Biology of Anthonomus testaceosquamosus Linell, 1897 (Coleoptera: Curculionidae)

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Although native to northeastern Mexico and southern Texas, the hibiscus bud weevil (HBW), *Anthonomus testaceosquamosus* Linell 1897, was recently discovered infesting hibiscus in south Florida in 2017. During outbreak events, HBW feeding on hibiscus buds has been found to significantly affect the marketability of the crop. Therefore, it is vital that an integrated pest management (IPM) program be developed for this pest in order to mitigate the economic loss to the hibiscus industry of south Florida.

invasive pest hibiscus bud weevil artificial diet

1. Introduction

The hibiscus bud weevil (HBW) (*Anthonomus testaceosquamosus*, Coleoptera: Curculionidae) is a small (≈4 mm) insect that infests China rose hibiscus (*Hibiscus rosa-sinensis* L., Malvales: Malvaceae). It originates in northeastern Mexico and southern Texas [1] and has been associated with multiple hosts within the family Malvaceae [1][2]. Female weevils oviposit their eggs inside hibiscus flower buds, inserted close to the anthers. Upon emergence, larvae feed on pollen and remain in the flower bud until they reach adulthood [3]. In Texas, heavy infestations on different varieties of tropical hibiscus resulted in bud drop, thereby decreasing the marketability of the plants [3]. In May 2017, HBW was detected infesting hibiscus in south Florida for the first time [4]; by the spring shipping period of 2019, HBW outbreaks were already responsible for large economic losses to the state's hibiscus industry.

The discovery of HBW in south Florida is of particular concern due to the importance of the hibiscus industry in the area. Florida is the number one hibiscus-producing state, of which most is grown in south Florida (including Miami-Dade County). Approximately 20% to 25% of plants sold from Miami-Dade County are hibiscus, and this ornamental is shipped throughout the North American continent. As of 2017, the market value of ornamental plants in the county was 697 million (farmgate price) [5]. Therefore, the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS-DPI), is now regulating this pest to curtail its spread. Currently, if HBW is detected at a nursery, the grower must sign a compliance agreement requiring that all plants be weevil-free prior to shipping. Hibiscus growers have a narrow shipping window of 3 months in the spring of each year, from March through June. Any losses incurred during this critical period can be devastating to these growers, and to the Florida industry as a whole.

Despite frequent insecticide applications and the implementation of sanitation practices (i.e., collection and destruction of fallen buds), hibiscus growers remain unable to control HBW populations. Therefore, it is vital that an integrated pest management (IPM) program be developed for this pest to mitigate economic losses to the hibiscus industry of south Florida. However, a comprehensive understanding of a pest's biology is critical for the development of such a program. Although close relatives of HBW such as the cotton boll weevil, *A. grandis*, and the pepper weevil, *A. eugenii*, have been studied extensively [6][7][8][9][10], little information is available for the HBW aside from an initial FDACS-DPI Pest Alert [4] and a University of Florida Fact Sheet [11]. Consequently, we investigated important biological parameters regarding the HBW life cycle. Specifically, we assessed the effects of temperature and diet on HBW development and fecundity. Here, we present for the first time a comprehensive study on the biology of this pest under different feeding regimes and rearing temperatures.

2.Biology of *Anthonomus testaceosquamosus* Linell

The HBW is a newly invasive pest in south Florida for which there is currently only one report that demonstrates its potential impact on the hibiscus industry [3]. Here we present the first comprehensive study on the biology of HBW reared at various temperatures and on various food sources, including its natural host (hibiscus buds), an artificial diet (pink bollworm diet), hibiscus pollen, and only water. Of the temperature regimes evaluated, 27 °C was the most favorable for weevil development. At this temperature, HBW successfully completed its life cycle within 15 days on its natural host (**Table 1**). These results are consistent with the high weevil populations observed in hibiscus nurseries between March and June. The abundance of flower buds in combination with favorable climatic conditions is conducive for weevil population growth during these months. Since hibiscus plants are shipped nationally and internationally from Miami-Dade County from March through June, the peak numbers of HBW during this critical period pose a serious threat to the Florida hibiscus industry. Growers must ensure that this regulated pest is absent from all hibiscus stock prior to shipment.

Table 1. Mean developmental time (days) ± SE of the hibiscus bud weevil (*Anthonomus testaceosquamosus*) under different temperatures and food sources at 60% RH and 12:12 h L:D. Within each column, different letters indicate significant differences (Tukey, $p < 0.05$).

Food Source	Temperature (°C)	Egg (*n*)	First Instar (*n*)	Second Instar (*n*)	Third Instar (*n*)	Pupa (*n*)	Egg to Adult (*n*)
Hibiscus buds	10	78.2 ± 0.55a (20)	-	-	-	-	-
	15	13 ± 1.33b (20)	4.9 ± 0.86a (10)	12.75 ± 2.46a (4)	87 ± 14.01a (3)	-	-
	27	3.35 ± 0.31d	2.6 ± 0.24a	3.73 ± 0.48a (19)	2.05 ± 0.19b	4.1 ± 0.27	15.78 ± 0.83

Food Source	Temperature (°C)	Egg (*n*)	First Instar (*n*)	Second Instar (*n*)	Third Instar (*n*)	Pupa (*n*)	Egg to Adult (*n*)
		(20)	(20)		(18)	(18)	(18)
	34	5.5 ± 0.29c (20)	2.53 ± 0.29a (19)	8.92 ± 1.3b (13)	25.5 ± 8.86ac (6)	-	-
Artificial diet	27	2.22 ± 0.05e (129)	1.94 ± 0.05b (128)	3.9 ± 0.08a (128)	4.25 ± 0.23b (128)	4.21 ± 0.07 (128)	16.47 ± 0.3 (128)

peratures … the field. During the win … and frost … ve growth … d 34 °C in July and August. During this time, hibiscus cuttings are kept in greenhouses prior to planting. Therefore, environmental conditions in combination with food availability may account for the fluctuations in weevil populations observed in nurseries. It remains unknown whether the HBW has an overwintering form and if yes, which form this is.

Our measurements of HBW head capsule width and length indicated the presence of three larval instars (**Table 2**), which agrees with other *Anthonomus* species such as *A. grandis* [7][9] and *A. eugenii* [10][12]. Due to the various challenges and the labor required to maintain a laboratory colony with hibiscus buds, we also evaluated an alternative, artificial diet for rearing HBW. We found that although HBW can develop and reproduce on the pink bollworm artificial diet (**Table 1**, **Table 3** and **Table 4**), its population growth was significantly lower than on hibiscus buds (**Table 4**). In the congeneric *A. grandis,* the pink bollworm diet was found to be an excellent rearing medium as the wheat germ within the diet stimulated oviposition [13]; this effect was not observed with HBW. Our results are more similar to those reported by Toapanta et al. [10] and Toba et al. [14], whereby *A. eugenii* required more time to develop when reared on an artificial diet than when it was reared on its natural host. Seal and Martin [15] used the artificial cotton boll weevil diet to successfully rear *A.eugenii*. The cotton boll weevil diet and pink boll worm diet are very similar. The main difference is that the former contains cholesterol [13][15]. However, we do not know whether the lack of cholesterol is responsible for the low oviposition of the HBW. Future experiments should test the HBW ability to develop and reproduce on the cotton boll weevil diet. Given these results, we conclude that the artificial pink bollworm diet can serve as an alternative food source for laboratory rearing when hibiscus buds are not available, but hibiscus buds remain the most suitable food source for HBW reproduction.

Table 2. Mean head capsule widths (µm) of larvae of the hibiscus bud weevil (*Anthonomus testaceosquamosus*) at 27 ± 1 °C, 60% RH and 12:12 h (L:D) photoperiod. Within each column, different letters indicate significant differences (Tukey, $p < 0.05$).

Instar	*n*	Width ± SE	Range	Dyar’s Constant
First	29	248.14 ± 8.57a	129–318.37	-
Second	26	383.31 ± 4.65b	318.38–461.38	1.48
Third	43	563.23 ± 5.07c	461.39–633	1.48

Table 3. Reproductive parameters for the hibiscus bud weevil (*Anthonomus testaceosquamosus*) when it developed, fed, and reproduced solely on hibiscus buds, on the artificial boll worm diet, or when it developed on the diet and reproduced on hibiscus buds. Within each column, different letters indicate significant differences (Tukey, $p < 0.05$).

Development, Feeding and Oviposition	Fecundity * (*n*)	Fertility ** (*n*)	Pre-Oviposition Period *** (*n*)	Oviposition Period *** (*n*)	Post-Oviposition Period *** (*n*)
Hibiscus buds	5.85 ± 0.48a (20)	55.2 ± 2.32 (10)	4.05 ± 0.4c (20)	40.35 ± 3.53 (20)	4.45 ± 1b (20)
Artificial diet	0.2 ± 0.04b (21)	NA	6.33 ± 0.3b (21)	32.29 ± 3.48 (21)	19.85 ± 3.94a (21)
Artificial diet + Hibiscus buds	0.73 ± 0.57b (20)	62 ± 0.03 (25)	11.35 ± 1.21a (20)	38.45 ± 3.15 (20)	19.85 ± 3.2a (20)

Values are mean ± SE. * (eggs/Female/Day). ** (% hatch). *** Days.

At 27 °C, population growth and net reproductive rate of HBW (**Table 4**) were higher than that of *A. grandis* [6][7][9] and *A. eugenii* [10] reared at a similar temperature and photoperiod. Moreover, HBW generation time and doubling time were much shorter in comparison to these other two important agricultural pests [7][10]. These results suggest [illegible] ustry. The [illegible] t injury to [illegible] However, [illegible] l, infested [illegible]

[illegible] ted for *A.* [illegible] [17][18], HBW [illegible] t up to 12 [illegible] riability in [illegible] that buds measuring 0.5–1.5 cm in length were more frequently infested than smaller or larger buds. Although HBW oviposits multiple eggs per bud, only a couple of adults will emerge from each bud. This result may be explained by larval cannibalism. Cannibalism is a common phenomenon across the animal kingdom [19][20], and is well-documented in insects [21] and in the family Curculionidae [22][23]. [illegible] food is scarce [24] which may be a common occurrence inside small hibiscus buds where the amount of food is limited, larvae cannot disperse in search of more food. In our experiments, it was not possible to observe larval cannibalism because to score daily oviposition we had to destroy the bud and remove all the eggs. Moreover, in weevil development experiments we only inserted one egg per bud or cell.

Table 4. Life table parameters and 95% confidence intervals of the hibiscus bud weevil (*Anthonomus testaceosquamosus*) when it developed, fed, and reproduced solely on hibiscus buds, on the artificial boll worm diet, or when it developed on diet and reproduced on hibiscus buds. Within each column, different letters indicate significant differences (Tukey, $p < 0.05$).

Development, Feeding and Oviposition	*n*	Net Reproductive Rate (Ro) *	Intrinsic Rate of Increase (rm) **	Generation Time (T) ***	Doubling Time (Dt) ***	Finite Rate of Increase (λ) ***
Hibiscus buds	20	136.73a 135.54–137.91	0.4547a 0.4522–0.4573	10.82a 10.76–10.88	1.52a 1.51–1.53	1.5758a 1.5717–1.5798
Artificial diet	21	7.65c 7.48–7.83	0.0578c 0.0572–0.0584	35.2c 35.03–35.3	11.99c 11.86–12.13	1.0599c 1.0588–1.0601
Artificial diet and Hibiscus buds	20	20.85b 20.46–21.23	0.0841b 0.0834–0.0847	36.09b 35.96–36.09	8.24b 8.18–8.30	1.0877b 1.0871–1.0885

* Female/female. ** Female/Female/Day. *** Day.

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