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Host selection and establishment of striped mealybug, *Ferrisia virgata*, on cotton cultivars

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Abstract The striped mealybug, *Ferrisia virgata* (Hemiptera: Pseudococcidae), is a polyphagous and cosmopolitan species, which attacks a wide variety of crops, including cotton. Lately, it has been found infesting colored fiber cotton and emerging as an important pest in the Northeast of Brazil. Therefore, this study investigated the effects of colonization, establishment, plant distribution and dispersal of *F. virgata* on cotton cultivars. Preference experiments were set up in the laboratory and in the greenhouse, in which cultivars were offered simultaneously to nymphs or females and the number of insects on each cultivar was counted after 24 h, 48 h, 22 days and 60 days, respectively. Meanwhile, attack of different plant structures and dispersal were investigated under field conditions. Results showed that *F. virgata* is able to become established on colored cotton fiber cultivars (BRS Verde, BRS Rubi, and BRS Safira) as well as on white cotton cultivars (BRS 201 and CNPA 7H) without showing any preference among these cultivars. Females produce a large number of offspring to enhance nymph survival and host plant colonization, since the number of first instars successfully established after migration is low (<10%). In addition, its short range dispersal can easily occur from plant to plant. Therefore, strategies to manage *F. virgata* will be needed to facilitate colored cotton production, especially in organic farms infested by this mealybug species.

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Introduction

Cotton (*Gossypium hirsutum* L.) is one of the most important crops in Brazil, and is cultivated by large as well as by small growers. However, the presence of arthropod pests can reduce yield and therefore profits in this commodity. About 250 species of herbivorous insects and mites can attack cotton plants in Brazil (Silva *et al.* 1968), and recently the striped mealybug, *Ferrisia virgata* (Cockerell) (Hemiptera: Pseudococcidae), has been added to this list (Ben-Dov 2005; Romano 2011). These cotton mealybugs have spread throughout the semiarid region of Brazil, where environmental conditions such as high temperature, low humidity, long dry season, and a large number of alternative host plants have favored their occurrence and population growth (Romano 2011).

They have been detected elsewhere and shown to be very harmful to cotton production (Nagrare *et al.* 2011; Wang *et al.* 2010). Both Bt-cotton and non-Bt white fiber cotton can successfully serve as hosts (Dutt 2007). However, there are still questions regarding the susceptibility of cotton cultivars of colored fiber that have been recently cultivated in the semiarid region of Brazil, since these colored cultivars have some different morphological characteristics in comparison with the usually cultivated white cotton cultivars, such as the presence of many trichomes on leaves (Breda 2011), which could impair the locomotion and initial settlement of mealybugs on plants.

Mealybugs are small insects that develop and feed continuously on plant tissue by sucking plant sap. Also, they can vector plant viruses and inject toxins into plant tissue resulting in chlorosis, stunting, deformation and death of plants (Ben-Dov 2005; Hoffman *et al.* 2011). Mealybugs can be found developing on different plant parts and can also move to the roots during the dry season (Schreiner 2000; Wang *et al.* 2010). Early growth cotton plants damaged by mealybugs appear distorted, with bushy shoots, crinkled and/or twisted bunchy leaves, and showing white cereous mass on various plant parts. Under severe attack, plants can dry completely.

The striped mealybug, *F. virgata*, is a cosmopolitan species that infests a wide variety of crops, such as sweet potato, cassava, coffee, cocoa, citrus, tomato, papaya, eggplant, cotton, etc., particularly on water-stressed plants, including weeds (Culik & Gullan 2005; Schreiner 2000). It also vectors plant pathogens such as the swollen shoot virus in cocoa, *Theobroma cacao* L. (Ben-Dov 2005), the piper yellow mottle virus in hot pepper, *Piper nigrum* L. (Bhat *et al.* 2003), and the bacterium *Candidatus* Liberibacter asiaticus (Las) in *Citrus* L. (Hoffman *et al.* 2011). The adult female is wingless, covered by a powdery white wax, with a pair of dark longitudinal stripes on the dorsum. The body is elongated, with white wax threads extending from the posterior end resembling tails. In cotton, *F. virgata* occur in patches and feed on all parts of a plant, particularly on growing tips or on leaves that join stems or along leaf veins, and they also attack squares and bolls (Hanchinal *et al.* 2011; Nagrare *et al.* 2011). When infestation occurs in the reproductive stage, it can reduce plant vigor; bolls will fail to open, and plant senescence is induced. In addition, feeding mealybugs also excrete a sugary solution called honeydew, which contributes to the development of black sooty mould (Nagrare *et al.* 2011).

The most common method used to control mealybugs is chemicals, but additional methods within an integrated pest management approach are required. Host plant resistance, when available, aims to reduce the infestation of pests and seems to be compatible with other control methods. Recently, the cultivation of cotton producing colored fibers such as BRS Verde, BRS Rubi, and BRS Safira has increased in the Northeast Region of Brazil, because these cultivars are well adapted to the local environmental conditions, and offer interesting options for organic growers beyond the higher marketing price of colored fiber cotton (*Jornal do Comércio*, 17 Oct. 2009). In addition, these cultivars differ morphologically from white fiber cotton cultivars, especially as regards plant height, trichome density, and number of bolls, all of which can affect their susceptibility to pests and hence their productivity (Carvalho 2006). For example, the cultivar BRS Safira has shown resistance to the cotton aphid *Aphis gossypii* Glover (Homoptera: Aphididae) (Carvalho 2006), and the cultivar BRS Verde reduced population growth of the cotton leafworm, *Alabama argillacea* (Hübner) (Lepidoptera: Noctuidae), by 44.6% in comparison with the white fiber cotton cultivar Acala 90 (Santos *et al.* 2008).

The objective of this study was to investigate the effects of colonization, establishment, plant distribution, and dispersal of mealybugs on cotton cultivars BRS Verde, BRS Rubi, and BRS Safira (all colored fiber varieties) compared with white fiber cultivars (BRS 201 and CNPA 7H), all of which are recommended for cultivation in the semiarid region of Brazil.

Materials and methods

Insects Ferrisia virgata was collected from cotton plants (cv. NuOpal) in Recife, State of Pernambuco, Brazil (8°01′39″S; 34°56′41.31″W; 4 m a.s.l.). Species identification was confirmed by specialists (Dr. Alessandra Rung—Plant Pest Diagnostics Branch, California Department of Food & Agriculture, EUA, and Dr. Douglas R. Miller—Agricultural Research Service, United States Department of Agriculture, USA).

Insects were reared in the laboratory according to Sanches & Carvalho (2010). The colony of *F. virgata* was maintained on plastic trays (40×30 cm) containing pumpkins (*Curcubita pepo* L.) (var. *j*acarezinho) as food for the mealybugs, and kept under controlled conditions of temperature of 25±1°C and photoperiod of 13 h:11 h L:D.

Plants Cotton seeds of the cultivars BRS Verde, BRS Rubi, BRS Safira (colored fiber) and BRS 201 (white fiber) were obtained from Embrapa Algodão—Paraíba State, while seeds of the cultivar CNPA 7H (white fiber) were obtained from the Pernambuco State Agricultural Agency. Regarding the white fiber cotton cultivar used, initial tests were conducted with cultivar BRS 201, but because of seed shortage during the experiments we had to replace it with another available white fiber cotton cultivar (CNPA 7H).

All plants used in mealybugs colony and experiments were cultivated in a greenhouse, under ambient environmental conditions. Plants were grown in 5-*l* plastic pots containing a mixture of soil and humus (2:1), and received 5 g per pot of N:P:K fertilizer (Yara Brasil Fertilizantes S.A., Porto Alegre, RS). Fifteen days after emergence, each plant also received a solution of 25 ml of ammonium sulfate (Fertine, Fertilizantes do Nordeste Ltda., Recife, PE), which was reapplied at 15-day intervals until experiments were initiated.

Preference of F. virgata for cotton cultivars in the laboratory To study whether mealybugs show any preference for white fiber or colored fiber cotton cultivars, preference tests were conducted with nymphs and adults of *F. virgata* in a controlled environment. Choice bioassays were carried out in the Insect Behavior Laboratory at UFRPE, under conditions of 24.9±1.46°C, 47.4±11.3% r.h., and a photoperiod of 12:12 h L:D.

Initially, host preference was studied using second instar nymphs, due to the small size of first instars (crawlers), which impaired observation (second instar nymphs also have dispersal ability, but with a slower locomotion rate). Whether dispersing nymphs have preferences for a particular cultivar was investigated by giving them a choice among four cotton cultivars: BRS Verde, BRS Rubi, BRS Safira, and BRS 201. Plants used in tests had 8–10 expanded leaves.

Free choice arenas consisting of the base of a glass petri dish (15 cm diam) were used in preference tests. Each dish was placed above a cylindrical PVC support (10 cm diam×20 cm height) and four plants—one of each tested cultivar—were placed around the arena (dish), with one expanded leaf of each cultivar (4th or 5th from the top) laid on the arena at equivalent distances from the center, without touching each other. Therefore, each choice arena had four cultivar options (leaves) to choose from. To prevent mealybugs climbing off the arena and spreading to other plant parts beyond the offered leaves, petioles of each offered leaf were covered with entomological glue (BioStop Cola, Biocontrole Métodos de Controle de Pragas Ltda, SP). Twenty second instar nymphs were released in the center of each arena with the help of a soft paint brush, in between leaves without touching any leaf, and the total number of nymphs found on each cotton cultivar leaf and petiole was counted after 24 and 48 h. In addition, each arena was dismantled and infested plants were kept separately under laboratory conditions for the next 15 days to check for the establishment of nymphs on chosen cotton cultivars. This experiment was replicated 30 times.

In addition, to investigate whether foundress mealybug females show any host preference regardless of nymphal choice during plant colonization, the methodology described previously for nymphs was also used with females. In this case, to reduce competition and interference among mealybug females, only four females were released in each arena (1 female: 1 plant), and because the locomotion of adult females is very slow, the number of mealybugs on each leaf was recorded/counted only at 48 h after release. This experiment was replicated 12 times. Data for the number of insects found per cotton cultivar 24 and 48 h after release were analyzed by a frequency test through the PROC FREQ of SAS.

Establishment of F. virgata nymphs on cotton plants in the laboratory Based on a previous test conducted in the laboratory to investigate establishment of second instar nymphs on cotton cultivars, results indicated a low number of insects becoming established on cotton plants. Therefore, we investigated the ability of crawlers, instead of second instars, to establish on different cotton cultivars under laboratory conditions, since they are the first to spread after foundress reproduction aiming to colonize new habitats. Using newly hatched nymphs from the stock colony, cotton leaf discs (3 cm diam) obtained from cotton plants of the corresponding cultivar (BRS Rubi, BRS Safira, BRS

Verde, and CNPA 7H) were artificially infested with 150 *F. virgata* first instar nymphs. Next, individual leaf discs were placed over the first top expanded leaf of the corresponding cotton cultivar, and held in place with a metal paper clip. The main stem of the plant received entomological glue on the base to avoid the possibility that released nymphs would escape from the plant as well as to prevent other insects from climbing onto the cotton plants. After infestation, plants remained in the laboratory for the following 22 days, under the conditions (± SE) of 26.5±0.02°C, 69.4±0.23% r.h., and 12 h:12 h (L:D). At the end of 22 days, the number of nymphs alive on each plant was counted. There were 20 replications per cotton cultivar.

Data analyses began by calculating the establishment rate per cotton cultivar [(number of nymphs found/150) × 100]. The data were submitted to normality (Kolmogorov-Smirnov) and homogeneity (Bartlett) tests. Because the data deviated from a normal distribution, they were transformed into square root (x+0.5) to meet ANOVA assumptions. Finally, the rate of establishment of mealybugs on the different cotton cultivars was submitted to an analysis of variance through the PROC ANOVA of SAS.

Colonization of different cotton cultivars by F. virgata in the greenhouse To understand how mealybugs establish on cotton plants in a semi-field condition, we investigated how different cotton cultivars were colonized by *F. virgata* in the greenhouse. Plants used in this experiment were 40 days old and cultivated as described previously. Tests were conducted in plastic-covered agricultural greenhouses (6 m length×2 m width×2 m height), with the sides closed with anti-aphid screen. During the tests, environmental conditions were monitored by DataLogger HOBO® set up to register at 30-min intervals. The average temperature was 28.8°C (min. = 20.95°C; max. = 48.49°C), r.h. was 54.07%, and the natural photoperiod was ~12 h of light.

Cotton leaf discs measuring 19 mm in diam were taken from the different tested cultivars (BRS Rubi, BRS Safira, BRS Verde, and CNPA 7H) and infested with 150 first instar *F. virgata* nymphs (crawlers), which were obtained from the laboratory stock colony. Each disc was placed individually on 15 cotton plants of each cultivar. Discs were placed on the first expanded top leaf and fixed with a metal clip. To prevent ants crawling on the plant, entomological glue was applied on the base of the plant's main stem. After 25 days, plants were carefully inspected for the presence of nymphs or adults, and the number of mealybugs was counted per plant to estimate their establishment rate in each cotton cultivar. After 50 days of infestation (90-d-old plants), plants were collected and packed in plastic bags carefully tagged, and brought to the laboratory where the total number of insects was counted per plant, inspecting all plant structures from top to bottom with the help of a stereomicroscope (OPTON–NTB 3A).

Data for the total number of mealybugs found per cotton cultivar were submitted to normality (Kolmogorov-Smirnov) and homogeneity (Bartlett) tests prior to analysis of variance through the PROC ANOVA of SAS.

Preference and dispersal of F. virgata among cotton cultivars in the greenhouse To verify whether previous laboratory results (experiment 1) applied also to a less controlled environment and to check for mealybug dispersal among plants, the preference and dispersal of *F. virgata* among cotton cultivars was studied also in the greenhouse. In this case, 30-day-old cotton plants of the studied cultivars were used. Uncontrolled greenhouse environmental conditions were monitored throughout the study period and revealed an average temperature of 31°C (min. = 22.1°C; max. = 45.4°C), r.h. of 55%, and natural photophase of ~12 h.

Four cotton plants, one of each cultivar, were randomly positioned in circular microplots (100 cm diam, 50 cm depth), and there were 15 replicates. Plants formed an equilateral triangle with the three colored cotton cultivars always at the corners and the white fiber cotton cultivar in the center. Cotton leaf discs (3 cm diam) infested with 350 *F. virgata* crawlers were placed on the top expanded leaf of the central plant of each replicate and attached with a metal clip. After 60 days, the whole plant was inspected and the total number of mealybugs per plant was counted with the help of a stereomicroscope.

Data on the number of insects found on each cotton cultivar after 60 days were submitted to chi-square test through the PROC FREQ of SAS testing the hypothesis of equal colonization among the colored fiber cotton cultivars.

Cotton plants infestation by F. virgata in the field As we understand that mealybugs can disperse and colonize plants in basically two different ways: from above—passive dispersal by wind, or from below—active dispersal by crawling, we decided to investigate how *F. virgata* infestation develops in the field, and how the insects distribute themselves on different plant parts, as well as whether there is any preference for plant structure by colonizing nymphs depending how they arrive on plants. Therefore, tests were conducted in a 600 m^2 experimental area at Recife-PE, Brazil (8°01′39″S; 34°56′41.31″W; 4 ma.s.l.), from March to June 2011. Environmental conditions were monitored by DataLogger WatchDog at 30-min intervals, registering an average temperature (±SD) of 27.45°C±4.37 (min. = 21.33°C; max. = 37.44°C), r.h. of 29.80%, and natural photoperiod of ~12 h.

Cotton plants of the cultivar BRS Rubi were planted directly into cylindrical microplots (100 cm× 50 cm), with a total of 40 replicates. Each microplot received four plants, with three forming an equilateral triangle, and the fourth in the center. Plants were 50 days old when the experiment began. For future reference and evaluation, each lateral plant was considered clockwise as lateral one, two or three, and this order was repeated at all microparcels.

Cotton leaf discs (3 cm diam) infested with 100 *F. virgata* crawlers were obtained from the laboratory stock colony. The central cotton plant in each microparcel received one infested leaf disc, which was placed on the main stem and secured with a clothes pin. Half of the plants (20 replicates) were infested on the top portion of the main stem, and the other half were infested at the base. The assessment of plant parts infested with *F. virgata* was conducted twice (30 and 60 days after infestation), when plants were 80- and 110-d old, respectively. To define the location of mealybugs on the plant structures and on each plant stratum (upper, middle and lower), recording the presence or absence of mealybugs on each structure/stratum, the position and the total number of nodes in the main stem were taken into account. All plant parts per stratum were fully inspected along the main stem (internodes) and the lateral stems, comprising leaves, petioles, squares, flowers, bolls and open bolls.

Data for the number of infested structures, the vertical distribution of mealybugs and infestation levels of the four plants in each replicate were transformed to log (x+1) and submitted to analysis of variance through PROC ANOVA of SAS, followed by Tukey's HSD test (P=0.05) for mean comparisons. The number of infested plant parts at 30 and 60 days after infestation was compared using the Student *t*-test (P=0.05) through PROC TTEST of SAS.

Results

Preference of F. virgata for cotton cultivars in the laboratory There was no significant difference in the choice/preference of cotton cultivars by second instar mealybugs 24 and 48 h after release (24 h: P=0.9982; and 48 h: P=0.9596). In addition, the establishment of nymphs on cotton plants in the laboratory during 15 days after release was similar across the four tested cotton cultivars (P=0.6727). Furthermore, adult females of *F. virgata* show similar colonization of the studied cotton cultivars 48 h after exposure, that is, there was no preference for the cotton cultivars offered to females as well as nymphs (P=0.0819).

Establishment of F. virgata nymphs on cotton plants in the laboratory The establishment rate of *F. virgata* on cotton plants up to 22 d after infestation was low, approximately 40%, regardless of cotton cultivar, with an average (± SE) of 33.3±5.17 for BRS Verde; 34.9± 5.06 for BRS Rubi; 41.5±4.91 for BRS Safira; and 47.7±4.05 for CNPA 7H mealybugs per plant. In addition, there was no significant difference in the colonization and establishment of first instar mealybugs on the different cotton cultivars offered in the laboratory (P=0.1471).

Colonization of different cotton cultivars by F. virgata in the greenhouse Similar to laboratory results, the establishment rate of *F. virgata* on cotton plants in the greenhouse was also low, with an average (±SE) of 18.7±1.62 surviving individuals after 25 d from 150 nymphs released, regardless of cotton cultivar. In contrast, after 50 d of infestation of cotton plants in the greenhouse, there was an increase in the number of mealybugs found per plant that was similar across all studied cotton cultivars (P=0.8013). This increase was a result of the reproduction of adult females developed from previously established nymphs and resulted in an average (±SE) of 7697.2±727.51 mealybugs per plant.

Preference and dispersal of F. virgata among cotton cultivars in the greenhouse Mealybug crawlers released on cotton plants in the greenhouse did not disperse readily on host plants. In contrast, after reproduction, mealybugs dispersed among cotton cultivars. Despite the high number of nymphs released, the number of surviving insects after the first 15 d after plant infestation was low (<10%), with no significant difference among cotton cultivars (P=0.6727). These results are similar to those of previous experiments conducted in both the laboratory and in the greenhouse.

Sixty days post infestation, when established insects had reproduced for one generation in the greenhouse, there was also a considerable number of established insects on field plot plants, with more than 7,500 mealybugs found per plant irrespective of cotton cultivar. Even though the second generation of crawlers dispersed to lateral plants in the microplot, there was no difference in the number of mealybugs found on the three lateral and the central plants of each microplot, that is, there was no difference among cotton cultivars (P=0.1651).

Cotton plants infestation by F. virgata in the field There was a low number of nymphs (zero to two nymphs per plant structure) present on field cotton plants 30 to 60 d after artificial infestation (Table 1). These were most frequently found on leaves with no significant differences in the numbers of mealybugs found on squares, bolls, internodes, petioles, flowers and open bolls (Table 1).

At 30 d after infestation, there was no difference in the number of plant structures infested with *F. virgata* among plants in the field plots (P=0.4317). On the other hand, in the 30 d between evaluations, there was a significant increase of 1.8 times in the number of plant structures infested by *F. virgata* (Fig. 1). Similarly, at 60 d after infestation, the number of structures infested with mealybugs in the central and lateral plants was not different (P=0.8948).

Considering the three plant strata (upper, middle and lower), when mealybugs were infested on the base of the plant there was a difference in their numbers recorded for the different strata, but only for 30 d after infestation (Table 2), with the largest number of mealybugs found on the middle stratum of the plant. This difference disappeared at the second evaluation (Table 2). If one looks at the overall vertical distribution of mealybugs, irrespective of place of initial colonization (top or base), the structures present in the middle stratum were more heavily infested by *F. virgata* at both 30 and 60 d after infestation (Table 3).

Table 1 Average number (±SE) of cotton plant structures infested with *Ferrisia virgata*, at 30 and 60 days post infestation

Plant structures	Days after infestation	
	30	60
Leaves	0.76±0.08bA[z]	1.17±0.12aA
Squares	0.05±0.02aB	0.03±0.01aC
Bolls	0.03±0.01bB	0.35±0.05aB
Internodes	0.01±0.00aB	0.006±0.00aC
Petioles	0.006±0.00aB	0.03±0.01aC
Flowers	0.03±0.03aB	0.00 aC
Open bolls	–[y]	0.0125±0.00C

[z] Means followed by the same lower case letter in the row and the same capital letter in the column do not differ statistically by Tukey's HSD test (P>0.05)

[y] At 30 days after infestation there were no open bolls on plants to be evaluated

Discussion

Loxdale *et al.* (1993) stated that dispersal at short range, within plants and between neighboring plants, has the same biological importance or even more agronomical importance than long-range dispersal. Therefore, understanding the dispersal capabilities of pest species within the environment, their capacity to locate and establish on unexploited hosts, and the factors associated with their dispersal is essential for predicting the course of pest infestations and for planning control procedures (Barrass *et al.* 1994; Rabkin & Lejeune 1954).

The lack of host preference showed by nymphs and adults supported our hypothesis that mealybugs colonization would be similar among different cotton cultivars, based on the fact that *F. virgata* has been previously described as a highly polyphagous species (Nagrare *et al.* 2011). *Ferrisia virgata* has been reported recently as an increasing problem for cotton cultivation in the semiarid habitats of Brazil (Romano 2011), being spotted on white cotton and occasionally on colored cotton cultivars and Bt-cotton (Dutt 2007). Our study is the first to report the plant colonization,

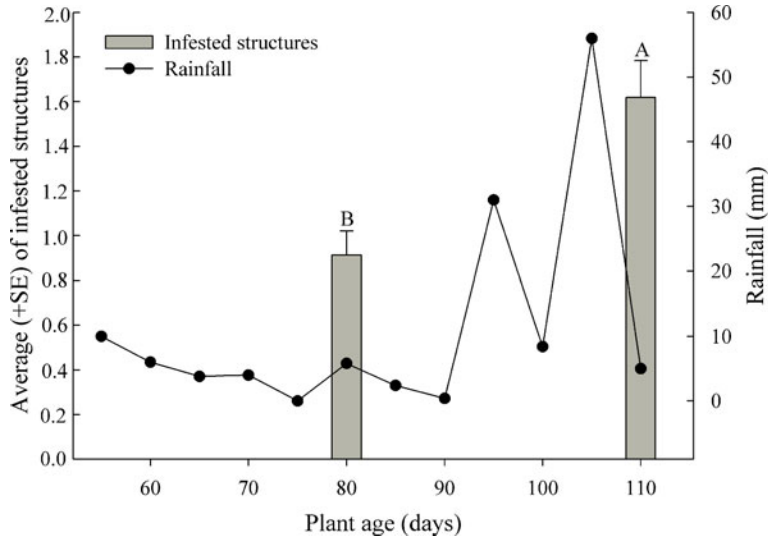

Fig. 1 Average number (±SE) of all cotton plant structures (leaves, petioles, stem, squares, flowers and bolls) infested by *Ferrisia virgata*, and rainfall during the field experiment from March to June 2011. Data for 80 and for 110 days differ statistically by the Student *t*-test ($P<0.05$)

capacity of reproduction and distribution of this pest species on colored cotton cultivars cultivated in Pernambuco, Brazil. In addition, results suggest that if not kept under control, *F. virgata* can rapidly achieve the status of a key pest in the region.

First instar mealybugs in general have a relatively low survival rate on cotton plants. After one month's infestation of grape leaves with 75 first instars of *Pseudoccocus maritimus* (Ehrhorn) (Hemiptera: Pseudococcidae), Grasswitz & James (2008) found fewer than eight nymphs per leaf. Additionally, Grasswitz & Burts (1995) showed a reduction of 65–75% in the population of *P. maritimus* nymphs on apple trees, after 3 weeks of infestation, even with the exclusion of natural enemies.

Many factors can reduce the establishment and survival of mealybug first instar larvae: (i) small size, which increases the risks of death by desiccation or consumption of energy (Moran *et al.* 1982); (ii) natural enemy attack, especially by ladybeetles, spiders and parasitoids (Grasswitz & Burts 1995); (iii) unsuccessful dispersal, in which nymphs do not reach a suitable host plant or feeding site. In our greenhouse experiment, even other factors could have affected the establishment of mealybugs on plants, such as the origin of the insects. The insects used to infest greenhouse plants were provided by the laboratory colony, where temperature conditions are more suitable than outdoors (25°C). In fact, the establishment rate in the laboratory was higher, about 40%. Females of *F. virgata* have a high reproductive potential, with average clutch sizes of ≅253 offspring per 16 days under

Table 2 Average number (±SE) of all cotton plant structures (leaves, petioles, stem, squares, flowers and bolls) infested with *Ferrisia virgata* on three plant strata in relation to the infestation site (top and base) at 30 and 60 days post infestation

Strata	Days post infestation			
	30		60	
	Top	Base	Top	Base
Upper	0.20±0.09A[z]	0.15±0.08AB	0.15±0.10A	0.3±0.12A
Middle	0.70±0.27A	0.55±0.18A	0.60±0.22A	0.6±0.11A
Lower	0.25±0.09A	0.10±0.06B	0.40±0.13A	0.4±0.18A

[z] Within columns, means followed by a common capital letter do not differ statistically by Tukey's HSD test ($P>0.05$)

Table 3 Average number (±SE) of all cotton plant structures (leaves, petioles, stem, squares, flowers and bolls) infested with *Ferrisia virgata*, irrespective of infestation spot (top and base) at 30 and 60 days post infestaon

Strata	Days post infestation	
	30	60
Upper	0.17±0.07B[z]	0.22±0.08B
Middle	0.62±0.16A	0.60±0.12A
Lower	0.70±0.06B	0.40±0.11AB

[z] Within columns, means followed by a common capital letter do not differ statistically by Tukey's HSD test ($P>0.05$)

laboratory conditions (M. D. Oliveira *et al.*, unpublished data), suggesting an adaptation of mealybugs to ensure offspring survival and plant colonization, since the chances of survival and success of finding a host plant in the field are low. This fact can be supported by the results obtained 60 d post infestation: after reproduction, the number of mealybugs established on the different cotton cultivars had increased similarly across cultivars, indicating no effect of the cotton cultivars on preference. Again, this result was somewhat expected based on laboratory results and the polyphagous feeding behavior of *F. virgata* described previously (Schreiner 2000).

Generally, dispersion occurs after reproduction when the first instar nymphs try to find new feeding sites before initiating feeding, regarded as an adaptation to reduce intra-specific competition, and because first instars have various characteristics that have been considered adaptations for dispersal, for instance long legs and antennae when compared with late instars (Beardsley & Gonzalez 1975; Gullan & Kosztarab 1997). Similar results were found for the carmine cochineal, *Dactylopius opuntiae* Cockerell (Hemiptera: Dactylopiidae), attacking giant cactus pear (*Opuntia ficus-indica* Mill), after 60 d of infestation from an original ten colonies and subsequent dispersal, when the insect population reached an average of 171 colonies per plant. The number of colonies per plant decreased as distance from the release point increased (Foxcroft & Hoffmann 2000; Lopes *et al.* 2009).

Adult female mealybugs cannot fly, but do have the ability to walk on the host plant; in general they have a sessile habit, usually staying and feeding for long periods on the same plant spot after establishment until death. Even in situations where leaves or other plant parts are dry, they do not tend to leave that feeding site (C.S.A. Silva-Torres, personal observation). In contrast, males have wings and can disperse to other areas. Nymphs are also more active, especially first instar nymphs, called crawlers, which are the main dispersal stage of mealybugs. Immature mealybugs (Barrass *et al.* 1994; Debach 1949; Furness 1976) typically disperse and colonize new host plants by walking and by traveling passively on the wind. Crawlers can walk quite fast and disperse throughout the plant, and could easily spread to adjacent plants if they are touching. For long distance dispersal, mealybugs could be spread through natural carriers such as raw cotton, linted cotton seeds, ornamental plants, wind, water, rain, birds, people, ants, farm animals, and equipment (CABI 2003; Grasswitz & James 2008; Nagrare *et al.* 2011; Strickland 1950). Mealybugs can also disperse by adhering via honeydew to falling leaves, as observed with the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) (Daane *et al.* 2006).

Experiments regarding field dispersal of *F. virgata* in cotton are lacking and necessary to understand how this pest can spread throughout semiarid cotton areas. Based on our results, nymphs were able to move short distances to establish elsewhere. However, any dispersal by wind would mask our results on host preference among cotton cultivars, since wind dispersal is an unintentional choice for the insect. Wind was negligible in the greenhouse, so all dispersal in this experiment was by walking.

Because mealybugs are generally sessile, they will be patchily distributed and concentrated at the place of initial colonization, with further spread to neighboring plants only after reproduction. These facts explain the larger number of mealybugs found on central plants of each microplot, which received the artificial infestation in our experiments. As the plants were positioned about 30 cm apart, mealybugs released could not easily reach neighbor plants, but after 60 d leaves from the central plant usually touched lateral plants, facilitating dispersal by nymphs of the F1 generation.

Again, a field experiment showed a low initial establishment of crawlers on cotton plants. However, established mealybugs reproduced and descending crawlers migrated to other plant parts, agreeing with results of our greenhouse experiments. Another factor affecting plant colonization by mealybugs was the amount of rainfall during the field test. Rainfall was intense after plant infestation and reached its peak between the 30 and 60 d evaluations. Possibly, the number of mealybugs found on different plant structures would have been higher if there had been no rainfall during the experiment. Other studies have also found that mealybug populations can be reduced after intensive rainfall because they can be easily washed off plants (Flores-Flores & Tekelenberg 2001; Goolsby *et al.* 2002; Lopes *et al.* 2009). Dhawan *et al.* (2009) found that the amount of precipitation was negatively correlated with cotton plants infestation by *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), another very important mealybug pest on cotton. In addition, Abbas *et al.* (2010) showed that during the peak of rainfall and associated lower temperatures, there was only a small production of *P. solenopsis* nymphs on cotton plants. Therefore, besides reducing mealybug populations by washing them off the plants, rainfall associated with low temperatures can potentially negatively affect the reproductive output of mealybugs.

Mealybug dispersal from central to lateral plants probably occurred right after plant infestation, before the first rainfalls. Moran *et al.* (1982) found that because of the small size and weight of crawlers, they can be easily carried by the wind and disperse between plants. After 60 d of infestation, the number of structures infested with mealybugs in all microplot plants was similar, confirming again the capability of short range dispersal of *F. virgata* by walking between neighboring plants.

Considering mealybug distribution on plants, and the three plant strata as well as the locale of infestation (top or base of plant), the highest number of mealybugs was found in the middle stratum, but this difference disappeared after reproduction. Therefore, the placement of initial infestation did not affect the vertical distribution of mealybugs on cotton plants. Conversely, by looking at the vertical distribution of mealybugs regardless of the locale of initial infestation, structures on the middle stratum were consistently more infested with mealybugs, indicating that even though the insects are able to disperse, their sessile behavior tends to keep them near the place of initial colonization.

Overall, we can conclude that *Ferrisia virgata* is capable of attacking colored fiber cotton cultivars as well as white cotton cultivars without showing any preference for colonization. Its short range dispersal can occur from plant to plant, by walking nymphs, especially first instar crawlers, which are more active than any other life stage. Therefore, strategies to manage *F. virgata* will be needed to achieve successful colored cotton production, especially in organic farms infested by this mealybug species. High 'crawler' mortality during dispersal, in addition to other natural mortality factors such as predation, parasitization and abiotic factors such as rain under field conditions, suggest that management interventions should be focused against reproducing adult females rather than crawlers if growers are to prevent the multiplication and spread of *F. virgata*.

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References

Abbas, G., Arif, M. J., Ashfaq, M., Aslam, M., & Saeed, S. (2010). The impact of some environmental factors on the fecundity of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae): a serious pest of cotton and other crops. *Pakistan Journal of Agricultural Sciences, 47*, 321–325.

Barrass, I. C., Jerie, P., & Ward, S. A. (1994). Aerial dispersal of first- and second-instar longtailed mealybug, *Pseudococcus longispinus* (Targioni Tozzetti) (Pseudococcidae: Hemiptera). *Australian Journal of Experimental Agriculture, 34*, 1205–1208.

Beardsley, J. W., & Gonzalez, R. H. (1975). Biology and ecology of armored scales. *Annual Review of Entomology, 20*, 47–73.

Ben-Dov, Y. (2005). ScaleNet, Pseudococcidae catalogue query results, *Ferrisia virgata*. http://www.sel.barc.usda.gov/catalogs/pseudoco/Ferrisiavirgata.htm. Accessed 9 March 2012.

Bhat, A. I., Devasahayam, S., Sarma, Y. R., & Pant, R. P. (2003). Association of a Badnavirus in black pepper (*Piper nigrum* L.) transmitted by mealybug. *Current Science, 84*, 1547–1550.

Breda, M. O. (2011). Efeitos letais e subletais de inseticidas botânicos sobre *Aphis gossypii* Glover (Hemiptera: Aphididae) e *Cycloneda sanguinea* (Linnaeus) (Coleoptera: Coccinellidae) em cultivares de algodão de fibra branca e colorida. [Lethal and sublethal effects of botanical insecticides on *Aphis gossypii* Glover (Hemiptera: Aphididae) and *Cycloneda sanguinea* (Linnaeus) (Coleoptera: Coccinellidae) on white and colored fiber cotton cultivars]. http://www.ppgea.ufrpe.br/novosite/images/Dissertacao/marianabreda.pdf. Accessed 7 August 2012.

CAB International [CABI]. (2003). *Crop protection compendium*. Wallingford, UK: CABI.

Carvalho, L. P. (2006). Cultivo do algodão herbáceo na agricultura familiar: Cultivares. [Cultivation of cotton in familiar agriculture: Cultivars]. http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Algodao/AlgodaAgricultura Familiar_2ed/cultivares.html. Accessed 10 February 2012.

Culik, M. P., & Gullan, P. J. (2005). A new pest of tomato and other records of mealybugs (Hemiptera: Pseudococcidae) from Espírito Santo, Brazil. *Zootaxa, 964*, 1–8.

Daane, K. M., Bentley, W. J., Walton, V. M., Malakar-Kuenen, R., Millar, J. G., Ingels, C. A., *et al.* (2006). New controls investigated for vine mealybug. *California Agriculture, 60*, 31–38.

DeBach, P. (1949). Population studies of the long-tailed mealybug and its natural enemies on citrus trees in southern California, 1946. *Ecology, 30*, 14–25.

Dhawan, A. K., Singh, K., Aneja, A., & Saini, S. (2009). Distribution of mealybug, *Phenacoccus solenopsis* Tinsley in cotton with relation to weather factors in South-Western districts of Punjab. *Journal of Entomological Research, 33*, 59–63.

Dutt, U. (2007). Mealy bug infestation in Punjab: Bt cotton falls flat. http://www.countercurrents.org/dutt210807.htm. Accessed 5 March 2012.

Flores-Flores, V., & Tekelenburg, A. (2001). Produção de corante Dacti (*Dactylopius coccus* Costa). [Production of Dacti dye (*Dactylopius coccus* Costa)]. In: P. Inglese, G. Barbera, & E.P. Barrios (Eds.), *Agroecologia, cultivos e usos da palma forrageira* (pp. 169–186). [*Agroecology, cultivation and use of cactus pear*] Jõao Pessoa, PB, Brasil: SEBRAE-PB.

Foxcroft, L. C., & Hoffmann, J. H. (2000). Dispersal of *Dactylopius opuntiae* Cockerell (Homoptera: Dactylopiidae), a biological control agent of *Opuntia stricta* (Haworth.) Haworth. (Cactaceae) in the Kruger National Park. *Koedoe, 43*, 1–5.

Furness, G. O. (1976). The dispersal, age-structure and natural enemies of the long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti), in relation to sampling and control. *Australian Journal of Zoology, 24*, 237–247.

Goolsby, J. A., Kirk, A. A., & Meyerdirk, D. E. (2002). Seasonal phenology and natural enemies of *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) in Australia. *Florida Entomologist, 85*, 494–498.

Grasswitz, T. R., & Burts, E. C. (1995). Effect of native natural enemies on the population dynamics of the grape mealybug, *Pseudococcus maritimus* (Hom.: Pseudococcidae), in apple and pear orchards. *Entomophaga, 40*, 105–117.

Grasswitz, T. R., & James, D. G. (2008). Movement of grape mealybug, *Pseudococcus maritimus*, on and between host plants. *Entomologia Experimentalis et Applicata, 129*, 268–275.

Gullan, P. J., & Kosztarab, M. (1997). Adaptations in scale insects. *Annual Review of Entomology, 42*, 23–50.

Hanchinal, S. G., Patil, B. V., Basavanagoud, K., Nagangoud, A., Biradar, D. P., & Janagoudar, B. S. (2011). Incidence of invasive mealybug (*Phenacoccus solenopsis* Tinsley) on cotton. *Karnataka Journal of Agricultural Science, 24*, 143–145.

Hoffman, M. T., Duan, Y., Zhou, L., Stocks, I., & Hall, D. (2011). Is the striped mealybug, *Ferrisia virgata*, a vector of huanglongbing bacterium *Candidatus* Liberibacter asiaticus? http://www.naa.ars.usda.gov/research/publications/publications.htm?seq_no_115=266184. Accessed 10 February 2012.

Lopes, E. B., Albuquerque, I. C., Brito, C. H., & Batista, J. L. (2009). Velocidade de infestação e dispersão de *Dactylopius opuntiae* Cockerell, 1896 em palma gigante na Paraíba. [Speed of infestation and dispersal of *Dactylopius opuntiae* Cockerell, 1896 on giant cactus pear in Paraiba.]. *Engenharia Ambiental—Espírito Santo do Pinhal, 6*, 196–205.

Loxdale, H. D., Hardie, J., Halbert, S., Foottit, R., Kidd, N. A. C., & Carter, C. I. (1993). The relative importance of short- and long-range movement of flying aphids. *Biological Review, 68*, 291–311.

Moran, V. C., Gunn, B. H., & Walter, G. H. (1982). Wind dispersal and settling of 1st-instar crawlers of the cochineal insect *Dactylopius austrinus* (Homoptera, Coccoidea, Dactylopiidae). *Ecological Entomology, 7*, 409–419.

Nagrare, V. S., Kranthi, S., Kumar, R., Jothi, B. D., Amutha, M., Deshmukh, A. J., *et al.* (2011). *Compendium of cotton mealybugs*. Shankar Nagar, Nagpur, India: CICR.

Rabkin, F. B., & Lejeune, R. R. (1954). Some aspects of the biology and dispersal of the pine tortoise scale, *Toumeyella numismaticum* (Pettit and McDaniel) (Homoptera: Coccidae). *Canadian Entomologist, 86*, 570–575.

Romano, D. (2011). Controle da cochonilha através de diferentes inseticidas, aplicados via foliar, na cultura do algodão adensado. [Control of mealybugs by different insecticides applied through foliar spray in narrow-row-cotton]. *Anais do 8° Congresso Brasileiro de Algodão & I Cotton Expo 2011* (São Paulo, Brasil), pp. 115–122.

Sanches, N. F. & Carvalho, R. S. (2010). Procedimentos para manejo da criação e multiplicação do predador exótico *Cryptolaemus montrouzieri*. [Procedure for colony rearing and multiplication of the exotic predator *Cryptolaemus montrouzieri*]. *Circular técnica n^o 99*. Cruz das Almas, Brasil: Embrapa Mandioca e Fruticultura.

Santos, R. L., Torres, J. B., & Bastos, C. S. (2008). Desenvolvimento e crescimento populacional de *Alabama argillacea* em algodoeiro de fibra branca e colorida. [Development and population growth of *Alabama argillacea* on white and colored fiber cotton]. *Pesquisa Agropecuária Brasileira, 43*, 457–463.

Schreiner, I. (2000). Striped mealybug [*Ferrisia virgata* (Cockrell)]. http://www.adap.hawaii.edu/adap/Publications/ADAP_pubs/2000-18.pdf. Accessed 5 March 2012.

Silva, A. G. A., Gonçalves, C. R., Galvão, D. M., Gonçalves, A. J. L., Gomes, J., Silva, M. *et al.* (1968). *Quarto catálogo dos insetos que vivem nas plantas do Brasil—seus parasitos e predadores*. [Development and population growth of *Alabama argillacea* on white and colored fiber cotton]. [*Fourth catalog of insects living on plants of Brazil—their parasitoids and predators*]. Rio de Janeiro, Brasil: Ministério da Agricultura—SDA.

Strickland, A. H. (1950). The dispersal of Pseudococcidae (Hemiptera-Homoptera) by air currents in the Gold Coast. *Proceedings of the Royal Entomological Society, London (Series A), 25*, 1–9.

Wang, Y., Watson, G. W., & Zhang, R. (2010). The potential distribution of an invasive mealybug *Phenacoccus solenopsis* and its threat to cotton in Asia. *Agricultural and Forest Entomology, 12*, 403–416.