using the "emmeans" function for multiple pairwise comparisons in the emmeans package in R (Lenth et al. 2020), and *P*-values were adjusted using Tukey's HSD post-hoc test ($\alpha = 0.05$). A multiple correlation analysis was conducted to determine the relationship between new vegetative growth and hibiscus mealybug abundance on sampled citrus trees and environmental factors (temperature, wind speed, relative humidity, and rainfall) in the sampling sites using Spearman's method, because the dataset was not normally distributed. All statistical analyses were conducted using R Statistical Software (v4.1.2; R Core Team 2021). Untransformed data are presented in the figures.

3 Results

3.1 Seasonal trend of hibiscus mealybug by absolute sampling

The population abundance of hibiscus mealybug varied significantly across the sampling months ($\chi^2 = 82.69$; $df = 11$; $P < 0.0001$) and among developmental stages ($\chi^2 = 118.42$; $df = 2$; $P < 0.0001$). Hibiscus mealybug completed multiple generations during the year, remaining active in low populations during winter as illustrated by the multiple peaks of first instars (crawlers), with three of the generations being well defined (Figure 1). Two of the generational peaks coincided with peaks of crawlers, resulting in high population densities (Figures 1 and 2). The second generation was

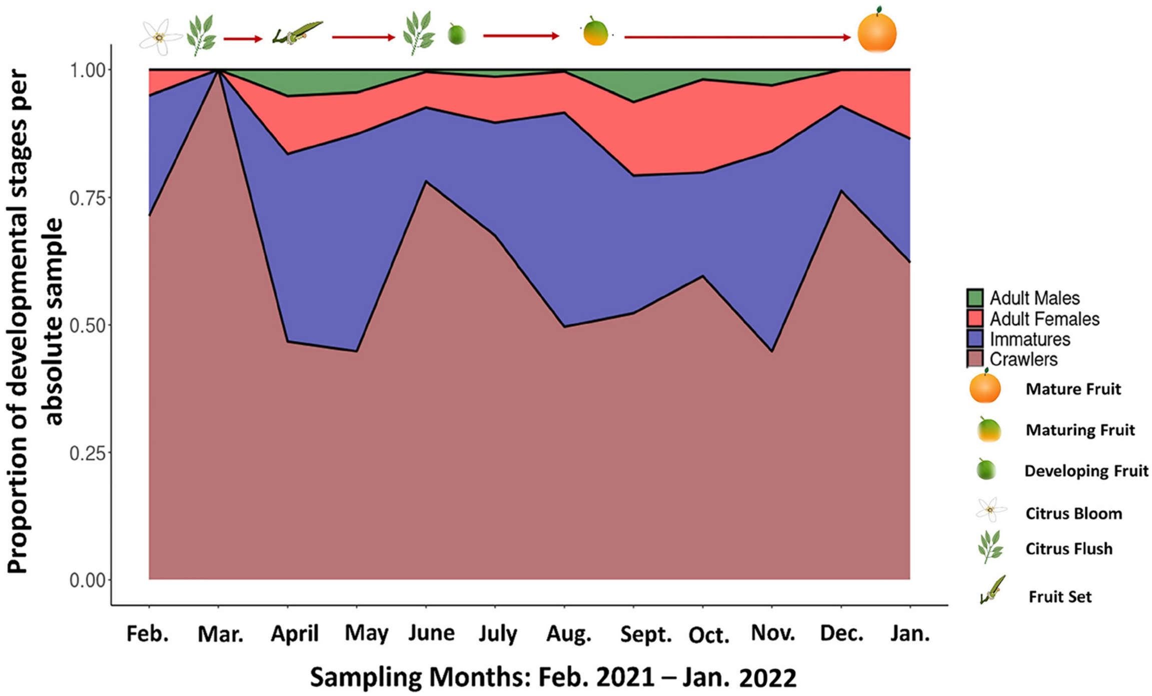

Figure 1: Seasonal relative abundance of developmental stages of hibiscus mealybug, *Nipaecoccus viridis*, in commercial citrus groves in central Florida. The proportion of each developmental stage per sample unit is presented. Crawler peaks represent generations. Overlayed with phenology of *Citrus sinensis* 'Valencia' in Florida (Tang et al. 2021).

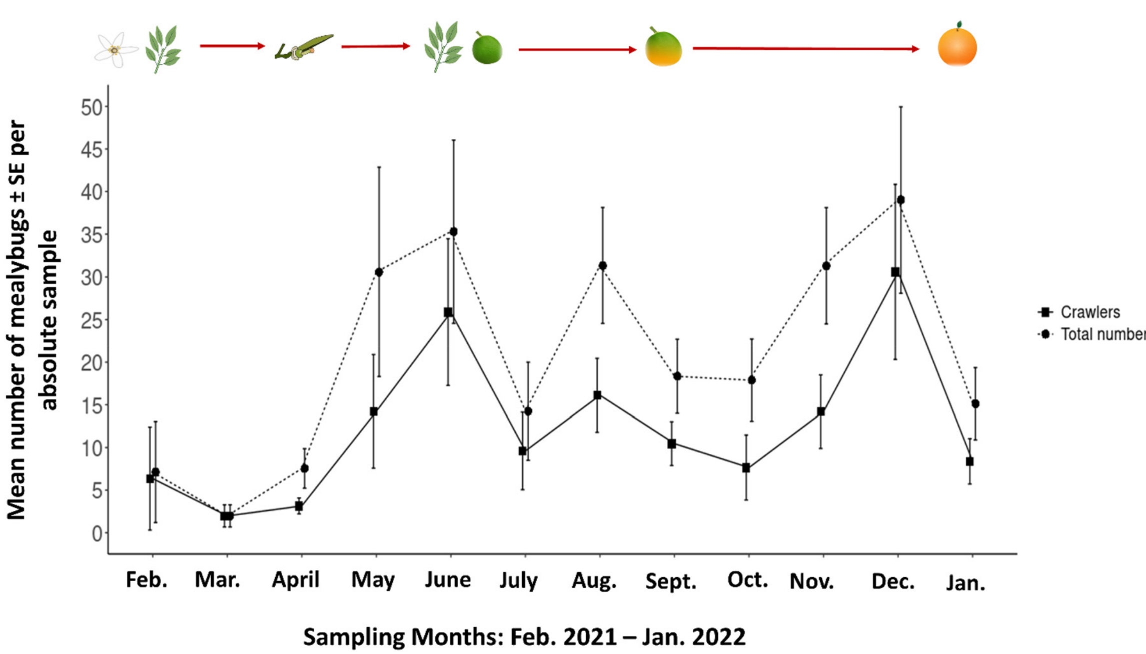

Figure 2: Seasonal trends of crawler and total hibiscus mealybug, *Nipaecoccus viridis*, populations using absolute sampling. Mean number of mealybugs ± SE collected per absolute sample. Our data are overlayed with phenology of *Citrus sinensis* 'Valencia' in Florida (Tang et al. 2021).

recorded between late spring and early summer of 2021, with crawlers comprising 78.1 % of total mealybug population. The last generation was recorded between late fall and early winter of 2021, with crawlers accounting for 76.3 % of total mealybug population (Figures 1 and 2).

Three population peaks were recorded for hibiscus mealybug (Figure 2). The number of hibiscus mealybugs per absolute sample started to increase in April and reached the first peak in late spring to mid-summer, between the end of May to the beginning of July 2021 (Figure 2). A second population peak was recorded in the summer, between late July and early September 2021. The population of hibiscus mealybug began to increase again in October and November, resulting in the formation of a third peak between late fall and winter of 2021 (Figure 2). The average numbers of all life stages of hibiscus mealybugs in the spring, summer, and fall peaks were 35.30 ± 10.74, 31.34 ± 6.79, and 39.02 ± 10.93 mealybugs per absolute sample unit, respectively (Figure 2). Mealybug populations decreased significantly in the winter months (January and February) (t = 4.13; df = 1; P = 0.0023).

The population of crawlers was relatively greater than other life stages monitored throughout the year. The correlation between crawler and total population abundance was positive and highly significant (ρ = 0.81; P < 0.0001), which indicates that crawler abundance drives the population growth of this pest (Supplementary Fig. 4). There was no significant correlation between crawler population abundance and environmental factors considered, including temperature (ρ = –0.01; P = 0.72), total rainfall (ρ = 0.10; P = 0.01), relative humidity (ρ = 0.11; P < 0.01) and windspeed (ρ = –0.05; P = 0.24) (Supplementary Fig. 4). Similarly, there was no correlation between total population abundance and environmental factors considered, including temperature (ρ = –0.01; P = 0.77), total rainfall (ρ = 0.07; P = 0.10), relative humidity (ρ = 0.10; P < 0.02), and windspeed (ρ = –0.03; P = 0.42) (Supplementary Fig. 4).

3.2 Seasonal trend of hibiscus mealybug by relative sampling

The number of mealybugs caught in corrugated cardboard band traps (CCBTs) varied significantly among the developmental stages and sampling months (χ^2 = 86.80; df = 18; P < 0.0001). Comparatively lower numbers of mealybugs were caught in the CCBTs than from absolute samples. Traps caught mainly immatures (2nd and 3rd instars), while adult females and crawlers were caught at relatively low levels. Three population peaks were observed for immature mealybugs, similar to the crawler population peaks observed from the absolute sampling method (Figures 3 and 4). The first, second, and third instar population peaks were recorded in June, August, and December 2021, with 1.50 ± 0.43, 0.90 ± 0.29, and 1.08 ± 0.47 immatures collected per CCBT, respectively (Figure 4).

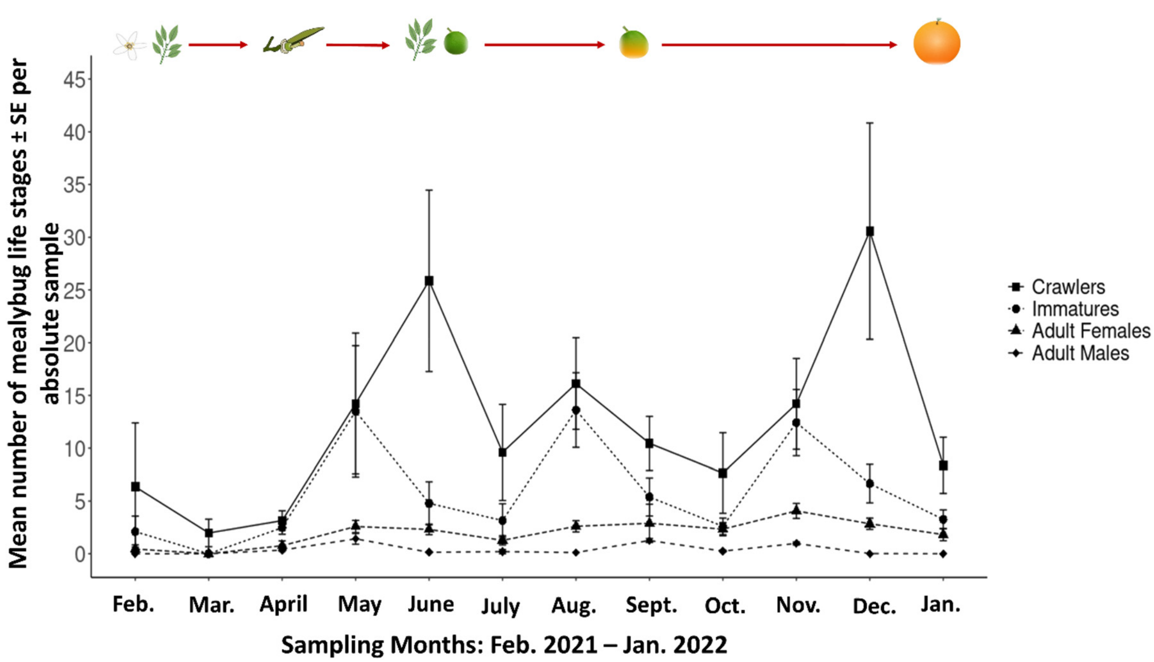

Figure 3: Seasonal trends of various developmental stages of hibiscus mealybug, *Nipaecoccus viridis*, populations using absolute sampling. Mean number of mealybugs ± SE collected per absolute sample. Our data are overlayed with phenology of *Citrus sinensis* 'Valencia' in Florida (Tang et al. 2021).

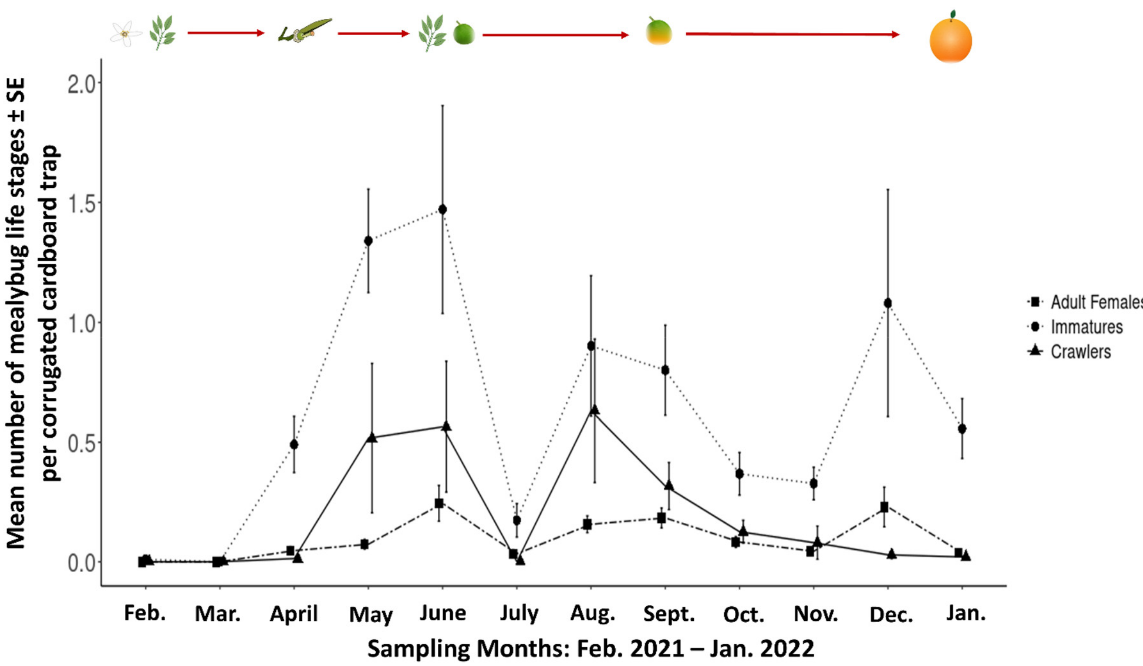

Figure 4: Seasonal trends of hibiscus mealybug, *Nipaecoccus viridis*, crawlers, immatures, and adult females captured in corrugated cardboard band traps (CCBTs). Mean number of mealybugs ± SE captured in CCBTs. Our data are overlayed with phenology of *Citrus sinensis* 'Valencia' in Florida (Tang et al. 2021).

The virgin female baited sticky traps (VFBSTs) attracted adult male hibiscus mealybugs, and significantly more adult males were collected in the VFBSTs than in blank traps ($\chi^2 = 78.28$; $df = 1$; $P < 0.0001$). However, the number of adult males trapped across sampling months did not differ significantly ($P = 0.48$) (Figure 5). We conducted adult male sampling through March 2022, but we did not collect any adult males in any of our sticky traps after January 2022,

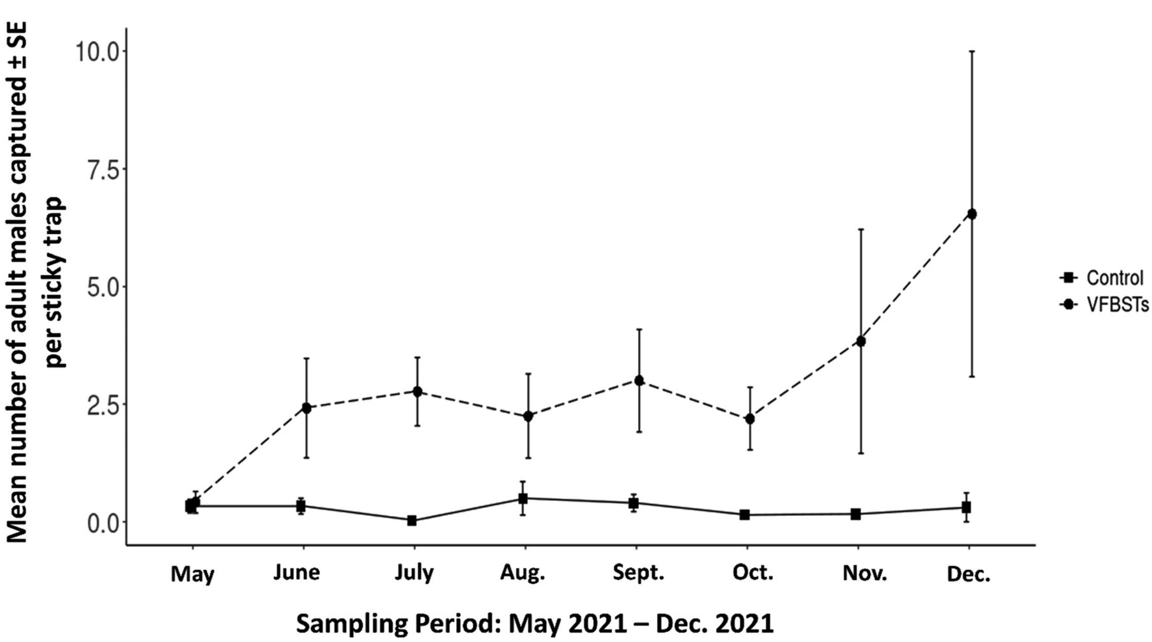

Figure 5: Seasonal trends of adult male hibiscus mealybug, *Nipaecoccus viridis*, captured in sticky traps baited with virgin female *N. viridis* (VFBSTs) or blank sticky traps (controls). Mean number of mealybugs ± SE captured in VFBSTs and controls.

which is likely because of the cooler weather (from 15 °C to −1 °C) that occurred in central Florida during the winter months of 2022 (January to early March) (Supplementary Table 1). There was no relationship between the number of adult males and total population of all life stages of hibiscus mealybug in the field (ρ = 0.10; P = 0.01) (Supplementary Fig. 4), an indicator that adult males captured in sex-pheromone baited traps cannot be used as an index to monitor abundance of hibiscus mealybug in citrus groves.

3.3 Flush phenology

Flush density of sweet orange trees, *C. sinensis*, varied significantly across sampling month (χ^2 = 705.73; df = 11; P < 0.0001) in the groves sampled. Two distinct flush peaks were observed. The first flush peak was recorded in the winter, between January and March 2021, and the second one was in the summer of 2021, between June and August (Figure 6). The second generation of hibiscus mealybug coincided with the summer flush cycle of sweet orange trees in central Florida. There was no relationship between new vegetative growth of citrus trees and abundance of mealybug crawlers (ρ = −0.05; P = 0.22) nor with the total abundance of all mealybug life stages (ρ = −0.07; P = 0.07) (Supplementary Fig. 4).

4 Discussion

This is the first quantitative description of trends in the seasonal phenology of hibiscus mealybug on citrus in Florida. We sampled the mealybug's population in six commercial citrus groves located in four counties in central Florida over a period of 12 months, which represents six site-years of data. All grower-cooperators involved with this project increased management inputs based on seasonal observations from our initial year of study, removing the opportunity for multiple years of data collection. Hibiscus mealybugs complete multiple generations per year on citrus trees in Florida, and three of the generations were clearly defined due to concentrated and homogeneous crawler emergence (Figure 1). The spring and late spring–early summer generations in March–April and May–June 2021, respectively, coincide with the periods of fruit setting and the early stage of fruit development of citrus in Florida. These generations are of major concern to citrus growers because feeding activity of hibiscus mealybug in and around the calyx during fruit set and development causes premature fruit abortion (L.M. Diepenbrock, personal observation). The abundance of hibiscus mealybugs varied seasonally. Mealybug populations began to increase in late March (early spring) and reached an initial peak in June (Figure 2). The mealybug population decreased rapidly in

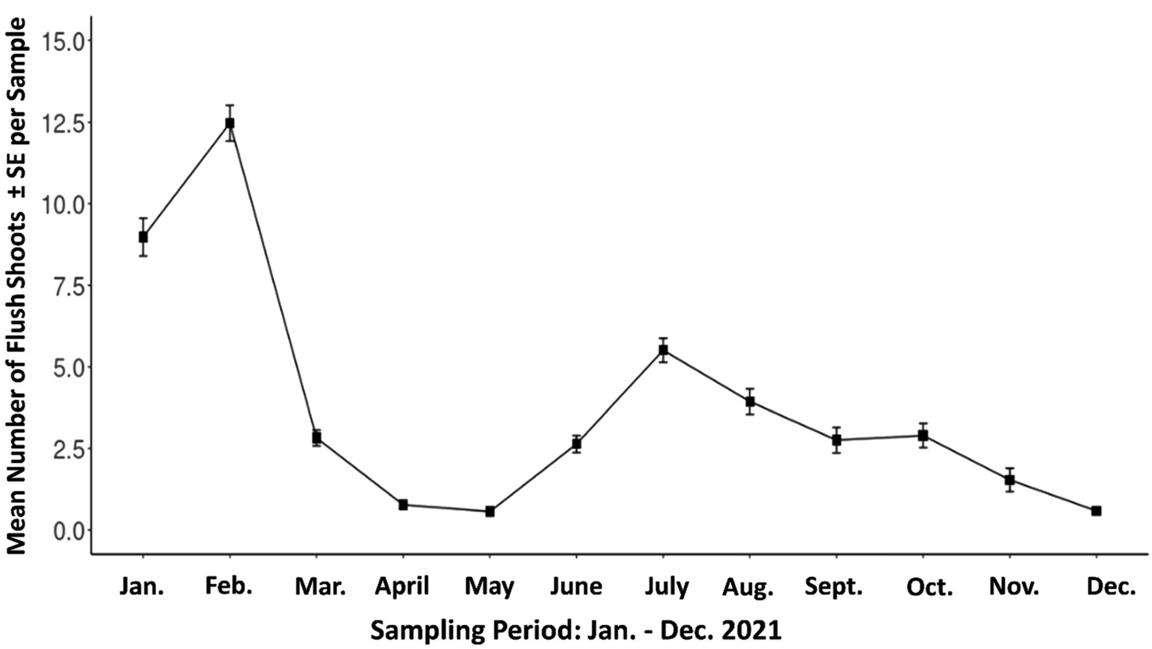

Figure 6: Shoot flushing (production of new vegetative growth) pattern of sweet orange trees, *Citrus sinensis* 'Valencia', at six commercial groves in central Florida.

July (mid-summer), probably because of strong winds and rain wash-off from storms associated with Hurricane Elsa in July 2021. The population then increased rapidly to reach another peak in August (late summer). However, the proportion of crawlers during the August peak (67.5 %) was comparatively lower than the population peaks in June (78.1 %) and December (76.3 %). This is probably due to dislodgement from strong winds and/or rain wash-off from tropical storms that occurred in Florida between July and September 2021 (FAWN). The total population of hibiscus mealybug was relatively low in September (early fall), and it gradually increased through November before reaching the third population peak in early December (winter) (Figure 2). The hibiscus mealybug population abundance declined rapidly and was below detectable levels during the winter months (late December–early March) (Figure 2). These results are similar to those of Zimmerman (1948) and indicate that hibiscus mealybug is multivoltine, occurring in multiple, overlapping generations (Bartlett et al. 1978; Sharaf and Meyerdirk 1987).

Monitoring the population levels of hibiscus mealybug by absolute sampling methods is a laborious and time-consuming process because it involves visual counts of live insects present on plant material. Corrugated cardboard traps wrapped around the trunks and branches of trees are commonly used to monitor populations of other mealybug species (Beltrà et al. 2013; Goolsby et al. 2002; Martínez-Blay et al. 2018). In this study, we compared trends in the seasonal abundance of hibiscus mealybugs based on absolute sampling with those obtained by simpler monitoring methods, i.e. corrugated cardboard band traps (CCBTs) and virgin female baited sticky traps (VFBSTs). Based on our results, similar trends were recorded in the population abundance of hibiscus mealybugs obtained from absolute samples (Figure 3) and those obtained using CCBTs (Figure 4). The CCBTs were able to detect and quantify immatures, crawlers, and adult females. However, unlike in the absolute sampling method where crawlers accounted for a larger proportion of mealybug captured, more immatures were collected from the CCBTs, which is probably because crawlers trapped in the CCBTs molted into second instars within the biweekly sampling interval, and/or because immature mealybugs (mostly males) use the bands as a shelter to build cocoons and develop into adults. In addition, the CCBTs can serve as a refuge for egg laying by gravid females (Beltrà et al. 2013).

Most importantly, the CCBTs were able to detect the most damaging mealybug generations that coincided with the period of fruit set (March) and early development (June). Based on our results, CCBTs represent a suitable, relatively simple, and less laborious sampling method than absolute sampling, to detect and quantify hibiscus mealybug population levels in citrus groves (Figure 4). Hibiscus mealybug crawlers have been reported to require between 16 and 25 days for development into adults (Olabiyi et al. 2023b). We began collecting immatures and adult female hibiscus mealybugs in March and crawlers in April. Taking the developmental rate of this pest into consideration, growers should apply systemic insecticides earlier in the year (February–March) when susceptible life stages (crawlers and immatures) will more likely be present in the field to prevent establishment of damaging populations of this pest in citrus groves.

As expected, virgin female hibiscus mealybugs were attractive to adult males. Significantly more adult males were captured in the virgin female-baited sticky traps than the blank traps (Figure 5). We presume that this capture was caused by sex pheromone released by females. In cases where the number of adult male mealybugs caught on baited traps correlates with total mealybug abundance in the field, as observed in citrus mealybug, *Planococcus citri* Risso (Franco et al. 2001) and vine mealybug, *Planococcus ficus* Ben-Dov (Walton et al. 2004), sex-pheromone baited traps can be used as an alternative to visual sampling techniques to estimate mealybug populations in the field (Millar et al. 2002). Based on our data, there was no significant relationship between the number of adult males captured and the total population of hibiscus mealybug (Supplementary Fig. 4). This is likely because the mealybug pest is parthenogenetic. A single parthenogenetic female has been reported to produce up to 750 eggs throughout its lifetime (Olabiyi et al. 2023b), which suggests that the population dynamics of hibiscus mealybug is not dependent on the number of adult males. Therefore, use of adult male populations as an index for monitoring population levels of hibiscus mealybugs in infested citrus groves is not recommended. Likewise, pheromone-based mating disruption or mass trapping of adult males are unlikely to provide effective control of hibiscus mealybug infestation as stand-alone tools.

Citrus flush is the new and tender vegetative growth of citrus trees. Citrus flush is produced multiple times per year in discrete cycles (Hall and Abrigo 2007) and is affected by environmental conditions (Carvalho et al. 2020). Several foliar pests of citrus, including Asian citrus psyllid, *D. citri*; citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae); brown citrus aphid, *Toxoptera citricida* (Kirkaldy; Hemiptera: Aphididae); and the Neotropical root weevil, *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae), are attracted to citrus flush by a combination of chemical and visual cues (Patt et al. 2014). Females of several of these pest species exhibit 'host fidelity' by preferentially feeding and laying their eggs on citrus flush because it provides optimal conditions for oviposition, survival, and development of juvenile life stages (Cifuentes-Arenas et al. 2018). Consequently, population dynamics of foliar citrus pests, such as *D. citri*, are synchronous with citrus flushing (Hall et al. 2008). Therefore, citrus growers can monitor for peak flush periods to effectively time deployment of management actions to control foliar pests of citrus (Hall and Albrigo 2007; Tsai and Liu 2000). However, there was no correlation between seasonal abundance of hibiscus mealybug and the production of new vegetative growth in citrus trees in Florida. This suggests that management of this mealybug will not be compatible with that of other common foliar pest of citrus, which are timed based on citrus flush phenology.

Here we provide the first description of the seasonal phenology of the invasive hibiscus mealybug in citrus groves in Florida. Our findings should be useful for development of effective management programs for controlling this damaging pest including predicting the timing of recently evaluated management actions (Demard and Diepenbrock 2023; Diepenbrock 2021; Diepenbrock et al. 2021; Middleton and Diepenbrock 2022a; Olabiyi et al. 2022). For example, immature instars are more susceptible to commercially available adjuvants than adults (Middleton and Diepenbrock 2022b). This is probably because the former has less protective wax coating or deposition on their bodies than the older life stages (Middleton and Diepenbrock 2022b). Therefore, growers should monitor for hibiscus mealybug crawlers and time management with contact insecticides to target juveniles given the susceptibility of these life stages. We have also shown that CCBTs can detect and quantify the relative population of hibiscus mealybugs, particularly during the spring–summer generation that poses the greatest risk to citrus production in Florida. CCBTs are a relatively simple tool for monitoring populations of hibiscus mealybug in groves that could forecast the need for insecticide sprays rather than relying on prophylactic and/or calendar-based treatments.

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