Phytoparasitica (2014) 42:311–316
DOI 10.1007/s12600-013-0361-6

Within-plant distribution of an invasive mealybug, *Phenacoccus solenopsis*, and associated losses in cotton

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Received: 6 June 2013 / Accepted: 25 October 2013 / Published online: 7 November 2013

Abstract The cotton mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), an invasive pest species, has appeared on a large scale on cotton in India since 2006. Its distribution within the plant, and associated yield losses in cotton, were studied over 2 years. Distribution of *P. solenopsis* was observed within the cotton plant from vegetative to boll formation stage. In the vegetative and square formation stages, the highest mealybug population was recorded on the upper portion of the stem, followed by the middle leaves of the plant. In the boll formation stage, there was no significant difference in distribution of the insect among plant parts. Losses in cotton due to the mealybug varied between 14.9% at Grade 1 and 53.6% at Grade 4, on a 0 to 4 severity index, with a mean reduction of 35% and 32%, during 2008 and 2009, respectively. There was a significant relationship between severity of infestation and decrease in seed cotton yield. The information generated from this study will help in the early detection of mealybug infestation and estimation of yield losses corresponding to the severity grade of the damage.

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Keywords Infestation · Severity index · Square and boll formation stages · Yield losses

Introduction

The invasive cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), caused economic damage, reducing yields by 50% in affected cotton since 2005 and 2006 in Pakistan (Abbas *et al.* 2005; Muhammad 2007; Hodgson *et al.* 2008) and 2006 in India (Jhala *et al.* 2008; Hodgson *et al.* 2008; Monga *et al.* 2009; Nagrare *et al.* 2009). The outbreak of *P. solenopsis* on cotton in Pakistan and India had a significant economic impact. *P. solenopsis* is an aggressive invasive species on agricultural and ornamental plants, and has spread rapidly among many countries across the globe. Its dispersal in Asia and beyond is a threat to the world's production of cotton and other crops (Wang *et al.* 2010). Its presence has been recently detected in South America (Watson & Chandler 2000), including Chile (Larrain 2002), Argentina (Granara de Willink 2003), and Brazil (Culik & Gullan 2005), but also in Hawaii (Kumashiro *et al.* 2001), the Caribbean Islands and Central America (Hodgson *et al.* 2008), Nigeria (Akintola & Ande 2008), Thailand and Taiwan (Hodgson *et al.* 2008), Sri Lanka (Prishanthini & Laxmi 2009), China (Wang *et al.* 2009; Wu & Zhang 2009), Australia (Charleston *et al*. 2010) and in Turkey (Kaydan *et al*. 2013). Specimens from India exhibited a wide range of variation in morphological and biological adaptations which were found to be environmentally determined (Hodgson *et al*. 2008). Widespread distribution of *P. solenopsis* was recorded in nine cotton growing states of India during 2007 and 2008 (Nagrare *et al*. 2009). *P. solenopsis* was found colonizing mainly young growth, including twigs, leaves, stems and fruiting bodies. Severely infested plants exhibited arrested growth covered with sooty mold, and developed partially opened small-sized bolls (Nagrare *et al*. 2009). Molecular characterization of *P. solenopsis* carried out at the Central Institute for Cotton Research (CICR) confirmed narrow genetic diversity among populations (ICAC Recorder 2008). A CLIMEX model (Wang *et al*. 2010) indicated that tropical regions worldwide are highly suitable for this species and the pest poses a serious economic threat to cotton and other agricultural crops in Asia and other parts of the world. The distribution of *P. solenopsis* is highly clumped, with a tendency to initially spread along rows rather than across rows, which is most likely aided by the mechanical movement of farm implements. In the absence of any typical damage symptoms, early infestation of *P. solenopsis* can easily go unnoticed.

In cotton no earlier reports are available, but Hara *et al*. (2001) mentioned that early root infestations by the mealybug *Paraputo ensete* (Williams & Matile-Ferrero) are unnoticed, as they live below ground and no visual symptoms are observable on above-ground plant parts until after extensive damage has been caused to the underground parts. Density, distribution and loss assessment based on non-destructive sampling methods and damage grades or severity indices have proved very useful in the case of this cryptic mealybug species (E. Tadesse, 2006, MSc thesis, Awassa Univ., Ethiopia) and that study inferred that the losses inflicted by mealybugs corresponding to damage severity indices, was useful in calculating yield losses in advance. The ability to predict pest damage based on earlier field counts is a valuable component of good control decisions (Geiger *et al*. 2001). Little or no information is available regarding within-plant distribution patterns of *P. solenopsis* on cotton in India. The aim of the current study was to determine the within-plant distribution of *P. solenopsis* to facilitate early detection of its incidence and to assess losses caused by different levels of mealybug infestations in cotton. The information generated would improve decision-making by researchers, extension specialists and cotton growers.

Materials and methods

Experiments were conducted on cotton planted at the Central Institute for Cotton Research, Regional Station, Sirsa (Haryana), India, during 2008 and 2009. Cotton cultivar RCH-134 Bt hybrid was planted during the second week of May. The plants were inspected weekly for mealybugs. Upon first detection, ten infested plants were tagged for later assessments of the number of mealybugs per plant at the vegetative, square, and boll formation stages of crop growth.

The periods of vegetative growth, square and boll formation occurred during June to July, August to September, and October to November, respectively. No pesticides were applied in the experimental plots. Initially, *P. solenopsis* infestation was noticed in patches in the field and showed a clumped distribution. Counts did not include crawlers, as they are active and tend to move from one plant to another. Counts of other stages including adults on vegetative parts such as stems and leaves were assessed in three strata, *viz*., upper, middle and lower portion of each selected plant, while counts on floral parts such as squares, flowers and bolls were assessed on the entire plant irrespective of their location in different plant strata. The total number and percentage of *P. solenopsis* mealybugs present (number present on specific plant parts/total count on whole plant X 100) were calculated.

Due to their clumped distribution and aggregation behavior, counting the exact number of individual mealybugs on a cotton plant was difficult. To overcome this difficulty, mealybug incidence was graded based both on their presence on different plant parts and the presence of damage symptoms. The resulting 'Severity Index' of mealybug infestation took into account the total number of mealybugs on infested plant parts as well as their density on the plant. Damaged plants were graded on a scale of 1–4: grade 1, 1–10 mealybugs scattered over the plant; grade 2, either the upper half portion of the stem or one branch infested with mealybugs; grade 3, more than one branch or stem infested; and grade 4, main branch plus side branches infested, and growth stunted. The correlation between different grades of mealybug incidence and crop yield losses due to mealybug infestation was calculated. At the end of the cropping season, 30 plants falling within each grade of mealybug infestation were tagged along with 30 healthy plants. The total seed cotton yield per plant from the infested plants in each grade was recorded and compared with the yield from healthy plants.

Data on the within-plant distribution of mealybugs were analyzed using the General Linear Model procedure for ANOVA (SAS 2009).

Results

The earliest mealybug infestation on the cotton plants was noticed at the onset of the season in the seedling stage. Significant differences in the distribution of mealybugs on different parts of the plant were recorded (Table 1). Throughout the crop growth period, the highest proportion of mealybugs was found on stems located in the upper and middle strata of the plant followed by leaves in the upper strata. In both 2008 and 2009, approximately 60% of the total population was found on stems located in the upper and middle strata. The population distribution on leaves in the upper and middle strata varied between 13.4% and 23.0% (36.4% in total) in 2008 and 12.2% and 27.5% (39.7% in total) in 2009, respectively. During the vegetative crop growth stage (June to July), the main stem and the branches in the upper portion, followed by leaves in the middle strata, harbored the highest proportion of the mealybug population. The stem in lower strata of the plant did not harbor any mealybugs during the vegetative phase. In the case of leaves, the highest mealybug population was recorded from the middle and upper strata of leaves during 2008 and 2009 at the end of the season (in June). In 2009, mealybug populations were low compared with 2008, but the pattern of distribution of mealybugs in different strata was similar. The highest proportion (37.5%) of the mealybug population was recorded on the upper stems followed by leaves in the middle strata (27.5%). The tender upper portions of the stem were the preferred site for feeding and reproduction by the mealybug. No confirmatory reports are available but dispersal of mealybugs to the middle strata is probably density-dependent and in response to adverse climatic conditions such as rainfall.

During the square formation stage (between August and September), the greatest percentage of the mealybug population was observed on the upper strata stems (44.9 and 27.9 average per plant, or 39.7% and 38.5% of the total plant population, respectively), whereas the populations on middle and lower strata were greatly reduced during this period.

In spite of the availability of the squares and flowers during this period, in both years the upper strata leaves contained the next highest concentration of mealybugs, followed by the squares and flowers (Table 1). This is probably due to an increase in the number of leaves available in the plant's upper strata during the square formation stage, providing additional sites for mealybug aggregation and feeding.

Observations during the full boll formation stage, which was characterized by the availability of more than 75% of mature bolls on the plant, were recorded between October and November. The observations on bolls were recorded from the whole plant without any differentiation between strata. During this period, the mealybug density recorded on bolls was not statistically different from that on upper stems during either year. Significantly lower proportions of the mealybug population were recorded on the other plant parts. The data showed that mealybugs preferred the upper portion of the stem during the vegetative and square formation stages, but showed an equal preference for bolls and the stems in the upper strata during the boll formation stage. Populations on the middle portion of the stem were not statistically different from those on the middle leaves.

Mealybugs suck sap from the plant cells and phloem, leading to loss of turgidity in the cotton plant, the growth of sooty mold, and ultimately loss in yield. The reduction in seed cotton yield varied between 14.9% in grade 1 plants and 53.7% in grade 4 plants, with a mean reduction of 35.4% and 32.7% during 2008 and 2009, respectively (Table 2). The severity of infestation and reduction in seed cotton yield were strongly correlated (Table 2).

Discussion

The study revealed that *P. solenopsis* has a clumped distribution on different plant parts. The majority of the *P. solenopsis* population was located on the stems of the plant, except at the end of the season, when the majority of mealybugs were recorded equally on bolls and upper stems, possibly because of nutritional status plus the absence of biotic (natural enemies) and abiotic (low temperature) stress factors at these sites. A similar

Table 1 Distribution of mealybugs on different plant parts at different stages of crop growth

Plant part	Strata	Mean number of mealybugs[z]					
		2008			2009		
		June, end	Aug. second week	Oct. first week	June, end	Aug. second week	Oct. first week
Stem	Upper	42.43[a] (40.2)[y]	44.87[a] (39.7)	45.17[a] (37.45)	24.5[a] (37.5)	27.9[a] (38.5)	35.1[a] (39.2)
	Middle	21.13[bc] (20.0)	6.13[d] (5.4)	1.17[d] (0.90)	13.5[c] (19.7)	1.9[d] (2.6)	0.0[d]
	Lower	0.00[e]	0.37[e] (0.3)	0.40[d] (0.3)	0.0[e]	0.0[ef]	0.0[d]
Leaf	Upper	14.17[c] (13.4)	29.40[b] (26.0)	20.80[b] (17.24)	8.4[d] (12.2)	17.5[b] (24.1)	13.7[b] (15.3)
	Middle	24.23[b] (23.0)	2.27[de] (2.0)	3.50[c] (2.98)	18.9[b] (27.5)	1.0[de] (1.4)	1.9[c] (2.1)
	Lower	3.47[d] (3.3)	0.00[e]	0.00[d]	3.4[e] (4.9)	0.0[f]	0.0[d]
Square	Whole plant		15.97[c] (14.1)			6.7[b] (9.2)	
Flower	Whole plant		13.07[c] (11.6)			17.4[c] (24.0)	
Boll	Whole plant	0.00[e]	0.83[e] (0.7)	49.57[a] (41.00)	0.0[f]	0.0[f]	38.9[a] (43.4)
CV (%)		22.76	20.73	13.51	12.37	9.38	9.86
F value (df = 8, 16)		47.67	45.55	190.39	138.66	253.93	386.98
LSD (P< 0.0001)		1.01	1.00	0.60	0.46	0.35	0.37

[z] Count data were subjected to square root (x+0.25) transformation to stabilize error variance and analyzed with the adopted general linear model procedure for ANOVA (SAS Institute, 2009). Within columns, means followed by a common letter do not differ significantly ($P < 0.0001$, LSD test)

[y] Figures in parentheses are percentage distribution values on respective plant parts

preference for microhabitats by other mealybug species has been reported by Flaherty *et al.* (1992), Clearwater (2000), and Geiger & Daane (2001). For example, abundance of the grape mealybug, *Pseudococcus maritimus* (Ehrhorn), on leaves and berries has been attributed to their providing a superior habitat compared to the bark (Clearwater 2000). Poor control of mealybugs may be partly due to a lack of knowledge about their feeding sites (Stewart 2005), so that the early stages of infestations go undetected and management decisions are initiated only after considerable damage has been done to the plants. At that stage alternative control options (cultural or mechanical) become difficult to implement because of the large number of plants affected by the infestation. The majority of the mealybug species show a clumped distribution, and the natural rate of movement between host plants is poorly understood. One study showed that the dispersal of the grape mealybug can be aided by wind, although there was a marked decline in numbers with increasing distance from the source plant (Grasswitz & James 2008).

Table 2 Effect of different levels of mealybug infestation (severity grades 1–4) on seed cotton yield

Infestation level	Mean reduction (%) in seed cotton yield*	
	2008	2009
Grade 1	16.63	14.87
Grade 2	33.47	30.09
Grade 3	37.83	34.53
Grade 4	53.65	51.16
Mean	35.39	32.66
CV (%)	3.25	3.85
R^2	0.995	0.994
F value (df 3, 27)	1751.3	1406.1
LSD (P<0.001)	1.05	1.15

*Data analyzed with general linear model procedure for ANOVA (SAS Institute, 2009)

Phenacoccus solenopsis was most exposed to pesticides and natural enemies during the vegetative and square formation stages of cotton growth. During the vegetative stage, the highest mealybug distribution was recorded on the upper portions of the succulent stems. *Pseudococcus maritimus* was also frequently found aggregated in masses on the stems or on the undersides of the leaves, difficulting insecticidal control of the mealybug (Geiger & Daane 2001). Schulthess *et al*. (1989) recorded a close relationship between the percentage of the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, living on shoot tips and the relative number of tips present on the cassava plants. The enset root mealybug utilizes root tissue for its food and consequently the majority (79%) of the mealybugs is found inhabiting the roots (Addis *et al*. 2008). In plantain, *Musca paradisiaca*, 94.5% of the total mealybugs – *Dysmicoccus* spp. – present on the plant were recovered on all (lower, middle and upper) portions of the pseudo-stem and a significant correlation was found between the number of mealybugs on the lower and middle pseudo-stems and the total number of mealybugs present (McLeod *et al*. 2002).

The distribution of vine mealybugs, *Pseudococcus longispinus* (Targioni-Tozzetti), *P. calceolariae* (Maskell) and *P. viburni* (Signoret) (=*affinis*), on different plant parts can vary greatly over the season, depending on climate and availability of food (Clearwater 2000). Distribution patterns may be different on perennial plants such as trees and shrubs compared with annual (herbaceous) plants. In our studies on cotton, large proportions of *P. solenopsis* were distributed on bolls and upper portions of the stem during the boll formation stage (October to November), possibly due to reduced night temperatures at the start of winter, together with the shelter provided by the spaces between the calyx and bolls. Geiger *et al*. (2001) reported that in California's Central Valley vineyards, distribution of mealybugs on vines varied greatly throughout the season, with a preference seen for concealed locations under the bark over the year. This combination of factors made sampling difficult and required a greater number of samples to be taken. In scale insects, it has been noted that spatial distribution patterns are the result of behavior, the morphological characteristics of the host-plant tissue, and the differential activity of predator and parasitoids (Nestel *et al*. 1995). Mealybugs prefer different locations for feeding and settling, which affects plant vigor and yield. Addis *et al*. (2008) observed that enset plants attacked by the root mealybug had fewer cord roots than healthy plants and were easily uprooted at the time of assessment. Geiger *et al*. (2001) evaluated damage to grapes due to the grape mealybug, *P. maritimus*, using a rating system, with 0 indicating no sign of mealybug damage, 1 if the bunch was salvageable but with some honeydew, 2 if the bunch was partially salvageable, and 3 if the grapes were unsalvageable. In the present study on cotton, higher severity grade corresponded to higher yield losses.

Acknowledgments We gratefully acknowledge the financial grant provided by World Bank through ICAR, New Delhi to carry out the present study as a part of the National Agricultural Innovation Project at CICR, Regional Station, Sirsa.

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