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Localizing the Puke Button of Grasshoppers: Tactile Stimulation of Different Body Regions Influences the Regurgitation Response of *Schistocerca Serialis Cubense* (Orthoptera)

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Abstract Regurgitation behavior is a defense strategy present in several insect orders where herbivorous prey may acquire secondary noxious metabolites from plants and store them to later orally excrete when threatened. Research on regurgitation has focused on its chemical composition, its relationship with diet, and its noxious effect on predators. However, virtually nothing is known about the proximal mechanisms eliciting this behavior. As regurgitation is expected to be closely connected with the structure and control of the gut, we aimed to determine whether pressure applied to different body regions (head, thorax, hind legs and abdomen) of the grasshopper elicited different responses. We found distinctly different regurgitation responses when stimulating different body regions. Stimulation on thorax triggered regurgitation most frequently, possibly due to the anatomical placement of the neural control of the gut. Additionally, we tested regurgitation responses between different instars and found that regurgitation was less likely to occur in adults than in the 4th and 5th nymphal instars. These findings suggest that the developmental differences between nymphs and adults contribute to the regurgitation behavior. Overall, our results provide a baseline for future research on the physiology behind the control of the regurgitation behavior.

Keywords Predator defense · Anti-predator behavior · *Schistocerca serialis cubense*

Introduction

Regurgitation is considered an antipredator defense strategy where insects excrete a mixture of salivary and digestive secretions that can also be accompanied by secondary noxious metabolites sequestered from plants (Zvereva and Kozlov 2016). This behavior has been observed in many phytophagous insects belonging to Blattodea (termites), Orthoptera, Thysanoptera, Hemiptera, Coleoptera, Diptera, Neuroptera, Hymenoptera, and Lepidoptera (Whitman et al. 1990; Grant 2006; Zvereva and Kozlov 2016; Rostás and Blassmann 2009) and even documented in Opiliones (Whitman et al. 1990). The majority of the literature investigating regurgitation in insects revolves around aspects such as the chemical composition of the regurgitant (Alborn et al. 2007), the developmental

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costs to the insect (Higginson et al. 2011; Zvereva and Kozlov 2016), and the aversion effect on predators (Eisner 1970; Sword 2001; Desurmont et al. 2017). Overall, excreted defenses such as regurgitation are suggested to be less effective for prey survival, when compared to strategies such as allelochemical sequestration and accumulation (Opitz and Müller 2009; Zvereva and Kozlov 2016). Furthermore, regurgitation could be nutritionally costly in some insects, as it has been shown to stunt development in lepidopterans (Higginson et al. 2011). However, excretion defenses also produce rapid aversion learning behavior of predators (Zvereva and Kozlov 2016; Sword 2001), and have shown to be effective predator repulsion against mammals, birds, reptiles, and other insects (Eisner 1970; Eisner et al. 1974; Peterson et al. 1987; Codella and Raffa 1995; Sword 2001). Despite these trade-offs, regurgitation has evolved repeatedly and independently in different taxa such as caterpillars (Lepidoptera), sawflies larvae (Hymenoptera), and grasshoppers (Orthoptera), suggesting its adaptive value as a defense mechanism (Zvereva and Kozlov 2016).

When handled, grasshoppers frequently regurgitate a mixture of salivary secretions, digestive enzymes, and partially digested food plant compounds (Eisner 1970; Freeman 1967, 1968; Lymbery and Bailey 1980; Ortego et al. 1997). This physiological defense mechanism appears to be a general feature for grasshoppers (as well as other orthopterans), regardless of whether the insects feed on toxic plants or not. It is assumed that regurgitant is typically distasteful, and in some cases, toxic for those insects that specialize on plants with strong defensive compounds (Sword 2001). For example, the regurgitant from those species feeding on toxic plants has caused significant distress when applied to or ingested by vertebrates (Freeman 1968; Curasson 1934; Lymbery and Bailey 1980; Sword 2001). Similarly, when the noxious regurgitant of grasshoppers has been presented to invertebrate predators, such as ants within the genera *Pogonomyrmex*, *Camponotus* and *Cremagaster*, they are repulsed by the regurgitant and do not advance toward prey items when the grasshopper regurgitant is present (Eisner 1970; Steiner 1981; Lymbery and Bailey 1980; Ortego et al. 1997; Calcagno et al. 2004). However, very little is known about the specific chemical properties of regurgitant for most grasshopper species.

Despite our partial understanding of the chemical components of the regurgitant, its relationship with the insect diet, and its noxious effect on predators, virtually nothing is known about the proximal mechanisms eliciting the regurgitation behavior. As the regurgitant is stored in and expelled from the foregut of insects, it can be expected that the neural physiology of the foregut is involved in the regurgitation behavior (Grant 2006). Previous studies illustrate that the insect gut is controlled by an interconnected network of ganglia and nerve plexuses that span across the different regions of the gut (Hartenstein 1997). Depending on where these ganglia are located, they contribute to regulating different processes such as swallowing, gut peristalsis, metabolism, or endocrine functions (Ayali 2004; Copenhaver 2007). The foregut, the region from where the regurgitant is expelled, is controlled by the stomatogastric nervous system (SNS), which is an important section of the enteric nervous system (ENS), and it contains interconnected ganglia, nerves, and stretch receptors that are distributed around the foregut musculature (Ayali 2004; Copenhaver 2007). On the other hand, the midgut ENS lacks ganglia but is formed by diffuse nerve plexuses that extend across the gut musculature, and in the hindgut, the ENS is primarily supplied by the proctodeal and rectal nerves that originate from the terminal ganglia of the central nervous system (CNS) (El Asrar et al. 2020; Copenhaver 2007). Previous studies have also described the molecules and neuromuscular pathways involved in the control of gut physiology (see El Asrar et al. 2020 for the review). Overall, gut-neural anatomy provides insights into how gut movement, and consequently regurgitation, are controlled.

Given the anatomical and physiological differences associated with the control of gut movement, testing whether tactile stimuli of different external body regions trigger different regurgitation responses would allow us to identify correlations between the external anatomy, the anatomy of the nervous system, and the regurgitation behavior. The existence of associations can ultimately help us to identify common physiological pathways behind the control and function of the regurgitation, and the gut as a whole. Additionally, understanding how this behavior changes along the individual's life history could also provide clues on how the environment and development of grasshoppers could be influencing this defense mechanism.

In the present study, we aim to describe how the regurgitation response is influenced by mechanical stimulation of different body regions (head, thorax, hind femur, and abdomen) of the grasshopper species *Schistocerca serialis cubense* (Orthoptera: Acrididae). As it is plausible that the regurgitation behavior is closely linked with gut control and structure, we hypothesize that a differential response will be elicited when we stimulate different body regions. Additionally, we hypothesize that the frequency of regurgitation will be different across the developmental stages product of the ontogenetic changes in the life history of nymphs and adults.

Methods

Study Insect

All individuals used in the experiments were from a long-term colony of *S. serialis cubense* maintained in the Department of Entomology at Texas A&M University. The colony started from individuals collected in 2011 from a population in Islamorada in the Florida Keys, FL. The colonies were maintained in wire-screened, aluminum cages (40×30×30 cm), at 30 °C, 30% relative humidity, and 14 h of light to 10 h of darkness. Colonies were offered rinsed Romaine lettuce, wheat grass, and wheat bran ad libitum.

Experimental Design

We used 61 fourth-instar nymphs, 60 fifth-instar nymphs, and 60 adults for a total of 181 experimental individuals. Each of the three age groups was divided into four even subgroups and was assigned to one of four body stimulus treatments. The body regions of the grasshoppers selected as treatments were the head, thorax, hind femur, and abdomen.

The grasshoppers were placed in individual plastic cages (10.6×10.6×25.4 cm) and were starved for at least 24 h prior to the start of the experiments. This action was taken to increase the probability that the grasshoppers would feed right before the start of our tests (Bernays and Chapman 1973), so we could quantify the amount of food they ingested prior to stimulation. As experimental individuals were pulled from their colony cage and separated into their individual cages, we recorded sex, life stage, and any notable body damage (i.e., missing a leg or antennae).

In order to have a better estimation of the amount of food eaten by the grasshoppers and how this relates to the regurgitation behavior, we offered each grasshopper five disks of lettuce of 17 mm in diameter that we cut using the open end of 15 mL Falcon tubes (Fisher Scientific, Waltham, MA), a minimum of 30 min before the trials. We prioritized testing first grasshoppers that had eaten the majority if not all of their lettuce disks after the 30 min passed. We recorded the amount of lettuce consumed by each individual upon its collection for testing. Grasshoppers were given a minimum of 30 min and a maximum of three hours to feed upon the five discs of lettuce. Thirty individuals rejected the food offered but were still included in the stimulation trials, due to the potential for them still having material to regurgitate from their meal 24 h prior to the trial. One individual was entirely excluded because not only did it not eat but during the feeding period they molted into an adult. With this observation, we hypothesize individuals that did not ingest any lettuce during the trials could be nearing ecdysis as previously observed by Bernays and Simpson (1982). Although we did not confirm that the individuals remained starved because they were nearing ecdysis, we included them because we did want to test if the amount of lettuce eaten had an impact on the regurgitation response.

The trials consisted of carefully transferring the grasshoppers from the isolation cage into a mesh chamber specifically designed for this study (Fig. 1), without directly touching the individuals to avoid triggering the regurgitation behavior before the formal trial. In order to reduce the stress of each grasshopper during the experimental stimulation, we designed a wire mesh chamber (Fig. 1) to allow them to move freely while confining them to a small area. The mesh cages were made of a 10×6.35 cm 19-gauge steel mesh that was cut and then fastened together with zip-ties to create a cylindrical chamber with a 2.54 cm diameter. At both ends of the mesh chamber, paper towels were folded and stuffed into the ends of the chamber to allow for an effective and efficient enclosure of each grasshopper. After placing the test subject into the mesh chamber, the chamber was placed upon a flat surface. Once the grasshopper was secure, the blunt ends of two wooden skewer sticks of

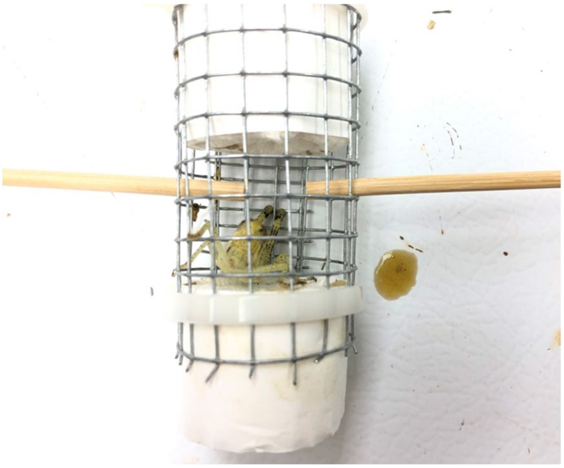

Fig. 1 The experimental design for each pinch encompassing the wire mesh chamber, the paper towels plugging the mesh chamber at both ends, and the blunt end of the skewers contacting the test subject

3 mm in diameter were used to gently apply simultaneous pressure to both sides of the assigned body part (either head, thorax, hind femur, or abdomen) of each grasshopper. The pressure applied lasted no more than three seconds. Using the blunt ends of the skewers provided a way to apply pressure to the insects with a uniform surface area for all individuals, without accidentally piercing their bodies. When applying the pressure, we tried to be as consistent as possible, and we did so by restricting the stimulation to three seconds and stimulating the grasshopper a minimum of once and a maximum of three times. Regurgitation responses were recorded as ‘yes’ or ‘no’, and if regurgitation occurred on the first stimulation, then the grasshopper was returned to the stock colony. If regurgitation did not occur on the first stimulation, we allowed a minimum of 10 s to transpire before stimulating the grasshopper again. This process did not exceed a total of three consecutive rounds of stimulation of the same individual even if the grasshopper never regurgitated, and the second or third round of stimulation was performed only when the first stimulation did not cause regurgitation. At the end of our experiments, we found that almost all experimental individuals regurgitated in the first stimulation, therefore those were used for all subsequent statistical analyses. There were also three instances recorded where during the transfer from the individual cages to the mesh chambers, grasshoppers had escaped and regurgitated upon recapturing, in these cases we had returned the grasshoppers to the individual cages and processed them at least 30 min after their recapture and after they had eaten more lettuce disks. Once the trial was complete, all individual grasshoppers were returned to their colony.

The independent variables within this study included the experimental individual’s life stage, sex, amount of lettuce eaten, and body region stimulated. The dependent variable was the presence (‘yes’) or absence (‘no’) of the regurgitation behavior after the stimulation.

Statistical Analyses

All statistical analyses were conducted in R version1.2.5042 (R Core Team 2021). To estimate our required sample size to calculate statistical significance prior to experimentation, we utilized the Chi squared power analysis in the ‘*pwr*’ package (Champely et al. 2020). This package contains functions for basic power calculations using effect sizes and notations from Cohen (1988). In this power analysis to estimate our required sample size, we used the function pwr.chisq.test with a medium effect size (Cohen suggested effect size of 0.3), the power was high (0.8), and the statistical significance was standard (0.05) (Champely et al. 2020). This same power analysis was recalculated post-experiment in order to calculate our true effect size.

We modeled the effects of life stage, body section, sex, and percent of lettuce eaten on the probabilities of regurgitation responses using generalized linear mixed models (GLM) from the ‘lme4’ package (Bates et al. 2015). As the dependent variable was the presence/absence of regurgitation, the model had a binomial error distribution. We used Akaike Information Criterion (AIC) to determine the best fit model in which we tested four models (Table 2). To further test whether the interactions among life stage, body section, and sex significantly influenced the regurgitation response, we performed an analysis of deviance. The predictors that significantly influenced the regurgitation responses were further analyzed as follows. To test for significant differences in regurgitation while stimulating different body regions of *S. serialis cubenes*, a two-tailed Fisher’s exact test was performed (due to some observation frequencies being less than five for some body regions), followed by an adjusted pairwise nominal independence post-hoc analysis with '*rcompanion*' package (Mangiafico 2022). The false discovery rate (fdr) method was used to correct for multiple comparisons for all statistical analyses. Differences in regurgitation among different life stages of *S. serialis cubense* were determined by Chi squared test. Chi squared analyses were also conducted to test for significant differences of regurgitation between sexes, and also between the first, second, and third stimulations on the test subject.

Results

Although a total of 190 individual grasshoppers were initially recruited for this study, nine individuals were removed from the statistical analyses due to missing life history data (e.g., sex or instar not specified in the dataset), molting during the trials, or the grasshopper's escaping during the trials with a final N of 181 grasshoppers. Demographics of the test subjects included in the final analyses were 115 females, 66 males, and of those 61 were 4th instar nymphs, 60 were 5th instar nymphs, and 60 were adults (Table 1). Prior to experimentation, a Chi-squared power analysis calculated at least 122 total observations were needed to identify a statistical significance, with high power (0.8) and medium effect size (0.3). Post experimentation, a Chi-squared power analysis indicated that with 181 total observations, there was an effect size of 0.25, at the same high power for comparisons between regurgitation and body section. Furthermore, results between life stages and regurgitation had an effect size of 0.23, at the same high power.

Each trial consisted of a minimum of one stimulation and a maximum of three stimulations, and we decided to do this in case the grasshopper had moved during the trial and contact between the grasshopper and skewers was compromised. In most of the trials, the first stimulation was successful in eliciting regurgitation and following stimulations were not conducted on that individual. In fact, we found it was significantly rare for an individual to regurgitate during the second or third stimulations ($X^2 = 47.703$, df = 2, $p < 0.001$, n = 181). Therefore, results from the first stimulation were used for all subsequent analyses.

Model 3, without interaction among the independent variables, is the best model fit with a lowest AIC (i.e., AIC = 155.56, Table 2, Table S1). Deviance analysis also indicated that two-way and three-way interactions among life stage, body section, and sex on the regurgitation response are not significant (Table S2). Life stage (deviance = 6.629, df = 2, p = 0.036) and body section (deviance = 85.32, df = 3, p < 0.001) strongly influenced the regurgitation response, while sex (deviance = 0.427, df = 1, p = 0.514) had a negligible impact (Table S2). Adding percentage of lettuce eaten (coefficient = 0.01, z = 0.77, p = 0.440, Table S1, Table S3) would not better explain the data (i.e., AIC = 156.96, Table S1).

Stimulations of different body regions resulted in different responses in regurgitation (df = 3, $p < 0.001$, Fig. 2, n = 181). When the thorax was stimulated, the

Table 2 A ranking of models for regurgitation responses based on the Akaike information criterion (AIC)

Model	AIC
1. Response ~ life stage × body section × sex	174.38
2. Response ~ life stage × body section + sex	161.32
3. Response ~ life stage + body section + sex	155.56
4. Response ~ life stage + body section + sex + percentage eaten	156.96

Table 1 Number of grasshopper regurgitation out of total number tested in each group

Body Sections Stimulated	Head	Thorax	Abdomen	Hind Leg	Total
Sex					
Female	16/30	20/29	0/30	3/26	39/115
Male	9/17	13/15	1/16	1/18	24/66
Life Stage					
4th instar nymph	11/16	15/16	1/15	2/14	29/61
5th instar nymph	5/16	10/13	0/16	1/15	16/60
Adult	9/15	8/15	0/15	1/15	18/60
Total	25/47	33/44	1/46	4/44	63/181

grasshopper regurgitated significantly more compared to when the abdomen ($p < 0.001$) and legs ($p < 0.001$; Fig. 2) were stimulated. We did not find significant differences in regurgitation when thorax and head were compared ($p = 0.0586$). Different life stages had significantly different responses in regurgitation ($p = 0.038$, Fig. 3). The 4th instars regurgitated significantly more when stimulated on any of the body regions compared with the 5th instars ($p = 0.029$) and adults ($p = 0.073$; Fig. 3). There were no statistical differences ($X^2 = 0.029$, df = 1, $p = 0.864$) between the sexes in their regurgitation response across all body sections.

Discussion

We describe for the first time that the regurgitation behavior of grasshoppers (*S. serialis cubense*) is dependent upon which body region is mechanically stimulated. We did not find significant interactions among life stage, body section, amount of lettuce

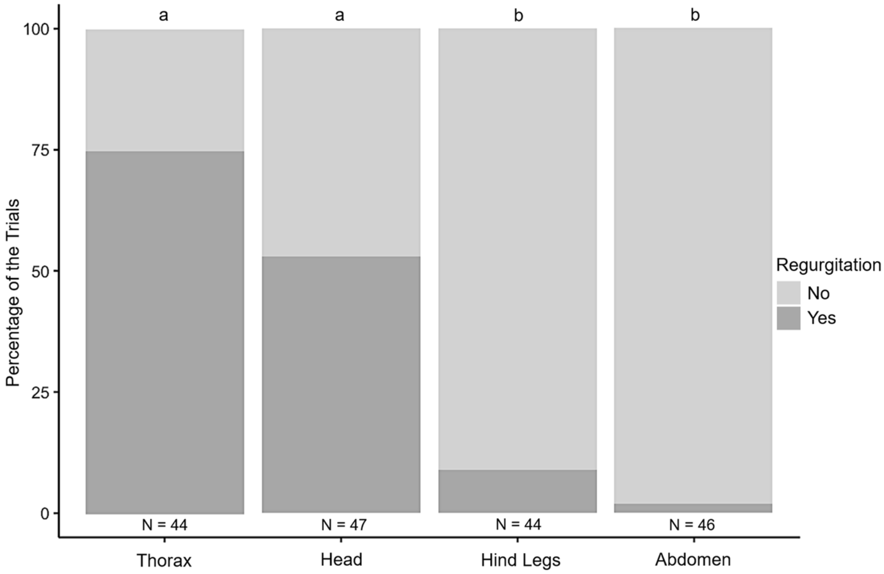

Fig. 2 The comparative proportions of regurgitation of 181 *Schistocerca serialis cubense* and which body section received the localized pinch stimulation. Significant differences are indicated by the differing lowercase letters above the bars. Total trials of each body section pinching are indicated below each bar for the corresponding body section

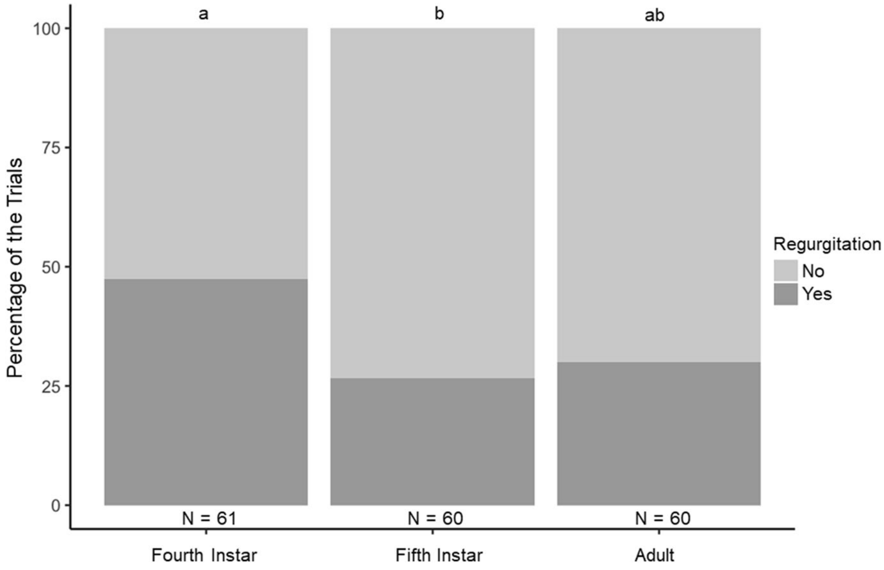

Fig. 3 The comparative proportions of regurgitation of 181 *S. serialis cubense* at different life stages. Significant differences are indicated by the differing lowercase letters above the bars. Total trials of each body section pinching are indicated below each bar for the corresponding body section

eaten, and sex on regurgitation. Our results indicate that while the stimulation of the thorax and head were not significantly different from one another, these two body regions are more likely to elicit the regurgitation behavior. Conversely, our data also suggest that the hind femora and the abdomen are body regions that are least likely to elicit regurgitation when mechanically stimulated. We propose that our results may be explained by the neuromuscular configuration concentrated in the thorax and the head regions. Additionally, we found that there are significant differences between the regurgitation response among different life stages, where 4^{th} instar individuals tend to regurgitate more often than 5^{th} instars or adults. Although our data limits our ability to draw exact conclusions, we speculate that regurgitation may have different efficiencies throughout the grasshopper development depending on the life history context of the instars. Together these results shed light on the regurgitation behavior being more complex than previously thought.

Pooled across the three developmental stages we tested, the differences in the regurgitation response between the body regions support the idea that regurgitation physiology is influenced by the anatomical heterogeneity of the insect gut and nervous system. The digestive tract of insects is divided into three distinct structures (foregut, midgut, and hindgut), each with specific functions and specializations (El Asrar et al. 2020). Each region interacts with a number of regulatory mechanisms that are governed by the interplay of the nervous system anatomy and the neuropeptides it releases (Robertson et al. 2012; El Asrar et al. 2020). In our experiments, the thorax and the head were the regions that induced more regurgitation response when stimulated, compared to the hind femora and abdomen. Anatomically, the head and thorax house the foregut, which is where the regurgitant is stored and expelled. The thorax, the region that contains most of the crop, is surrounded by a dense neuromuscular network of interconnected ganglia, nerves, and stretch receptors that conform the SNS (Hartenstein 1997; Copenhaver 2007; Wipfler et al. 2015; El Asrar et al. 2020). The proximity of the SNS with the foregut, the head, and thorax likely influences the regurgitation patterns observed. The previous claim is also supported by a previous work with caterpillars in which a strong link between the regurgitation response and foregut morphology was found (Grant 2006). On the other hand, we found that the abdomen, which houses the midgut and hindgut, was the region that was least likely to elicit a regurgitation response when mechanically stimulated. A possible explanation for these differences is that the midgut ENS lacks ganglia, and it just contains a diffuse network of nerves, whereas the hindgut is controlled by the CNS through the abdominal ganglia (Copenhaver 2007; El Asrar et al. 2020). Here, it is important to note that a question remains on whether these differences are maintained across life stages.

The principal components of the SNS are the frontal ganglion, hypocerebral ganglion, and ventricular ganglion that are interconnected and usually located below the esophagus. These ganglia innervate the musculature and the stretch receptors of the foregut (Copenhaver 2007) and have been linked to the control of the gut movement through the release of several neuropeptides that promote or inhibit gut peristalsis (Copenhaver 2007; El Asrar et al. 2020). For example, the allatoregulatory peptides/allatostatins (ASTs) inhibit foregut peristalsis in *Locusta migratoria* (Robertson et al. 2012), proctolin stimulates the contraction of the foregut and midgut in *Schistocerca gregaria* and *L. migratoria* (Banner et al. 1990; Wood et al. 1992; Lange and Orchard 1998; Clark et al. 2006), and the corticotropin-releasing factor (CRF) and related diuretic hormones (DH) have been linked to the gut movement inhibition and muscle contraction (Van Wielendaele et al. 2012; Lee et al. 2016; Urbański et al. 2019; El Asrar et al. 2020). Although the regurgitation patterns we found correlate with general differences in the neuromuscular organization of the gut, our understanding on how the anatomy and physiology of the gut regulate regurgitation behavior is practically non-existent, so further investigation is needed to elucidate how the regurgitation process is controlled.

Our results show that none of the body regions that we stimulated prompted a regurgitation response in 100% of the trials (Fig. 2). In the case of the thorax and the head treatments, we speculate that our findings can be related to the presence of stretch receptors in the foregut that sense the crop load. The stretch receptors are multipolar neurons that are present in the foregut and hindgut, and signal peristalsis through the release of diuretic hormones when the gut wall in these regions is expanded, promoting the discharge of its content (Ayali et al. 2002; Van Wielendaele et al.

2012; El Asrar et al. 2020). Thus, it is possible that these receptors could also influence the regurgitation behavior by signaling the crop to discharge only when the gut wall is expanded to a certain level. This hypothesis could also explain why the grasshoppers sometimes defecate when they are handled (personal observation). However, our GLM results indicate that adding the percentage of lettuce eaten as one of the predictors for regurgitation did not help in better explaining our data (Table 2, Table S1, Table S3). In order to understand the influence of ingested food on the regurgitation response, it may be necessary to conduct further research to test whether the stretch receptors are involved in regurgitation behavior in grasshoppers, and whether the stretch receptors maintain a consistent level of excitability as the grasshoppers age.

As our study is a baseline to explore the possible causes of regurgitation behavior, the fact that we did not observe regurgitation in all individuals across the developmental stages can also be due to other intrinsic factors such as developmental stage, self-control of the behavior, or extrinsic context-dependent factors such as the orientation, time of the stimuli, or plasticity as noted by Bateman and Fleming (2009, 2013). From the reasons mentioned above, we tested how the regurgitation response changed across the developmental stages, which can provide a baseline to explore how development influences this behavior. We found that the 4th instar nymphs regurgitated significantly more often than the 5th instar nymphs and, although there was no statistical difference, adults did seem to regurgitate less often than the two younger life stages. A possible explanation for this pattern may have to do with the differences in the life histories among different developmental stages and other available defense strategies. Regurgitation is one of several defense strategies against predation that a grasshopper can utilize. Depending on the species, life stage, and context, a grasshopper can either show aposematic coloration, startle behaviors, hide, camouflage, jump, kick, self-amputate, bite, or fly away prior to the physical encounter with a predator (Waldbauer 2012; Sugiura 2020). The use of any of these strategies instead of regurgitation to avoid predation could be dependent on the context and developmental stage of the individual. For example, younger nymphal grasshoppers are more vulnerable to predation because they lack wings, are smaller, and are weaker than older nymphs and well-developed adults, which could significantly impact their fitness in the future (Bateman and Fleming 2005; Fleming and Bateman 2007; Fleming et al. 2007). The change in regurgitation patterns across the development of juveniles has been also observed in caterpillars, in which regurgitation stopped at the cutoff between the 3rd and 4th instar (Cornell et al. 1987). Further research is needed to understand the mechanisms behind the ontogenetic changes in the regurgitation patterns in juvenile insects.

Overall, our results showed that there is a differential regurgitation response associated with stimulation of the head, thorax, abdomen, and hind legs of *S. serialis cubense*. Also, the 4th instar nymphs are more likely to regurgitate than the 5th instar nymphs and adults. The patterns we found could be partially explained by the control and proximity of the SNS to the foregut, the lack of ganglia or stretch receptors in the midgut ENS, and the control of the midgut by the CNS. Our results are not enough to determine whether this behavior is just a reflex product of physiological responses to the application of pressure to the body, or if there is some sort of voluntary control by the individual. Further research is necessary to understand in detail the neuromuscular pathways involved in the regurgitation response and to shed light on what causes the phenomenon of regurgitation.

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Author Contributions JHMD, AJD, and HS contributed to the conception and design of the research. Material preparation and data collection was done by JHMD, AJD, CL, and JS. Data analysis was conducted by CL and JS. All authors contributed to the writing and preparation of the manuscript for submission. All authors read and approved the final manuscript.

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Data Availability The dataset is included as supplementary information of this article.

Code Availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate All authors consent to participate in the present work.

Consent for publication All authors consent to submit the work for publication to the Journal of Insect Behavior.

Conflict of interest The authors declare no conflict of interest.

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