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Effects of Host-plant Density on Herbivores and Their Parasitoids: A Field Experiment with a Native Perennial Legume

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

EFFECTS OF HOST-PLANT DENSITY ON HERBIVORES AND THEIR PARASITOIDS: A FIELD EXPERIMENT WITH A NATIVE PERENNIAL LEGUME

A thesis submitted in partial fulfillment of the
requirements for the degree of
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Andrea Salas
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To: Dean Michael R. Heithaus
College of Arts, Sciences and Education

This thesis, written by Andrea Salas, and entitled Effects of Host-plant Density on Herbivores and Their Parasitoids: A Field Experiment with a Native Perennial Legume, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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The thesis of Andrea Salas is approved.

Dean Michael R. Heithaus
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Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2016

DEDICATION

I dedicate this thesis to all who provided unconditional support to complete this study. I thank my family, professors, co-workers, friends and the department of Earth and Environment whose motivation and contributions were the engine to achieve success during this journey. I want to thank my research assistants whose love, support, and appreciation for this project encouraged me to keep on working in this rewarding field.

ABSTRACT OF THE THESIS

EFFECTS OF HOST-PLANT DENSITY ON HERBIVORES AND THEIR PARASITOIDS:

A FIELD EXPERIMENT WITH A NATIVE PERENNIAL LEGUME

by

Andrea Salas

Florida International University, 2016

Miami, Florida

Professor Krishnaswamy Jayachandran, Major Professor

Senna mexicana chapmanii (Fabaceae: Caesalpinoideae), an attractive and threatened species native to pine rocklands of southern Florida, is consumed by folivorous caterpillars of Sulfur butterflies (Lepidoptera: Pieridae). Caterpillars may be deterred or eaten by predators, but also very important are parasitoids, both flies and wasps. This study investigated the effects of plant density on Sulfur caterpillar numbers and rates of parasitization.

Senna mexicana chapmanii plantations were established at agricultural and urban areas; both sites are adjacent to protected pine rockland areas. Sulfur butterfly immature stages were collected and reared to glean information regarding number of herbivores and rates of parasitization. Continuing this weekly monitoring protocol over the course of a year provided data to determine that higher plant density has an effect on levels of parisitization and is correlated with the number of herbivores. Elucidating these patterns has important implications understanding the factors that regulate interactions in this plant/herbivore/parasitoid system.

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1. INTRODUCTION

Climate change is expected to affect the productivity of plants of anthropogenic importance, particularly through fluctuations in the population distribution of beneficial insects such as parasitoids (Ali, 2013). Parasitoids play a key role in agro-economies and complex ecological interactions of a variety of ecosystems. Because of their dependence on a host for growth, survival and reproduction, parasitoids have developed abilities to recognize cues elicited from their hosts and its habitat. Host-location abilities have categorized parasitoids as effective biological control agents for important pests in agricultural and conservation settings. Despite the importance of parasitoids as beneficial insects, few studies address the influence of vegetation spatial attributes on the effectiveness of parasitoids' host-location strategies.

Spatial attributes of plant patches can affect parasitoids' host-location strategies. According to the resource concentration hypothesis, number of herbivores would increase with increasing plant patch size because these folivores may be more likely to find or less likely to leave large plant patches (Sheehan and Shelton, 1989). Few studies, however, have associated plant spatial attributes such as size and isolation, with parasitoids' host-location strategies (Fenoglio et al., 2013). One of these studies demonstrated that a parasitoid's ability to detect visual, chemical and mechanical cues derived from its host and its substrate is enhanced as a result of enlarged signals from high host-plant density patches (Sheehan and Shelton, 1989). Also, herbivores and their parasitoids' host-plant patches distribution may resemble metacommunities because of insect dispersal, which can be affected by physical barriers (Fenoglio et al., 2013). The migration, extinction and abundance of insects can be affected by the density and spacing of host-plants (Barbosa and Barbosa, 1998).

As intensity of urban and agricultural development increases, natural areas tend to become reduced, isolated and difficult environments for insects to maintain viable populations (Luck and Wu, 2002). In addition to host-plant density, habitat structure or diversity can influence herbivore feeding behavior and parasitoid abilities to find hosts (Tscharntke et al., 2002). For example, intra- and interplant movement of herbivores can be affected by abiotic factors such as wind speed, relative humidity, and precipitation, thereby affecting a parasitoid's ability to recognize visual and chemical stimuli elicited by the host and its host-plant (Barbosa and Barbosa, 1998; Bezemer et al., 2010; Godfray, 1994). On the other hand, diverse plant communities may provide sources of nutrition that favor herbivore survival over adult parasitoids (Barbosa and Letourneau, 1988). Therefore, because patterns of distribution and abundance of a species can vary in time and space, long-term field observations are crucial to the study of herbivores and their parasitoids.

The current study aims to understand the effect of plant density on folivorous caterpillar numbers and rates of parasitization in urban and agricultural environments. Particularly, this study investigated if higher host-density leads to greater sulfur butterfly caterpillar (folivore) numbers and greater levels of parasitization, or if host-plant density has a different, or no effect, on pierid herbivores and their biocontrol agents. Elucidating the patterns will be important in understanding the factors that regulate the numbers of folivores and their parasitoids.

Here we specifically ask: i) Does host-plant density affect the number of caterpillar herbivores? ii) Does host-plant density affect the rate of parasitization of the herbivores? and, if so, iii) Does the number of caterpillar herbivores affect the rate of parasitization? and iv) How do all of the above differ between urban and agricultural sites?

We hypothesize that (Figure 1): i) the number of herbivores will be greater in high density host-plants than in medium or low density of plants; ii) the rate of parasitization will be greater in high density of plants than in medium or low density of plants; iii) the rate of parasitization will be positively correlated with number of herbivores; and that iv) the number of herbivores will be greater in agricultural than urban sites.

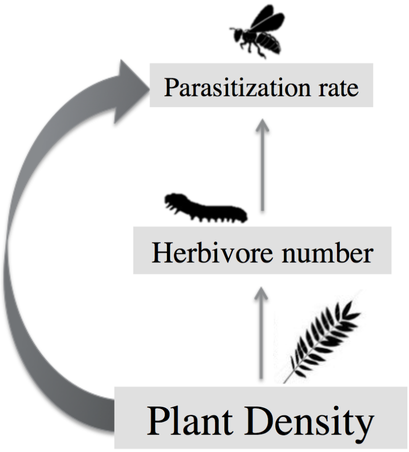

Figure 1: Diagram of parasitoid/herbivore/host-plant interaction

2. LITERATURE REVIEW

2.1 Introduction to Host-location Mechanisms

In an effort to discover and describe natural enemies that have the potential to control populations of lepidopteran pests, scientists have engaged in the study of parasitoids. Biological control (biocontrol) schemes originated as a result of the need to cope with environmental, health and economic issues linked to agricultural and conservation settings such as pest resistance to pesticides, economic loss, biological and environmental pollution, and loss of biodiversity (Menalled et al., 2003). Therefore, parasitoids have been frequently studied through laboratory experiments, where their ability to locate hosts has been examined.

Parasitoid-host relationships occurring in the class Insecta have been observed by numerous researchers; many studies have shown that certain species from the orders Diptera, Hymenoptera, Neuroptera (Redborg, 1982), Coleoptera (Weber et al., 2008), Strepsiptera (Kathirithamby, 2008), Trichoptera (Wells, 1992), and Lepidoptera (Pierce, 1995) interact as parasitoids. Among the orders studied for their numerous parasitoid species and host-location strategies are the orders Diptera and Hymenoptera (Godfray, 1994).

As a result of natural selection, parasitoids have evolved diverse strategies to find hosts contributing to their success in growth, reproduction and survival. Parasitoids are defined as intermediates between predators and true parasites as a result of their complex ecological relationships (Godfray, 1994). Parasitoid-host relationships occur because of the dependency on a host for nutrition and shelter for completion of the development of immature parasitoids' offspring. Diverse strategies used by parasitoids to achieve successful parasitization can be described through several steps.

Host-location strategies used by parasitoids belonging to the orders Diptera and Hymenoptera can be described as a "hunt cycle" (Figure 2). During this cycle, a naïve (without previous laying egg experience) female parasitoid emerges from a pupa, searches for food, and mates with a male parasitoid. The gravid (full of eggs) female then engages in host searching by sensing chemical cues from the habitat in which the host could potentially reside. Once the female observes the form and structure of a plant on which the host may be located, she explores it to perceive vibrations that signal the presence of an active host. The chemical cues that originate directly and indirectly from the host stimulate the parasitoid to visualize and to oviposit (lay eggs) near or inside the host. The larva that emerges from the parasitoid egg feeds on and subsequently kills the host (at a certain stage of host development) and develops into a new adult parasitoid that repeats the mechanisms of host location (Price, 1975). While parasitoids' host location strategies vary among species, it is possible to identify cues that contribute to their capacity to find a host (Godfray, 1994; Hawkins, 1994; Price, 1975).

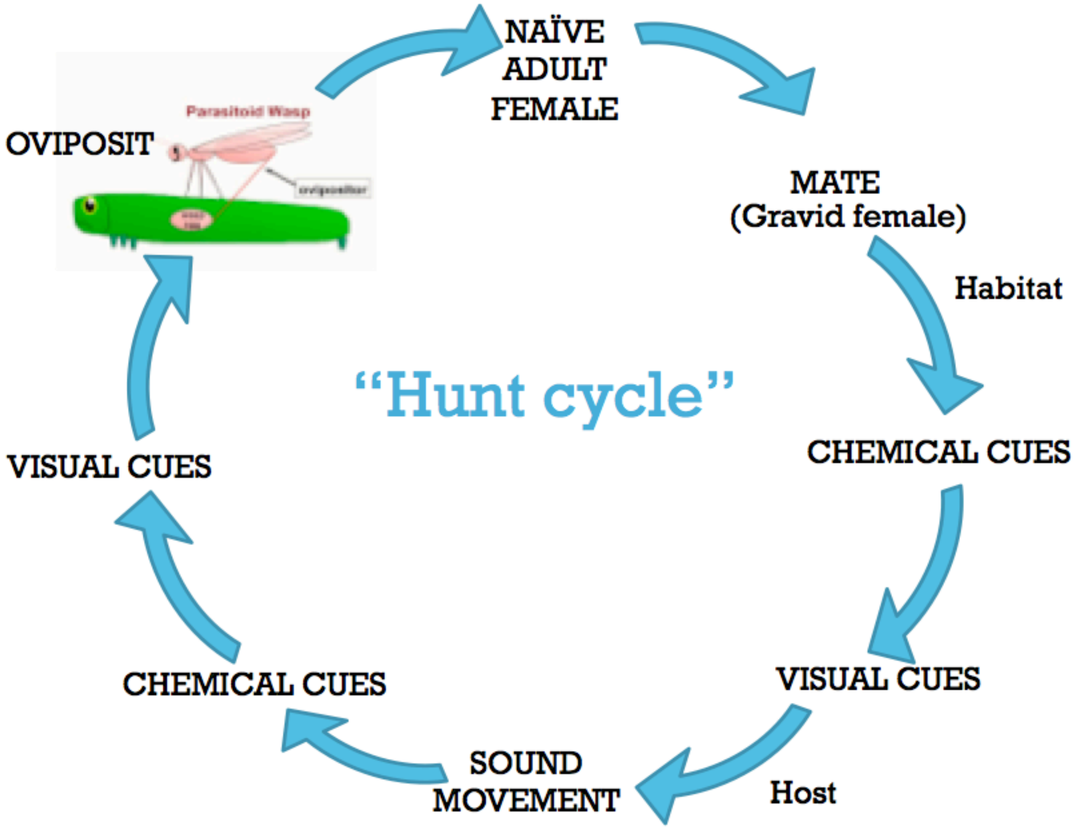

Figure 2: Mechanisms of Host-location. Drawing from website www.polatrec.com.

Host location strategies contribution to biocontrol methods may vary according to the species of the individuals and environmental factors affecting their performance. Hosts possess certain size, color, odor, shape and behavior that would provide information regarding their species and life stage. For example, a certain species of the order Hymenoptera may respond to volatiles (substances easily evaporated at normal temperatures) from a specific host species or species closely related to its host (De Rijk et al., 2013), while other species of the same order may attack multiple species of hosts (Henry and Roitberg, 2009). Also, abiotic factors may interfere with parasitoids' ability to respond to stimuli associated the host-plant, microhabitat and the host itself (Godfray, 1994). Therefore, results from studies performed in laboratories may not accurately measure the effectiveness of parasitoids' host location strategies. In this literature review, we highlight some of the host location strategies studied in laboratory settings, and speculate about factors that could influence the results of the experiments conducted.

2.2 Laboratory studies of Host-location Mechanisms

2.2.1 Visual Cues

Visual cues allow parasitoids to recognize the size, color or shape of hosts or host substrates (host's source of nutrition). Lobdell et al. (2005) indicated that the egg parasitoid, *Trichogramma ostriniae,* using color as a visual cue, has the potential to control the density of the lepidopteran *Ostrinia nubilalis* in maize fields. The parasitoid's behavior towards yellow, black, white, and green beads representing the eggs was analyzed using a Petri dish arena with green background. Based on the proportion of *Trichogramma ostriniae* that attempted to insert their ovipositors into the beads, the most preferred color was yellow and the least preferred was black. Parasitoids' color preference, however, could be linked to information regarding hosts' identity, developmental stage, substrate, or previous parasitization (Lobdell et al., 2005). In addition, parasitoids' behavior towards stimuli is affected by mechanical cues that evoke further responses.

2.2.2 Mechanical Cues

Parasitoids react to mechanical stimuli that originate from their hosts. A study whose purpose was to develop a control for leafminers (*Phyllonorycter* sp.), a pest that affects apple orchards, demonstrated that parasitoids have the ability to detect vibrations (Meyhöfer et al., 1997). Scientists identified vibrations emitted by moving, feeding, and wriggling behavioral states of leafminers and observed the behavioral responses of their parasitoids, *Sympiesis sericeicornis*, in artificial mines. The results indicated that *Sympiesis sericeicornis* reacted by increasing their probing (insertion of egg laying organ) frequency, pausing, and extending the time spent searching for food in response to the mechanical signals (Meyhöfer et al.,1997). Furthermore, parasitoids recognize vibrations and other cues to obtain information regarding the quality of their hosts (Casas et al., 1998)

2.2.3 Chemical Cues

Chemical cues are among the most frequently studied mechanisms of host location used by parasitoids. Parasitoids' bodies are covered with chemoreceptors, which aid in the recognition of kairomones. Kairomones are defined as chemical stimuli secreted by a signaler who benefits the receiver (Price, 1975). For example, host-derived products such as frass and saliva leave traces that can be recognized by parasitoids' sensory setae. The importance of chemical stimuli relies on the variety of information it provides regarding the hosts, as well as the habitat of the hosts.

Stemborer larvae are widely studied because of their destructive nature in maize and sorghum fields of Africa. The ability of *Cotesia flavipes* to recognize chemical cues elicited by stemborer larval hosts was determined in a study (Obonyo et al. 2010) , in which the recognition of kairomones from body extracts and feeding activity secretions derived from host and non-host were compared through ovipositor (egg laying organ) insertions and antennating (touching with the antenna) by parasitoids. Both hosts and non-hosts washed with distilled water elicited no response from the parasitoid. In contrast, when the host and non-host were washed with hosts' body extracts, the parasitoids displayed ovipositor insertion behavior. Regurgitations and frass (excreta) of the host induced antennating behavior at short distances of exposure. The study indicated that *Cotesia flavipes* uses chemical cues to recognize and accept its host, depending on the proximity of the host and the product of the feeding activity of the stemborer larvae (Obonyo et al., 2010). While laboratory experiments demonstrate that parasitoids can recognize cues originating from their host, parasitoids' host-location strategies can be affected by factors not considered during these experiments, such as the host response to attack or changing characteristics of the host-plants and the environment

2.3 Factors that affect Host-location Strategies in the Field

Findings regarding host-location strategies used by parasitoids under controlled conditions of laboratories may differ from results obtained from field studies (Casas, 1989). For example, in agricultural, urban, or natural areas, parasitoids' abilities to discriminate among suitable hosts may be affected by wind, rain, light availability, or temperature, subsequently affecting the ability of parasitoids to locate their host (Bezemer et al., 2010). Therefore, it is important to consider multiple factors that can affect the ability to recognize visual, mechanical and chemical cues can be affected by the hosts and its environment.

2.4.1 Host-derived Factors

Stimuli may be limited by hosts' defense mechanisms to avoid detection of host-derived cues. For example, cryptic coloration allows hosts to blend with their surroundings and avoid being visualized by parasitoids (Godfray, 1994). In addition, there are a few field studies that show that herbivore partial consumption of leaves or plant part preference can affect strategies to avoid parasitization (Stamp and Casey, 1993). Also, hosts, such as stemborers, can attack parasitoids in stem tunnels by taking advantage of its restricted movement (Casas, 1989). Therefore, when studying host-location strategies it may be relevant to consider the influence of host-plants on parasitoids response to cues.

2.4.2 Plant-derived Factors

Field studies recognize that cues from plants may be crucial in the recognition of stimuli. For example, plant spatial attributes can vary according to the habitat that harbor hosts (Fenoglio et al., 2013). There are few studies, however, that consider the effects of host-plant spatial attributes on host abundance and rates of parasitization. One of these studies present evidence that recognition of visual and chemical cues by parasitoids can be positively affected by high host-plant density and reduced plant spacing, suggesting that cues derived from plants may be easier to detect that cues elicited by its host (Sheehan and Shelton, 1989). In addition, other insects that have mutualistic relationships with plants may attack beneficial visitors of plants such as parasitoids (Barbosa and

Letourneau, 1988). Parasitoids may encounter challenges, however, when relying on plant-derived signals.

2.4.3 Physiological Factors

Parasitoids mechanisms of host-location can be affected by physiological changes in host-plants. Most information regarding chemical stimuli recognized by parasitoids using olfactometer tests may not be reliable. Indeed, olfactometer tests in which parasitoids are stimulated while walking do not consider that response when walking could vary from a response to the same cue when flying and it is unlikely to identify responses to cues at long distance from these tests (Tscharntke et al., 2002). Furthermore, parasitoids may be attracted to volatiles that plants release as a result of herbivory from non-hosts or suitable hosts (Bukovinszky et al., 2012). Some studies have shown that conflict between defense against herbivores and attraction to parasitoids can occur when allelochemicals sequestered by herbivores affect the development or survival of immature stages of parasitoids (Barbosa and Letourneau, 1988). At the same time, physiological changes in the parasitoids can influence their host-searching behavior according to the environmental conditions in which it inhabits.

Physiological conditions of parasitoids can be affected by their habitat. Some habitats, such as Urban areas, may be highly fragmented, restricting movement of female parasitoids to sources of nutrition (Bianchi et al., 2006), which could provoke hunger or impede finding hosts when ovaries are mature, decreasing the opportunity for oviposition (Lewis et al., 1990). Also, the life cycle of certain parasitoid species and hosts may not occur simultaneously, especially when diapause is observed (Bale et al., 2008).

Therefore, considering the influence of the habitat conditions on parasitoids can provide a better understanding of the effectiveness of parasitoids' host location strategies.
Studies conducted in laboratory conditions can identify possible visual, mechanical, and chemical cues elicited by hosts which subsequently could be recognized by parasitoids; however, they cannot guarantee the effectiveness of parasitoids' to recognize these cues when subjected to other factors in the field (Lewis et al., 1990). Studies that consider parasitoids' ability to parasitize hosts as learned, and also genetically determined and heritable, suggest that parasitoids that demonstrate better use of host-location strategies should be selected to breed and establish a population that would enhance the overall performance in the field (Henry et al., 2010). Also, the abiotic and biotic factors such as altered nutritional quality, defensive capabilities and morphological characteristics of host-plants, light intensity, and temperature that influence the recognition of cues (Godfray, 1994) should be integrated during studies of mechanisms of host-location. Further studies should incorporate the environment of the habitat that parasitoids have colonized, or the habitat in which they will be released as to find hosts (Bale et al., 2008).

3. METHODS:

3.1 Study site

The study was carried out at two field sites: the University of Florida's Tropical Research and Education Center (UF-TREC), in Homestead, Florida, and the USDA Subtropical Horticulture Research Station, in Miami, Florida. The first site (UF-TREC) is located in the midst of agricultural areas in southern Miami-Dade county, and the second site (USDA) is in the midst of urban/suburban development. The regional climate of these sites is classified as subtropical, with average annual temperatures fluctuating between 3.2 – 24.8°C in January and 22.7 – 32.4°C in July. The mean annual precipitation is around 1496 mm. The sites elevations are close to sea level, and the substrate consists of flat calcareous limestone rocklands. At each of these sites, there is an adjacent fragment of pine rockland habitat, an imperiled ecosystem with remnants only in protected natural areas of south Florida (Koptur et al., 2015).

3.2 Study species

Senna mexicana chapmanii (Fabaceae: Caesalpinoideae) is an attractive ornamental and threatened species native to southern Florida. Currently state-listed as threatened (Atlas of Florida Plants), this species grows naturally as an upright or sprawling subshrub, up to 1.2 m in height, spreading broader than tall in the pine rocklands and rockland hammock edges of Miami-Dade and Monroe counties, as well as in the Bahamas and Cuba. Plants of *S. mexicana chapmanii* are visited by beneficial insects that feed on pollen from flowers and nectar from extrafloral nectaries that occur on the pedicels of flowers or throughout the foliage between basal leaflets. Foliage of *S. mexicana chapmanii* is consumed by folivorous caterpillars of sulfur butterflies (Lepidoptera: Pieridae). Caterpillars may be deterred or eaten by predators (such as ants, vespid wasps, spiders, and coccinellid beetles), but also very important are parasitoids (Koptur et al. 2015), both flies (Diptera) and wasps (Hymenoptera).

3.3 Experimental design

Senna mexicana chapmanii plants were propagated from scarified seeds, soaked and then planted in trays, and potted up to three gallon-sized pots under greenhouse conditions. After 6 months, 288 plants were transplanted into agricultural and urban sites adjacent to protected pine rockland areas. Plantations were established at experimental sites according to a modified plant spacing design known as Nelder's wheel (Nelder, 1962). In three areas at each site, and three replicate plots per area, seedlings were planted in a semicircular array with 16 plants each at distance of 1 m, 5 m, and 10 m from other individuals, representing high, medium, and low host-plant densities, respectively (Figure 3). At each site, each of the three plots contained three semicircular arrays and 16 plants per semicircle. Irrigation was necessary to aid the establishment of plants during the hot and dry month of May 2015. Field observations began in June 2015 and continued until April 2016.

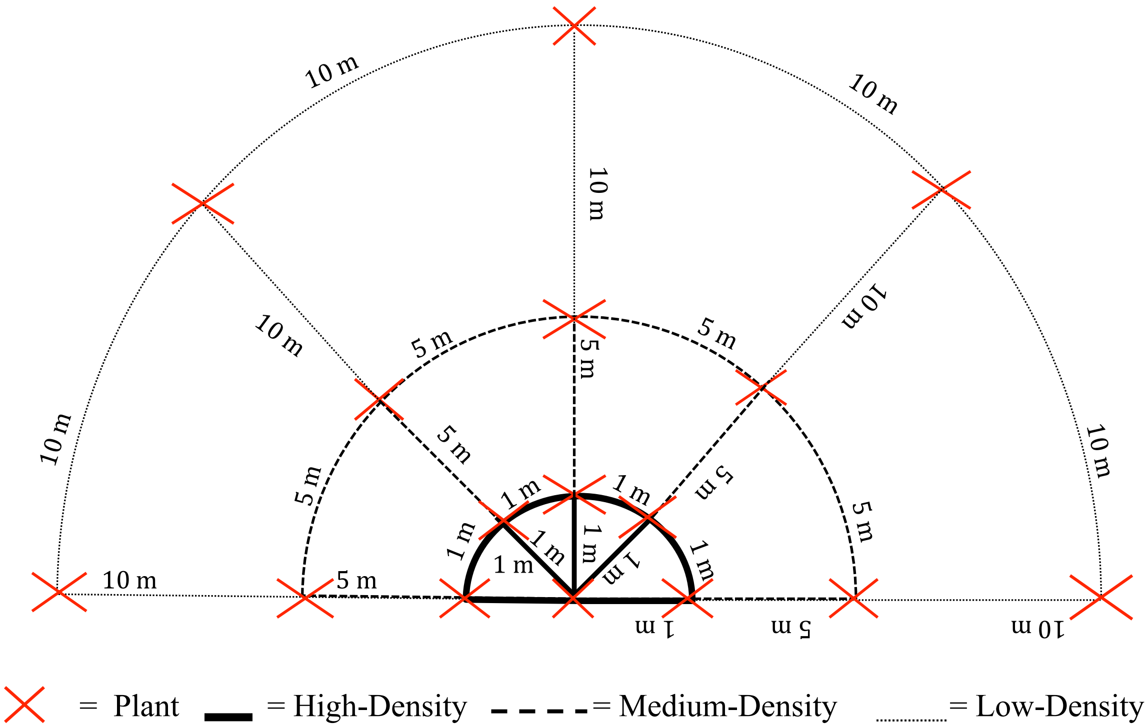

Figure 3: Experimental design (Semicircular array)

3.4 Data collection

During the experimental period, each plant from high, medium and low densities was sampled using a weekly monitoring protocol. The number of sulfur folivores in immature stages (eggs and 1st, 2nd, 3rd, 4th and 5th instar larvae) was recorded, and all herbivores were collected, during morning hours. Herbivores were reared following the procedure described in Koptur et al. (2015), using 1-gallon plastic bags per herbivore to avoid spread of disease, and feeding and monitoring in the laboratory until complete development, death, or emergence of parasitoid larvae. Parasitoid larvae that pupated were placed in glass vials with loosened caps to allow the emergence of adult parasitoids. Parasitoid specimens were preserved in a 75% ethanol solution for further identification.

Statistical analysis

Sampling plants from high, medium, and low host-plant densities was performed to assess the effect of host-plant density on the number of herbivores and rates of parasitization at urban and agricultural sites. Because several species of sulfur butterflies (*Phoebis sennae*, *Phoebis philea*, and *Abaeis nicippe*) using *Senna mexicana chapmanii* as a host-plant have caterpillars with similar morphology, it is impossible to discern the exact species attacked by parasitoids. Consequently, we refer to the caterpillar herbivores collectively as sulfurs. From herbivores collected at the second instar of development, parasitoids of the order Hymenoptera were observed emerging at the second instar of herbivore development, while from an herbivore collected at the third instar of development, a parasitoid of the order Diptera emerged from the pupa stage of development. In addition, though further identification is required for parasitoids, a few of them belong to the genera *Apanteles, Brasema, Ceraphron*, and *Encrateola*. Agricultural areas represented 81% and urban areas represented 19% of the overall herbivore collection. Analyses were performed using general linear model (GLM) with *post hoc* comparison of means, for which normal distribution was approximated via bootstrapping. Because there was presence of herbivores at both sites but parasitoids were found only at the agricultural site (Table 1), the effects of sites, plots, semi-circles, host-plant density, and the interaction of density and sites on the number of herbivores at both sites were analyzed separately from the effects of semi-circles, host-plant density, number of herbivores, and the interaction of host-plant density and number of herbivores on the number of parasitoids at agricultural sites. Analyses for parasitoids also investigated the relationship between the number of parasitoids and the number of herbivores at the agricultural site. In addition, an Analysis of Variance (ANOVA) was carried out to compare mean number of herbivores across sites and to determine the difference on the mean number of herbivores at different developmental stages (egg and 1st, 2nd, 3rd, 4th, 5th instars) across host-plant densities of both sites. Statistical analyses were performed using SPSS 22.

4. RESULTS

In this study, a total of 2904 individuals resulted from the sum of herbivores (immature developmental stages) collected from different host-plant densities at both experimental sites. Although the effects of sites, plots, semi-circles, and host-plant density were not significant, and the relationship between number of herbivores and host-plant density did not differ between sites (Table 2), the mean number of herbivores differed significantly between experimental sites (Figure 1). On average there were 12.653 more herbivores per plant at the agricultural site than the urban site.

An Analysis of variance (ANOVA) showed a significant difference in the mean of number of specific developmental stages of herbivores across host-plant densities (Table 4). According to the post hoc analysis, there was a difference in the number of third instars between high host-plant density and other host-plant densities at the agricultural site. On average, high host-plant density had 1.400 more third instars than medium host-plant density, and 1.044 more third instars than low host-plant density (Figure 5). Also, the mean number of fourth instars differed significantly between high and low host-plant densities, and on average, high host-plant density had 0.581 more fourth instars than low density at the agricultural site (Figure 6). In addition, there was a difference in the number of fifth instars between medium and low host-plant densities, and on average there were 0.897 more fifth instars on medium host-plant density than low density at the agricultural site (Figure 7).

While the effect of semi-circles, host-plant density, and number of herbivores were not significant, and the relationship between number of parasitoids and number of herbivores did not differ between host-plant densities, the number of herbivores had an effect on the number of parasitoids (Table 3). Overall, the relationship between the number of herbivores and parasitoids showed a significant moderate correlation between the number herbivores and number of parasitoids at the agricultural site (Figure 8); that is, number of parasitoids increases with an increase in number of herbivores. However, breaking down for each planting density, there was a moderate correlation for high density while for medium and low host-plant density there was a low correlation (Figure 9).

5. DISCUSSION

We first present experimental evidence that the density of *S. mexicana chapmanii* does not significantly affect number of herbivores. While some studies suggest that host-plants growing at shorter plant spacing would grow smaller in size and would be less preferred for oviposition than plants growing at larger distance (Finch and Skinner, 1976), *S.*

mexicana chapmanii growing at different plant spacing did not exhibit prominent differences in plant size in this study. Indeed, plants reached a similar size, and minimal differences observed may reflect differences in genotype or light availability. Because *S. mexicana chapmanii* did not differ greatly in size, and there was no significant difference in herbivore numbers among host-plant densities, female butterflies may maximize their own fitness by laying eggs singly in low or medium density host-plants, rather than high host-plant density already occupied by greater number of competitor herbivores, thereby avoiding competition among females for oviposition sites (Thompson and Pellmyr, 1990) and attack from natural enemies (Mousseau and Fox, 1998). Therefore, patterns of plant density and spacing can affect the behavior of sulfur butterflies, thus affecting the ability of natural enemies to inflict mortality on herbivores by consuming and parasitizing their immature stages.

Results show a correlation between number of herbivores and rate of parasitization in high host-plant densities. This could be merely a result of maximized host-location stimuli with increasing herbivore activity in high host-plant densities (Bezemer et al., 2010). The proximity of neighboring host-plants in high densities probably provided favorable changes in light intensity, moisture, and temperature for herbivores (Casey and Stamp, 1993). Contact between branches of host-plants located at shorter plant spacing and microcrimate conditions may have facilitated interplant movement and distribution of active hosts, increasing herbivore consumption of plant tissues, and production of frass and saliva. These may have in turn strengthened visual or chemical cues that stimulated parasitoids to find or exploit hosts in high host-plant density at a shorter amount of time, decreasing discovery time of hosts (Barbosa and Letourneau, 1988).

Rather than conspicuous and gregarious behavior, sulfur folivores may exhibit cryptic behavior to hide from parasitoids, probably by incorporating *Senna mexicana chapmanii* host-plant chemicals (Hawkins, 1994). There is no certainty regarding the exact stage in which caterpillars were attacked by parasitoids, just when the parasitoid adults emerged. Different parasitoids likely attacked their caterpillar hosts at different stages: Hymenoptera parasitoids emerged from second instars may have come from eggs or tiny larvae parasitized. Diptera parasitoids that emerged from pupae may have come from eggs laid on leaves and consumed by caterpillars, or oviposition by female flies on caterpillars. Also, at the field sites, it was possible to observe that yellow eggs were laid singly by female butterflies on yellow flower buds and edges of light green new leaves of host-plants, yellow early instars (first and second instars) that resembled the color and shape of flower parts were feeding from flowers and new leaves, while late instars (third, fourth and fifth instars) caterpillars exhibit coloration such as green or yellow with black stripes or green with blue dots, which resembles the dark green color of the older leaves or yellow color of flowers consumed; perhaps this was camouflage that helped them to avoid detection by predators or parasitoids that rely on visual cues to find hosts. However, hosts feeding on small new leaves rather than large old leaves may be more vulnerable to attack because less surface area may increase searching efficiency of parasitoids (Barbosa and Barbosa, 1998). On the other hand, while parasitoids may have used host-derived chemicals (e.g. frass) as host-location cues (Godfray, 1994), herbivores undergoing a certain larval developmental stage may have released incorporated host-plant odors as chemocryptic strategies in the absence of motion to avoid being attacked by parasitoids that use chemosensory organs to find hosts (Casey and Stamp, 1993). Also, it is possible that parasitoids attack or emergence occurred at specific instars to avoid detrimental effects of allelochemicals sequestered by herbivores, such as egg encapsulation, which could affect the development or survival of immature stages of parasitoids (Barbosa and Barbosa, 1998). Therefore, parasitoid/host/plant interactions mediated by chemicals can induce multiple responses.

Senna mexicana chapmanii, like many plants in the Fabaceae family, is likely to form associations with plant symbionts such as mycorrhizal fungi and rhizobia which can aid the development of mycelial networks that allow communication between neighboring plants (Scheublin et al., 2006; Babikova et al., 2013), thereby affecting parasitoid/host/plant interactions. It is possible that herbivores feeding on high host-plant densities could induce the release of allelochemicals through roots and that mycelial networks could have transported these allelochemicals as warning signals inducing infested and uninfested neighboring plants to the release of volatile organic chemicals to recruit parasitoids. While host-plants can play an important role in defense against herbivory and complexity of food webs, there are other factors that may have affected the results of this study.

Differences between areas may reflect differences in the habitat landscape surrounding the experimental sites. The effect of host-plant density on the number of herbivores was similar in both sites, but there was no parasitization observed and the number of herbivores was lower in the urban site. While high temperatures in urban areas can favor insects' development (Youngsteadt et al., 2015), this may have occurred because the population abundance of predators may have been greater in urban areas (Holt, 1996), probably increasing interspecies competition or inflicting mortality on parasitoids (Aars and Ims, 1999). For example, some studies show that ants visiting the extrafloral nectaries of *S. mexicana chapmannii* can reduce herbivore numbers (Jones and Koptur, 2015) and also reduce the numbers of other beneficial insects on herbivores (Koptur, Jones and Pena, 2015). Likewise, greater fragmentation of habitats in urban areas (McKinney, 2006) could have restricted access to alternative host-plants (e.g. *Cassia javanica*, *Senna polyphylla* and *Senna surrattensis*) to parasitoids because their small body size may allow shorter dispersal than their hosts in isolated patches (Daoust et al., 2012; Tscharntke et al., 2004). In addition to the diversity and distribution of host-plants (Kareiva, 1987) and perhaps artificial lighting (Davies et al., 2012), there may be other differences between sites that could affect herbivores and their parasitoids' abilities to find hosts or nectar sources, favorable microclimate and protection from predators (Fenoglio et al., 2013), which in turn may influence their longevity (Dyer, 1996), fecundity and survival (Landis et al., 2000;).

Agricultural practices such as fertilization, irrigation, and pesticide application can affect the quality or morphology of host-plants (Barbosa and Barbosa, 1998). While there was no size difference between plants among different densities, plants in agricultural sites grew bigger in size and a greater number of herbivores were observed. Favorable conditions for plant growth in agricultural sites may suggest changes in plant canopy structure and higher levels of nutrients such as nitrogen, which in turn can affect oviposition preferences, and feeding behavior of sulfur caterpillars (Casey and Stamp, 1993). Furthermore, while insects can be susceptible to pesticides (Coats et al. 1979), ambient levels of pesticides in areas non-populated by humans may be lower than in urban areas where application of aerial pesticides for mosquito control may have a greater effect on other organisms (Dhang, 2014). In addition, plant physical attributes such as size, shape, light intensity, color, and herbivory damage may have influenced parasitoid abilities to locate hosts by recognition of visual cues by host-plants (Barbosa and Letourneau, 1988). Although we found that the number of herbivores differed between sites and the number of herbivores affected rates of parasitization, other factors deserve further exploration for understanding of parasitoid/host/plant interactions.

6. CONCLUSIONS

Findings of this study underline the importance of host-plant physical attributes and the surrounding landscape in the study of parasitoid-host interactions. The results presented clearly indicate that parasitoids host-location strategies were greatly influenced by the number of herbivores. The lack of significant difference in the number of herbivores harbored by different densities of host-plants and the absence of natural enemies in the urban site suggest that long-term studies of parasitoid/host/plants interactions are needed in varying environmental field conditions. Moreover, given the importance of *Senna mexicana chapmanii*, a threatened species native to Florida, and parasitoids for its potential control the population of other herbivores that could be pests, we expect that these findings promote further studies that contribute to the preservation of native plants and their beneficial insects.

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TABLES

Table 1. Number of herbivores and percentages of parasitization at both sites categorized according to host-plant density. Significant differences are indicated with different letters.

Matrix	Host-plant density	Number of herbivores	% parasitized
Agricultural	High	968	6^c
	Medium	709	3^b
	Low	686	2^b
Urban	High	249	0^a
	Medium	156	0^a
	Low	136	0^a

Table 2. The effects of plots, semi-circles, host-plant density, number of herbivores and host-plant density*sites interaction on the number of parasitoids. Results based on samples from host-plants within the urban and agricultural sites.

Factor	**DF**	**F**	**p**
Sites	1	42.900	.000
Plots	1	1.939	.165
Semicircles	1	2.109	.148
Host-plant density	2	2.052	.130
Host-plant density * Sites	2	.154	.857

Table 3. The effects of semi-circles, host-plant density, number of herbivores and host-plant density*number of herbivores interaction on the number of parasitoids. Results based on samples from host-plants within the agricultural site.

Factor	DF	F	P
Semi-circles	6	1.759	.113
Host-plant density	2	.068	.934
Number of herbivores	1	25.604	.000
Host-plant density * Number of herbivores	2	.897	.410

Table 4: Analysis of variance (ANOVA) for the effect of plant density (low, medium, and high) on mean number of herbivores at urban and agricultural sites:

Factor	**DF**	**Eggs**		**1st instar**		**2nd instar**		**3rd instar**		**4th instar**		**5th instar**	
		F	***p***	**F**	***p***	**F**	***p***	**F**	***P***	**F**	***p***	**F**	***p***
Host-plant density	2	.103	.902	.166	.847	2.346	.098	3.820	.023	3.022	.050	5.427	.005
Sites	1	115.987	.000	93.943	.000	54.583	.000	21.931	.000	16.160	.000	22.330	.000
Host-plant density * Sites	2	.909	.404	.144	.866	.252	.778	2.926	.065	1.501	.225	2.106	.124
Error	282												

FIGURES

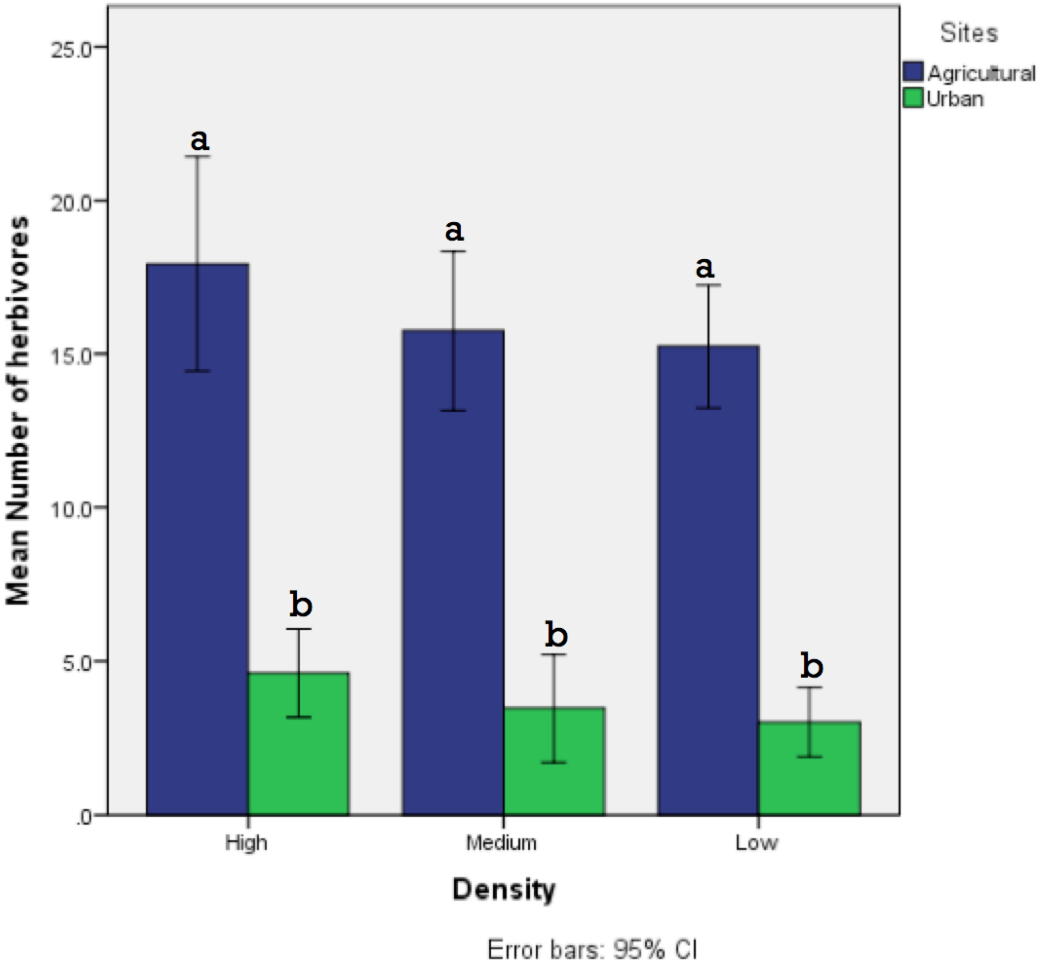

Figure 1. Mean number of herbivores at both sites categorized according to host-plant density. Significant differences are indicated with different letters.

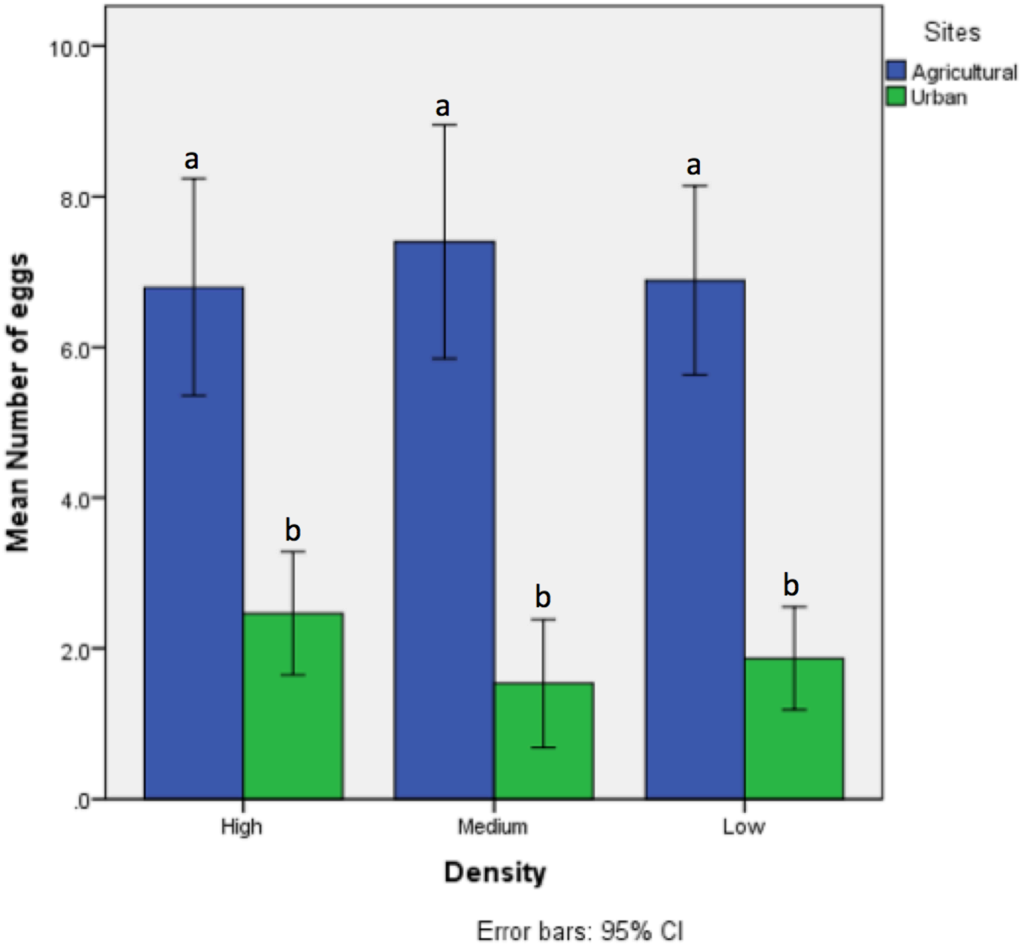

Figure 2. Mean number of eggs at both sites categorized according to host-plant density. Significant differences are indicated with different letters.

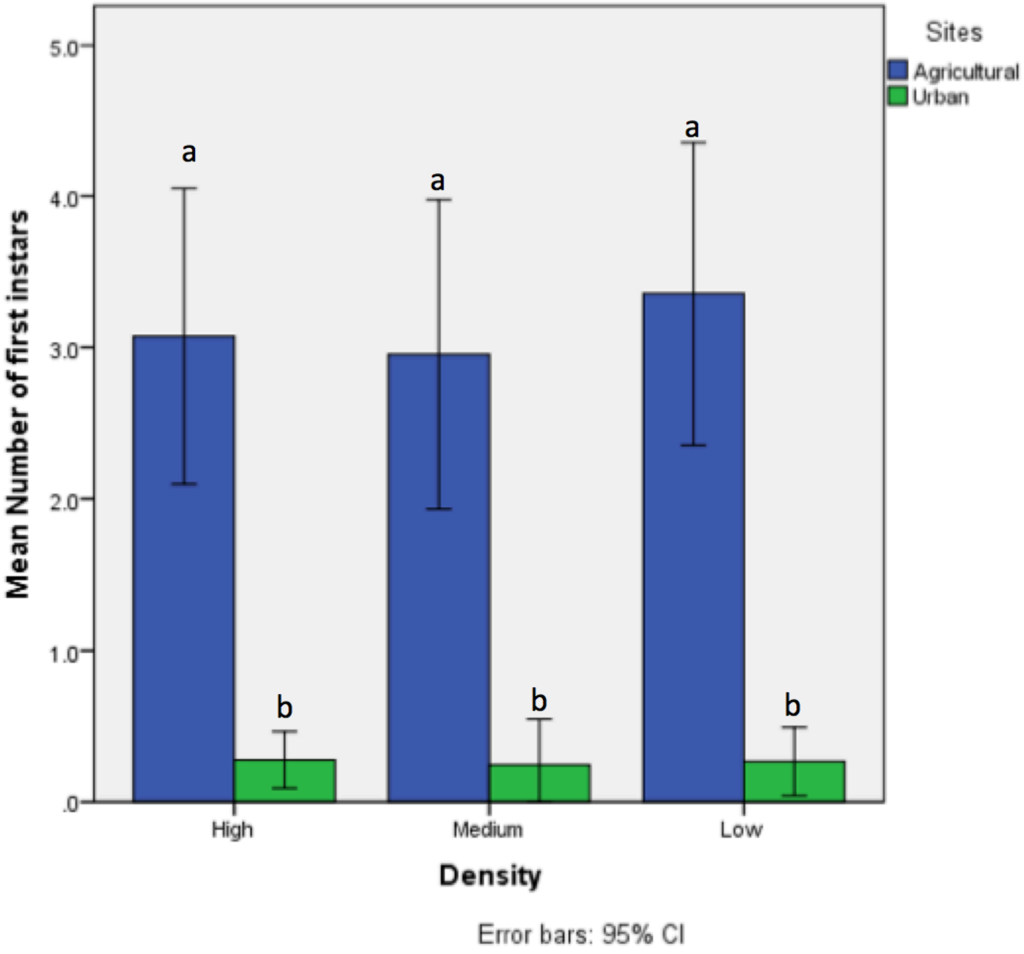

Figure 3. Mean number of 1st instars at both sites categorized according to host-plant density. Significant differences are indicated with different letters.

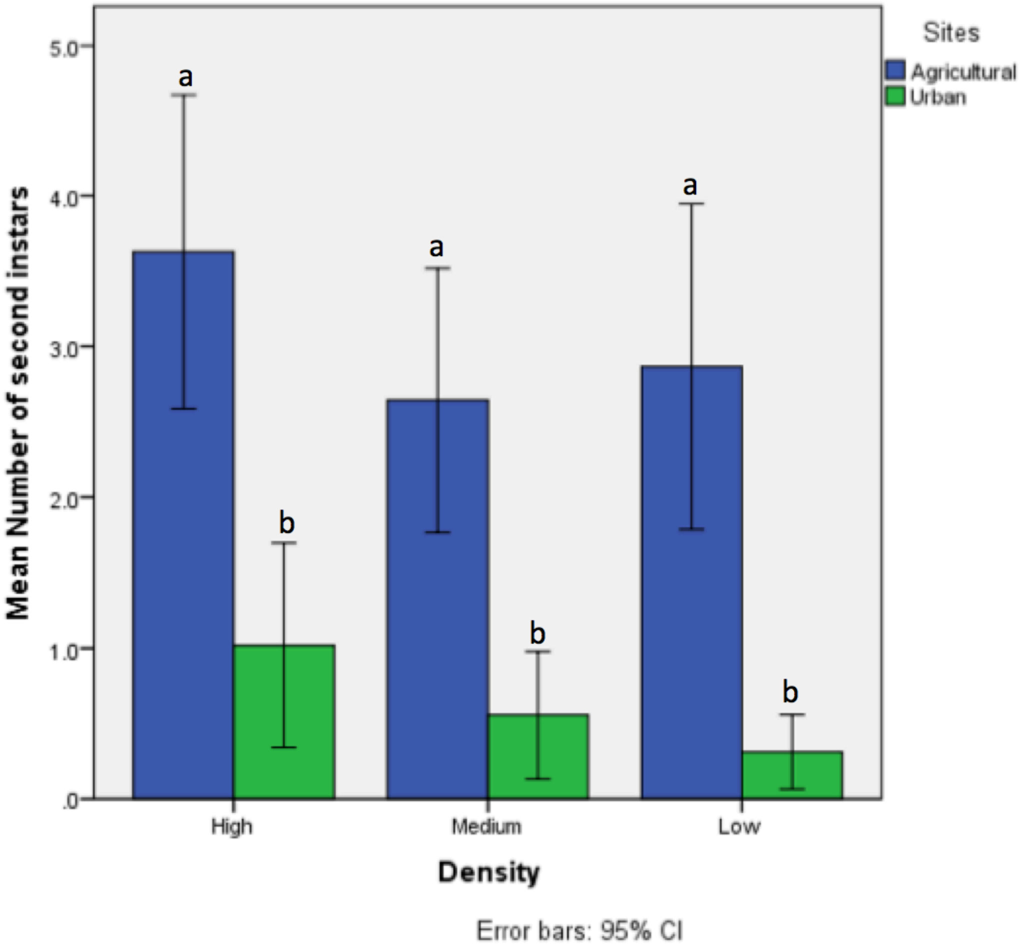

Figure 4. Mean number of 2^{nd} instars at both sites categorized according to host-plant density. Significant differences are indicated with different letters.

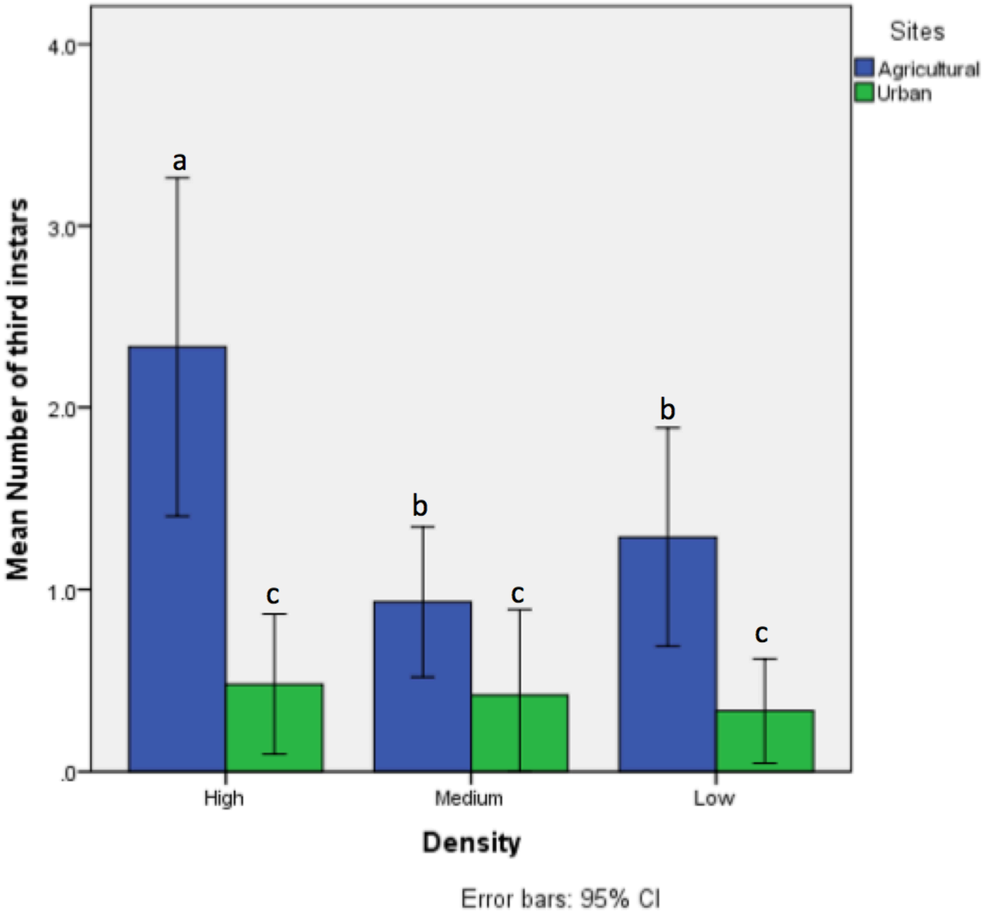

Figure 5. Mean number of 3rd instars at both sites categorized according to host-plant density. Significant differences are indicated with different letters.

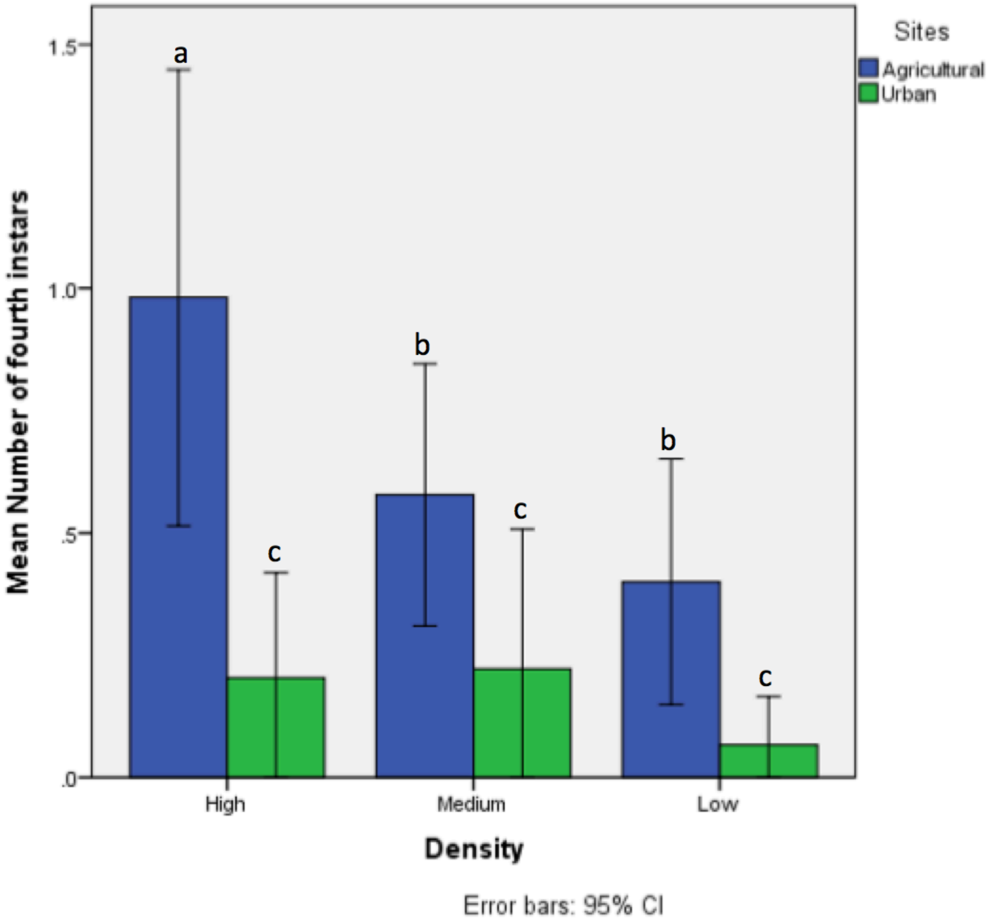

Figure 6. Mean number of 4th instars at both sites categorized according to host-plant density. Significant differences are indicated with different letters.

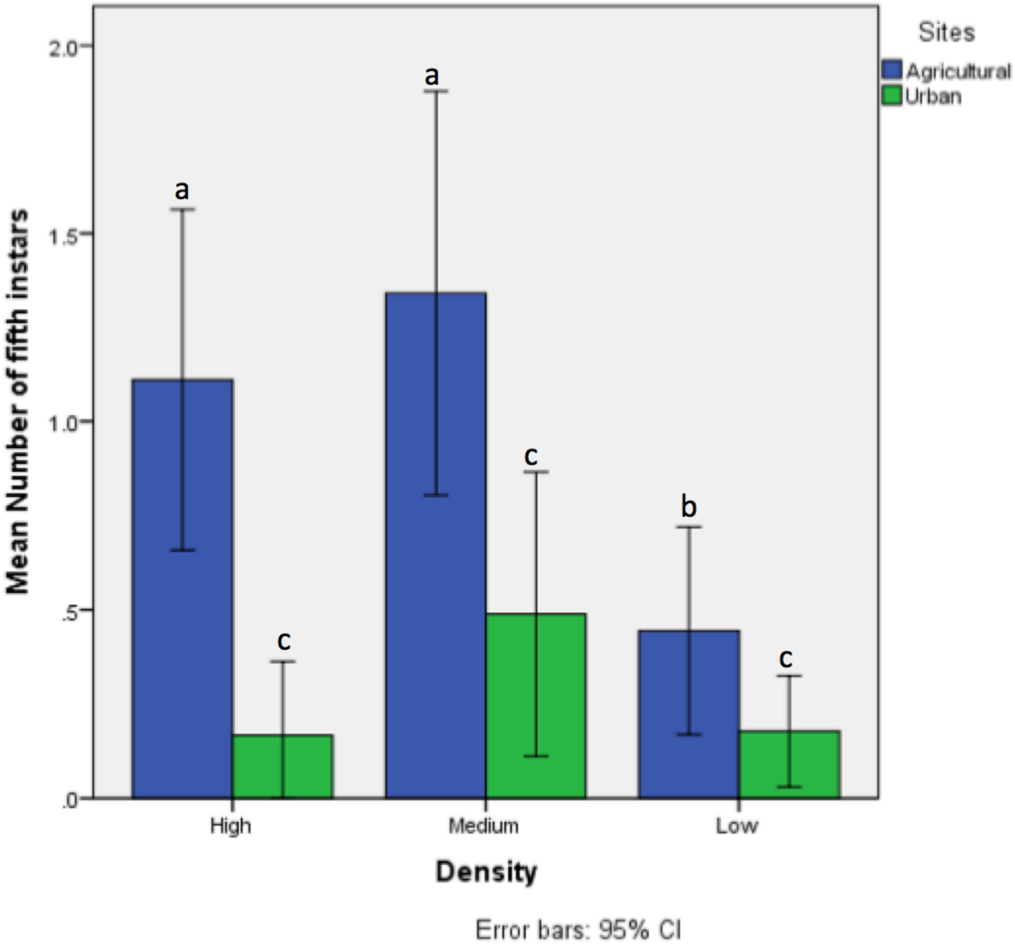

Figure 7. Mean number of 5th instars at both sites categorized according to host-plant density. Significant differences are indicated with different letters.

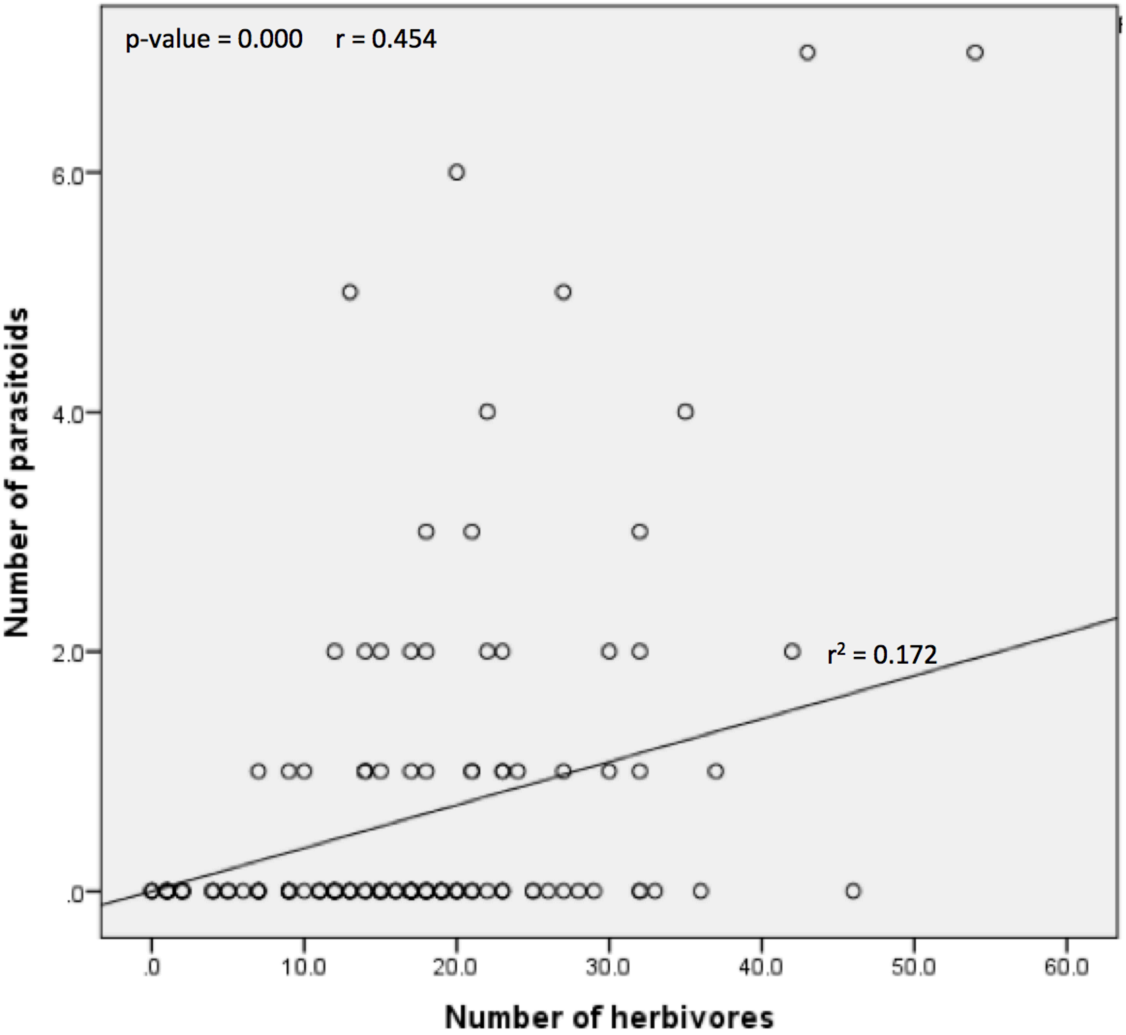

Figure 8. Number of herbivores vs. number of parasitoids at the agricultural site. The correlation coefficient r is indicated with its level of significance.

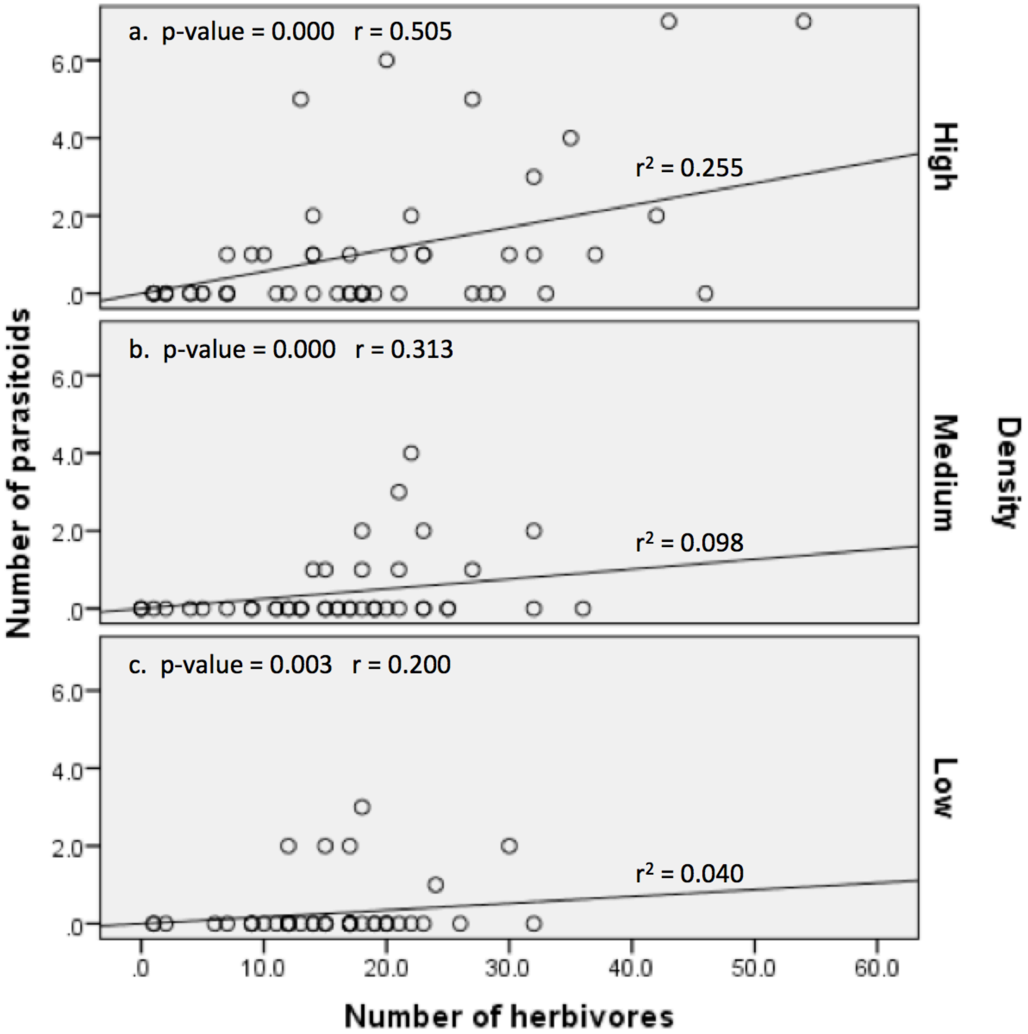

Figure 9. Number of herbivores vs. number of parasitoids at the agricultural site categorized according to host-plant density. The correlation coefficient r, is indicated for each, with its level of significance.