

Survival pattern of the boll weevil during cotton fallow in Midwestern Brazil

Carmen Sílvia Soares Pires⁽¹⁾, Mayra Pimenta⁽²⁾, Renata Alves da Mata⁽³⁾,
Lucas Machado de Souza⁽¹⁾, Débora Pires Paula⁽¹⁾, Edison Ryoiti Sujii⁽¹⁾ and Eliana Maria Gouveia Fontes⁽¹⁾

⁽¹⁾Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Avenida W5 Norte (Final), Caixa Postal 02372, CEP 70770-917 Brasília, DF, Brazil. E-mail: carmen.pires@embrapa.br, lucas.souza@embrapa.br, debora.pires@embrapa.br, edison.sujii@embrapa.br, eliana.fontes@embrapa.br ⁽²⁾Instituto Chico Mendes de Conservação da Biodiversidade, EQSW 103/104, Complexo Administrativo, Bloco C, Setor Sudoeste, CEP 70670-350 Brasília, DF, Brazil. E-mail: mayrapimenta@yahoo.com.br ⁽³⁾Instituto Federal de Educação, Ciência e Tecnologia de Goiás, Campus Formosa, Rua 64, s/nº, Expansão Parque Lago, CEP 73813-816 Formosa, GO, Brazil. E-mail: alvesdamata@gmail.com

Abstract – The objective of this work was to determine the survival pattern of the cotton boll weevil during fallow in Midwestern Brazil. The percentage of adults that remained in the cotton reproductive structures, the percentage of adults searching for shelters, and the longevity of adults fed on pollen and nectar as alternative food sources were determined. For this, four populations were sampled in cotton squares and bolls, totaling 11,293 structures, from 2008 to 2012. The emergency of cotton weevil adults was monitored from the collection of the structures until the next cotton season. In the laboratory, newly-emerged adults were fed on hibiscus or Spanish needle, and their life span was monitored individually. Most adults (85.73%) left the reproductive structures, regardless of the cotton plant phenology, up to 49 days after the structures were collected. One individual (0.0002%) from 5,544 adults was found alive after the fallow period. The diet with hibiscus and Spanish needle provided adult longevity of 76±38 days, which was enough time for adults to survive during the fallow period. Most of the boll weevils leave the cotton structures at the end of harvest, survive using alternative food sources, and do not use cotton plant structures as shelter during the legal cotton fallow period in Midwestern Brazil.

Index terms: *Anthonomus grandis*, *Gossypium hirsutum*, alternative food, cotton bolls.

Padrão de sobrevivência do bicudo-do-algodoeiro durante o pousio na região Centro-Oeste do Brasil

Resumo – O objetivo deste trabalho foi determinar o padrão de sobrevivência do bicudo-do-algodoeiro durante o pousio no Centro-Oeste do Brasil. Foram determinadas as percentagens de adultos das populações que permaneceram nas estruturas reprodutivas do algodoeiro, as percentagens de adultos que saíram para os refúgios, e a longevidade dos adultos alimentados com pólen e néctar como fontes de alimentos alternativos. Para tanto, foram amostradas quatro populações em botões florais e maçãs do algodoeiro, que totalizaram 11.293 estruturas, de 2008 a 2012. A emergência de adultos do bicudo-do-algodoeiro foi monitorada desde a coleta das estruturas até a próxima safra de algodão. Em laboratório, adultos recém-emergidos foram alimentados com hibisco ou picão, e sua longevidade foi monitorada individualmente. A maioria dos adultos (85,73%) saiu das estruturas reprodutivas, independentemente da fenologia do algodoeiro, até 49 dias após as estruturas terem sido coletadas. Um indivíduo (0,0002%) entre 5.544 adultos foi encontrado vivo após o período da entressafra. A dieta de hibisco e picão permitiu uma longevidade de 76±38 dias, tempo suficiente para manter os adultos vivos durante a entressafra. A maioria dos bicudos-de-algodoeiro deixa as estruturas reprodutivas do algodoeiro no final da colheita, sobrevive com alimento alternativo e não usa as estruturas da planta como abrigo durante o período legal de pousio na região Centro-Oeste do Brasil.

Termos para indexação: *Anthonomus grandis*, *Gossypium hirsutum*, alimento alternativo, maçãs do algodoeiro.

Introduction

The cotton boll weevil, *Anthonomus grandis* Boheman, 1843 (Coleoptera: Curculionidae), has

become one of the main phytosanitary problems affecting the cotton (*Gossypium hirsutum* L.) crop in Brazil since it was first recorded in 1983 (Nakano, 1983). The weevil is difficult to control, because of

the endophagous habit of the larvae and the cryptic behavior of the adults, which find shelter within flower bracts. In the Midwestern region of the country, the control of the cotton boll weevil is based on the intensive use of chemical insecticides, which account for 28% of total production costs (Conab, 2017), with spray applications also during harvest. Other types of associated management practices include: pheromone traps to monitor adults; completely clearing the cotton fields according to Brazilian legislation; and destroying stumps and regrowth in periods that are fixed by law (Azambuja & Degrande, 2014).

In Midwestern Brazil, cotton is grown from November–January to April–June and harvest occurs from May to August, followed by a fallow period of three to four months. During the cotton-growing season, populations of boll weevils increase and, in Brazil it was estimated, based on life cycle (Gabriel et al., 1986), that there are four to seven generations per year. At the end of the crop cycle, harvesting operations begin, such as drying of leaves, collection of fibers, and destruction of crop residues, when many boll weevil individuals are caught in pheromone traps placed around the cotton fields (Ribeiro et al., 2010). A similar behavior has also been observed for boll weevils in the temperate and subtropical regions of North America (Showler, 2003, 2006). In the tropics and subtropics, males and females go into reproductive dormancy (Spurgeon & Raulston, 2006; Paula et al., 2013), but remain active throughout the year (Guerra et al., 1982; Macêdo et al., 2015) due to alternative foods that can sustain them in the absence of cotton (Hardee et al., 1999; Ribeiro et al., 2010).

In the Cerrado and Caatinga biomes in Brazil, Ribeiro et al. (2010) and Macêdo et al. (2015) observed the movement of boll weevils in areas near cotton fields throughout the growing and fallow periods, even when squares and bolls were available. Ribeiro et al. (2010) analyzed the digestive tract of adults captured in areas of uncleared vegetation around these fields and found pollen from more than 20 plant families and from Pteridophyta, as well as fungal spores and algal cysts. In cotton fields, Ribeiro et al. (2010) and Macêdo et al. (2015) captured boll weevils at low densities even after the plants had been defoliated and harvested, and the crop residues destroyed and incorporated into the soil. Showler (2003) also captured a large number of boll weevils in these fields after the crop was harvested and

the residues had been removed, which was attributed to cotton crop residues, including dry and damaged bolls, left on or in the soil. Braga Sobrinho & Lukefahr (1983) had already suggested that bolls damaged at the end of the cycle probably serve as shelters during the fallow period, whereas Greenberg et al. (2003) and Macêdo (2014) reported the survival of boll weevils within dry bolls in the field and in the laboratory, respectively. In favorable temperature and humidity conditions, survival in the laboratory reached 105 days (Macêdo, 2014).

The role played by cotton crop residues and alternative food sources in maintaining much of the boll weevil population during the fallow period has still not been fully clarified under the prevailing environmental conditions in Midwestern Brazil. It is still necessary to determine, for example: if the emergence pattern of adults changes over the cotton cycle, i.e., if, at the end of the season, most adults remain within the structures to withstand the drought conditions prevailing in cotton fallow; what percentage of adults stays within the cotton reproductive structures that fall onto the ground; what percentage of adults leaves for the surrounding environment to seek refuge and alternative foods during the fallow period; and what is the survival rate of the adults that leave the cotton reproductive structures at the end of harvest and feed exclusively on alternative host plants.

The objective of this work was to determine the survival pattern of the cotton boll weevil during fallow in Midwestern Brazil.

Materials and Methods

Four populations of boll weevils (I to IV) originated from the Distrito Federal and the state of Goiás, both located in Midwestern Brazil, were studied between 2008 and 2012 (Table 1) in order to determine: changes in the emergence pattern of adults over the cotton (*Gossypium hirsutum* var. *latifolium* Hutch.) cycle; the percentage of adults that stayed within the cotton reproductive structures that fell onto the ground; and the percentage of adults that left for the surrounding environment.

For populations I and II, squares with 5–10-mm diameter and bolls with 15–30-mm diameter, with signs of boll weevil oviposition, were randomly collected every two weeks in the upper third of the plants from

30 (beginning of flowering) up to 180 days after planting (beginning of boll opening). For populations III and IV, the last generation of adults was evaluated by randomly collecting, on three occasions at the end of harvest, dry bolls that already had fibers formed and signs of boll weevil oviposition. A total of 11,293 reproductive structures were sampled in the four populations (Table 2) and were conditioned in 500-mL transparent plastic pots, covered with organdy to allow air circulation, containing one to five randomly-chosen structures. To confirm if the structures kept on their own would dry out and make the emergence of adult boll weevils difficult, the squares and bolls sampled in one of the populations were conditioned in groups

(about 80 squares and 40 bolls per pot) in 2-L plastic pots. The cotton structures were kept in the laboratory in conditions that would favor the development of boll weevil larvae ($25\pm 2^{\circ}\text{C}$, $60\pm 10\%$ relative humidity, and 13-hour light period), from the time of their collection until the beginning of the flowering period in the next cotton crop; the exception was the structures collected in 2008, containing population I, which were only monitored until the beginning of the fallow period. The pots were observed every two days to record the number of emerged adults. At the end of the incubation period, all the structures were opened to check for the presence of immature or adult individuals, dead or alive, within the squares and bolls.

Table 1. Characterization of boll weevil (*Anthonomus grandis*) populations sampled in cotton (*Gossypium hirsutum* var. *latifolium*) reproductive structures, in Midwestern Brazil.

Population	Location	Coordinates	Management	Sampled period	Incubated cotton structures
I	Experimental area of Cenargen ⁽¹⁾ in Brasília, Distrito Federal	15°43'45"S 47°54'1"W	No use of pesticides, growth regulators, and desiccants	February–June 2008; March–June 2009; January–July 2010; January–May 2011	Square and boll
II	Pamplona farm in Cristalina, Goiás	16°13'21"S 47°38'57"W	Use of pesticides, growth regulators, and desiccants	March–June 2009; March–April 2010	Square and boll
III	Macaé farm in Cristalina, Goiás	16°16'41"S 47°41'2"W	Use of pesticides, growth regulators, and desiccants	July–August 2010	Dry boll
IV	Água Limpa farm in Brasília, Distrito Federal	15°57'4"S 47°56'3"W	No use of pesticides, growth regulators, and desiccants	June–September 2012	Dry boll

⁽¹⁾Embrapa Recursos Genéticos e Biotecnologia.

Table 2. Number and percentage (in parentheses) of different populations of adult boll weevils (*Anthonomus grandis*) that left or stayed (dead or alive) within cotton (*Gossypium hirsutum* var. *latifolium*) reproductive structures during the fallow season, in Midwestern Brazil, from 2008 to 2012.

Population	Sampling year	Number of incubated structures	Emerged adults (%)	Adults found within the structures at the end of the incubation period		
				Dead (%)	Live (%)	Adults observed
I	2008	329	386 (-)	-	-	329
I	2009	268	329 (-)	-	-	268
I	2010	4,712	2,169 (90.19)	236 (9.81)	0	2,405
I	2011	2,746	1,307 (99.39)	8 (0.61)	0	1,315
II	2009	312	79 (82.29)	17 (17.71)	0	96
II	2010	101	64 (100)	0	0	64
III	2010	1,901	197 (50.13)	196 (49.87)	0	393
IV in the laboratory	2012	519	221 (55.80)	174 (43.94)	1 (0.25)	396
IV in the field and laboratory	2012	405	103 (64.38)	57 (35.62)	0	160
Total		11,293	4,855	688	1	5,544

⁽¹⁾Data were not registered because the structures were not opened at the end of the incubation period.

In the present study, the term “emergence” was used to characterize the adults leaving the reproductive structures of the cotton plant, i.e., squares, bolls, and dry bolls. A pattern was observed for adult boll weevil emergence when populations from different locations, years, and areas under varying management conditions were analyzed. In order to assess adult longevity after leaving the cotton reproductive structures at the end of harvest and when feeding on alternative sources of pollen and nectar, two populations of adult boll weevils were assessed: one from the Macaé farm, located in the municipality of Cristalina, in the state of Goiás, and the other from an experimental farm (15°32'18"S; 54°11'49"W) belonging to Instituto Mato-grossense do Algodão, in the state of Mato Grosso, both also in Midwestern Brazil.

For all of the studied populations, the time from the collection of the reproductive structures in the field to the emergence of each individual was determined. Time of emergence was expressed as the number of days that the individual took to leave the structure, taking into account the date on which the cages were set up and incubation started in the laboratory. The pattern of adult emergence was analyzed separately for each of the studied populations and sampled years. For each sampling year and population, the dates were grouped into “start”, “middle”, and “end” of the crop cycle, and curves were constructed for the cumulative percentage of emergence throughout the monitoring period. In these curves, the cumulative percentage of emergence is shown for each seven-day interval. For each population, the mean times of emergence for the adults from the cotton structures collected at the start, in the middle, and at the end of the crop cycle were compared using the analysis of variance (Anova), at 5% probability, or the Kruskal-Wallis nonparametric test, when the data did not fit normal distribution. Each adult was considered as one replicate, and the number of emerged adults of the first two populations at the start, in the middle, and at the end of the crop cycles was, respectively: 134, 153, and 99 for population I in 2008; 29, 270, and 30 for population I in 2009; 744, 827, and 598 for population I in 2010; 274, 705, and 328 for population I in 2011; 3, 37, and 39 for population II in 2009; and 14, 8, and 42 for population II in 2010.

To check if the pattern of adult emergence could be affected by the manual removal of reproductive structures, cotton squares and bolls with signs of boll

weevil oviposition, found on the ground below the cotton plants, were also collected for the assessment of population I, in the 2008 and 2009 crop seasons. The mean emergence times of the adults from the structures collected from the soil and from the plant were compared using Student's t-test. Each adult was considered as one replicate, and the number of emerged adults for these treatments and years was: 244 and 142 for the structures collected from the ground and from the plant, respectively, for population I in 2008; and 148 and 181 for population I in 2009.

To assess if the emergence pattern could have been affected by the constant conditions in the laboratory, the time to emergence was also evaluated in the field. A total of 500 bolls with signs of boll weevil oviposition were selected from the cotton plants of Água Limpa farm, in the Distrito Federal (2012 harvest). These structures were put in bags on 6/21/2012, near the end of the cotton cycle. Even after collecting cotton fiber on 7/5/2012, the plants that contained bagged structures were kept in the field for two more months until 9/5/2012, when all the crop residues were destroyed, according to Regulation no. 44/2008/MAPA (Brasil, 2008), which refers to the elimination of cotton plant residues for phytosanitary reasons. The monitoring of the bagged structures was done weekly to record the number of adults emerged. The emerged boll weevils were removed, and the structure was bagged again, in a way that the mechanical disturbance would be as slight as possible. In this case, emergence time was expressed as the number of days that the individual took to leave after the reproductive structure had been isolated on the plants kept in the field. When the crop residues were removed from the field, all the bagged structures, by now dry bolls, were collected and kept in the laboratory for another five months, in order to monitor periodically the emergence of adults, as done with the other populations (Table 1).

To verify if the crop practices adopted during cotton harvest affected boll weevil emergence from the cotton structures, two samples of population III from Macaé Farm, located in the municipality of Cristalina, in the state of Goiás, were collected in July 2010: one right before desiccant application, and the other right after and before harvest. The structures were incubated in the laboratory under the conditions previously described, and the number of emerged adults was registered periodically until February 2011. Each adult

was considered as one replicate, and the number of emerged adults varied among the two treatments: 163 and 69, respectively, before and after the application of desiccants in the field. The mean emergence times of boll weevils in the structures containing population III, collected before and after the application of desiccants in the field, were compared using Student's t-test.

To monitor the mortality of the adults found inside the dry bolls throughout the period equivalent to cotton fallow, between June and September 2012, a total of 1,749 dry bolls were collected weekly, stored in plastic pots, and kept in the laboratory at $25\pm 2^{\circ}\text{C}$, $60\pm 10\%$ relative humidity, and a 13-hour light period. Every two weeks, 100 of these structures were randomly selected and opened to record the number of live and dead boll weevil adults.

The longevity of adult boll weevils emerging from squares and dry bolls was evaluated using alternative sources of pollen and nectar during the fallow period under laboratory conditions. Both adults emerging from squares and those emerging from dry bolls were subjected to the following treatments: flowers of Spanish needle [*Bidens pilosa* L. (Asteraceae)] and of hibiscus [*Hibiscus rosa-sinensis* L. (Malvaceae)] + water; cotton squares + water; and only water (control). The cotton flowers and squares were offered ad libitum and changed every two days, and water was offered by soaking a hank of cotton. Flowers of Spanish needle and hibiscus were used because they are known to be rich sources of pollen and nectar, besides the fact that Spanish needle is widely found in the cotton growing areas and hibiscus belongs to the same family as cotton. The adults from dry bolls (65 individuals) were obtained at the beginning of June 2011, at the end of the crop cycle, from Macaé farm and those from squares (231 individuals) from an experimental farm belonging to Instituto Mato-grossense do Algodão, where the cotton plants were irrigated. The damaged structures were kept in the laboratory under controlled temperature of $24.56\pm 0.51^{\circ}\text{C}$, relative humidity of $55.21\pm 13.00\%$, and 12 hours of light. The treatments were set up three days after the structures were collected in the field. The adults, totaling 20 up to 91 individuals per treatment, were kept in cages, consisting of 500-mL transparent plastic pots covered with organdy, with a maximum of five individuals in each one. The number of dead individuals was recorded daily. To assess the survival time of the cotton boll

weevil in the different treatments, the mean longevity (in days) of the adults was compared using the Anova, and the adjusted model was subjected to the analysis of residuals to evaluate its fit and distribution of errors. The Kruskal-Wallis test was used when it was not possible to perform the Anova. In the construction of survival curves, the Kaplan-Meier (Kaplan & Meier, 1958) test was used. All the statistical analyses were carried out with the R software, version 2.14.0.2011 (R Core Team, 2011).

Results and Discussion

The populations of boll weevils, from the two sites of origin, evaluated during the reproductive cycle of cotton, from 2008 to 2011, presented a similar emergence pattern, with 100% of the emergences from the cotton structures taking from 5 to 49 days after incubation in the laboratory (Figure 1). The mean time of emergence of the adults from the cotton structures collected at the start, in the middle, and at the end of the crop cycle did not differ significantly for any of the populations studied, as shown by the obtained results: SD=2, F=0.78, and p=0.841 for population I in 2008; SD=2, F=0.82, and p=0.468 for population I in 2009; SD=2, F=0.50, and p=0.618 for population I in 2010; SD=2, F=1.57, and p=0.296 for population I in 2011; SD=2, F=0.31, and p=0.745 for population II in 2009; and SD=2, F=0.99, and p=0.418 for population II in 2010.

Apparently, the proximity of the end of the cotton cycle did not affect the emergence pattern of the boll weevils (Figure 2), since adults from the two populations from dry bolls collected from June to September, soon before cotton harvest, did not differ regarding mean incubation time, as observed for population III from Macaé farm in 2010 and for population IV from Água Limpa farm in 2012. The boll weevils from the dry bolls at the end of the cotton crop cycle emerged sooner than those from squares in the middle of the cycle (populations III and IV). This result could be attributed to the collection of dry bolls that already had adults formed and ready to emerge. Considering that the larval cycle of the boll weevil lasts 18 to 21 days on average (Gabriel et al., 1986), it might be inferred that the incubation time of eggs and larvae in an initial development stage in the cotton reproductive structures collected in the field

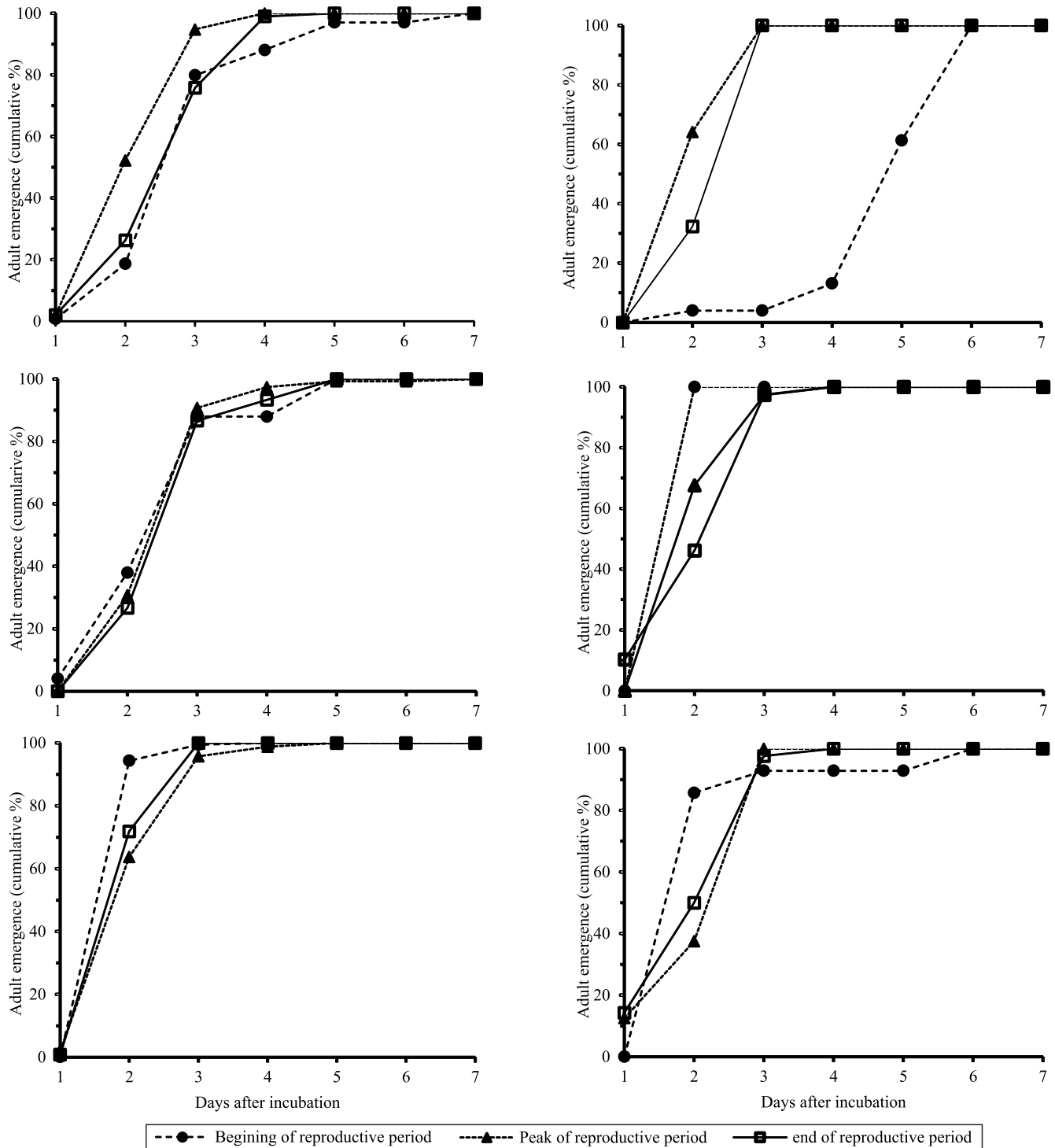


Figure 1. Emergence pattern (cumulative percentage) of adult boll weevils (*Anthonomus grandis*) from cotton (*Gossypium hirsutum* var. *latifolium*) reproductive structures (squares and bolls) collected during different reproductive stages of the plants. The emergence pattern was monitored in two boll weevil populations, during different years: one from the experimental area of Embrapa Recursos Genéticos e Biotecnologia, located in Brasília, in the Distrito Federal, from 2008 to 2011; and the other from Pamplona farm, located in Cristalina, in the state of Goiás, during 2009 and 2010. Emergence time was expressed as the number of days that the individual took to leave the cotton structure after its collection in the field and incubation in laboratory conditions ($25\pm 2^{\circ}\text{C}$, $60\pm 10\%$ relative humidity, and 13-hour photophase).

was of 10 to 15 days. However, for the structures that contained fifth-stage larvae, pre-pupae, and pupae, the incubation times were shorter than five days. The act of removing the dry bolls from the cotton plant and keeping them in the laboratory may have been a mechanical stimulus for the emergence of adults from

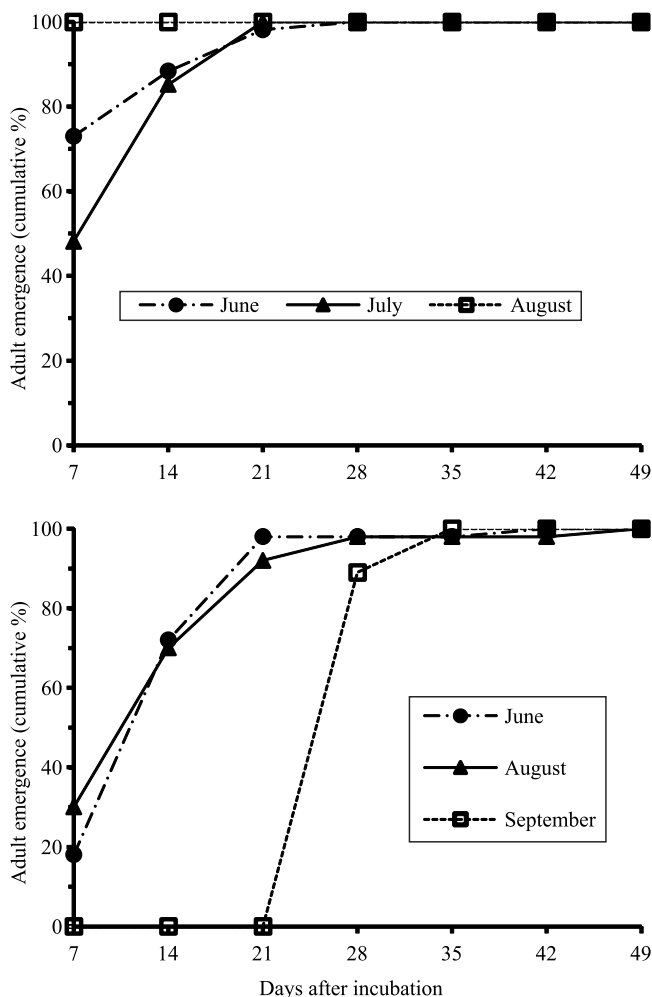


Figure 2. Emergence pattern (cumulative percentage) of two populations (III and IV) of adult boll weevils (*Anthonomus grandis*) from cotton (*Gossypium hirsutum* var. *latifolium*) bolls collected at the end of the reproductive stage of the plants. Population III was obtained from Macaé farm, located in Cristalina, in the state of Goiás (Kruskal-Wallis, $\chi^2=1.92$, $SD=2$, and $p=0.38$); and population IV from Água Limpa farm, located in the Distrito Federal ($F=0.93$, $SD=2$, and $p=0.35$). Emergence time was expressed as the number of days that the individual took to leave the cotton structure after its collection in the field and incubation in laboratory conditions ($25\pm 2^\circ\text{C}$, $60\pm 10\%$ relative humidity, and 13-hour photophase).

these structures, as happens during harvest on farms (Showler, 2003; Lima Jr. et al., 2013). These results are indicative that the crop management at the end of the cycle, with desiccant application and harvesting, could disturb the adults and force them to leave the cotton structures.

Regarding adult boll weevils, the mean incubation time in the structures sampled at the end of the cotton cycle was shorter soon after the application of desiccants (2.5 ± 1.29 days), than right before their application (10.33 ± 4.52 days) ($t=3.32$, $SD=11$, and $p=0.006$). This may explain the high number of adults captured in the traps, as noted at the end of the cotton cycle (Showler, 2003; Lima Jr. et al., 2013).

No significant difference was found for the mean emergence time (days) of boll weevil adults in the structures collected from soil surface and from the plant, respectively, in the two years when population I was studied: 16.54 ± 5.66 and 20.63 ± 8.24 days in 2008; and 14.73 ± 6.30 and 11.98 ± 4.64 days in 2009. This result shows that, in the present work, the sampling method of collecting reproductive structures from the plants did not affect larval development and the emergence pattern of the adults.

Adults emerging at the end of harvest from dry bolls, kept in bags on cotton plants from June 21 to September 5 (when the plants were removed from the field) and then incubated in the laboratory, presented an emergence pattern similar to that of those emerged from the reproductive structures (squares and bolls) kept in the laboratory throughout the crop cycle (Figure 3). After 49 days of observation in the field, 68.3% of the adults had already left the dry bolls, whereas, after 70 days of observation, 98% had left these structures. The remaining 0.02% of adults emerged from the dry bolls in the laboratory just seven days after incubation. Therefore, by the beginning of September, 100% of the adults had left the reproductive structures of the cotton plant.

On all the sampling dates for squares and bolls, almost all adults found inside the cotton reproductive structures in February of the following sampling year were dead (Table 2). However, one live adult was found in February 2013, that is, 153 days after incubation, in the bolls collected at the end of the 2012 cycle, in Água Limpa farm. This represented 0.25% of the boll weevils of population IV and 0.0002% of the boll weevils of all four sampled populations (5,544 adults),

a number that is not negligible considering the high biotic potential of the insect. For the dry bolls that were bagged on the plants from June to September 2012, the last live adult was found within one of the structures at the beginning of December 2012, 90 days after the structure had been removed from the plant and incubated in the laboratory. The number of adults found dead within the cotton reproductive structures varied from 0.61% for population I to 43.94% for population IV.

In all laboratory and field observations, most adults (85.73%) of the four evaluated populations left the structures in which they had developed during the different phases of the cotton crop cycle in up to 49 days. Most adults (14.25%) that had not left by the beginning of the reproductive period of the next cotton crop were found dead (Table 2). The high percentages of adults found dead inside the structures may be a consequence of the management strategy practiced at Pamplona farm (population II) and Macaé farm (population III), which included applying desiccants and insecticides for the control of boll weevils just

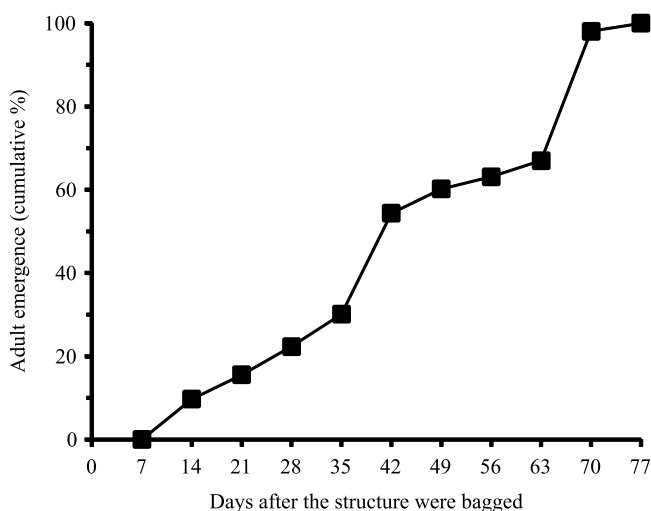


Figure 3. Emergence pattern (cumulative percentage) of the fourth (IV) evaluated population of adult boll weevils (*Anthonomus grandis*), obtained from cotton (*Gossypium hirsutum* var. *latifolium*) bolls monitored in the field at the end of the cotton season (June, July, and August 2012) and in the laboratory during the fallow season (from September to February 2013). Population IV was obtained from Água Limpa farm, located in the Distrito Federal, under laboratory conditions (25±2°C, 60±10% relative humidity, and 13-hour photophase).

before harvest. Desiccants act on the physiology of the plant, accelerating its metabolism, which could indirectly interfere with the development process of boll weevil larvae and pupae. According to Greenberg et al. (2004a), based on field observations, the effects of defoliant on insects have been suggested, but the mechanisms involved are poorly known, indicating that more detailed studies are necessary. However, in the experimental area at Água Limpa farm (population IV) desiccants and insecticides were not applied, and the number of dead boll weevils inside the structures was higher than the number that emerged, without any apparent cause being identified.

In the municipality of Cristalina, in the state of Goiás, the legal fallow period is of 80 days, usually from August 8 to October 30 (Goiás, 2014). In the present study, it was noted that boll weevils spent less time in the reproductive structures than the duration of the fallow period in the region under study. Considering that the cotton flowering period starts between 30 and 45 days after planting (Rosolem, 2001), the squares in the new planted areas will become available for the boll weevil about 125 days after the previous crop has finished. This shows that the time that the boll weevil stayed within the structures was not enough for it to protect itself during the whole fallow period until the cotton reached its reproductive phase in the next crop cycle. In the Distrito Federal and in the state of Goiás, nearly all boll weevils at the end of the crop did not remain protected within the dry bolls for more than 50 days, but probably left in search of shelter and food in the surrounding areas (Figure 3). This result reinforces the importance of destroying cotton stalks for boll weevil management, since, at any time, adults who have left the structures are able to colonize the regrowth of cotton plants after harvest (Paula et al., 2013; Ribeiro et al., 2015).

Under the conditions for cotton cultivation prevalent in the subtropical regions of the United States, a substantial number of boll weevils may remain in cotton fields, even after defoliation, harvesting, and incorporation of crop residues into the soil, because some food sources can still be found (Showler, 2006). Greenberg et al. (2004b) pointed out that a combination between soil type, temperature, and humidity, as well as the position of the bolls, on the soil or buried in it, can be decisive factors for the survival of the boll weevil in empty cotton fields. Although the boll weevils can

remain in empty fields, Showler (2006) found that 98% of them leave the reproductive structures of the cotton plant. In the present study, practically 100% of the boll weevils left the dry bolls soon after completing their development.

The adults fed on cotton squares presented greater longevity than those fed on pollen from alternative plants or on only water, surviving for a mean of 101 ± 51 days (Figure 4). The longevity of adults emerging from dry bolls was greater than that of those emerging from squares, both for the treatment with water (log-rank test: $z=6.22$ and $p<0.001$) (Figure 4 A) and for that with cotton squares (log-rank test: $z=-2.21$ and $p=0.020$) (Figure 4 B). The development of larvae in bolls generates larger adults with greater lipid reserves (Rolim, 2014), which may favor survival during the cotton fallow period. The boll weevils from the bolls at the end of the crop cycle allocated energy to survive in detriment of developing their reproductive system (Paula et al., 2013). This pattern is associated with the phenology of the cotton plant and may be considered the main adaptive strategy of the boll weevil to overcome fallow periods in Midwestern Brazil.

Independently of the food source for larvae, no significant difference was observed in the survivorship pattern of adults fed with pollen and nectar from hibiscus and Spanish needle (Figure 4). This diet produced mean longevity of 76 ± 38 days, and only one individual, from a total of 87 adults, reached 225 days. The adults kept only on water survived a mean of 16.91 ± 14.97 days, with two individuals reaching 60 and 96 days. Of all the adults fed on flowers from these alternative plants, 5.7% presented longevity over 120 days, which is the time necessary to reach the start of the flowering period in the next crop cycle. The longevity of adults fed on hibiscus and Spanish needle flowers confirmed that sources of nectar and pollen from alternative plants can be enough for boll weevils to survive during the period when cotton is not available. Similar results were reported by Gabriel (2002) for adult boll weevils from cotton squares, when fed with the species *Hibiscus tiliaceus* L. The author observed that boll weevils fed with hibiscus lived longer (225 days for males and 253 days for females) than those fed with *Malvaviscus arboreus* Cav. (42 and 95 days for males and females, respectively) and *Abutilon striatum* G.F.Dicks. ex Lindl. (17 and

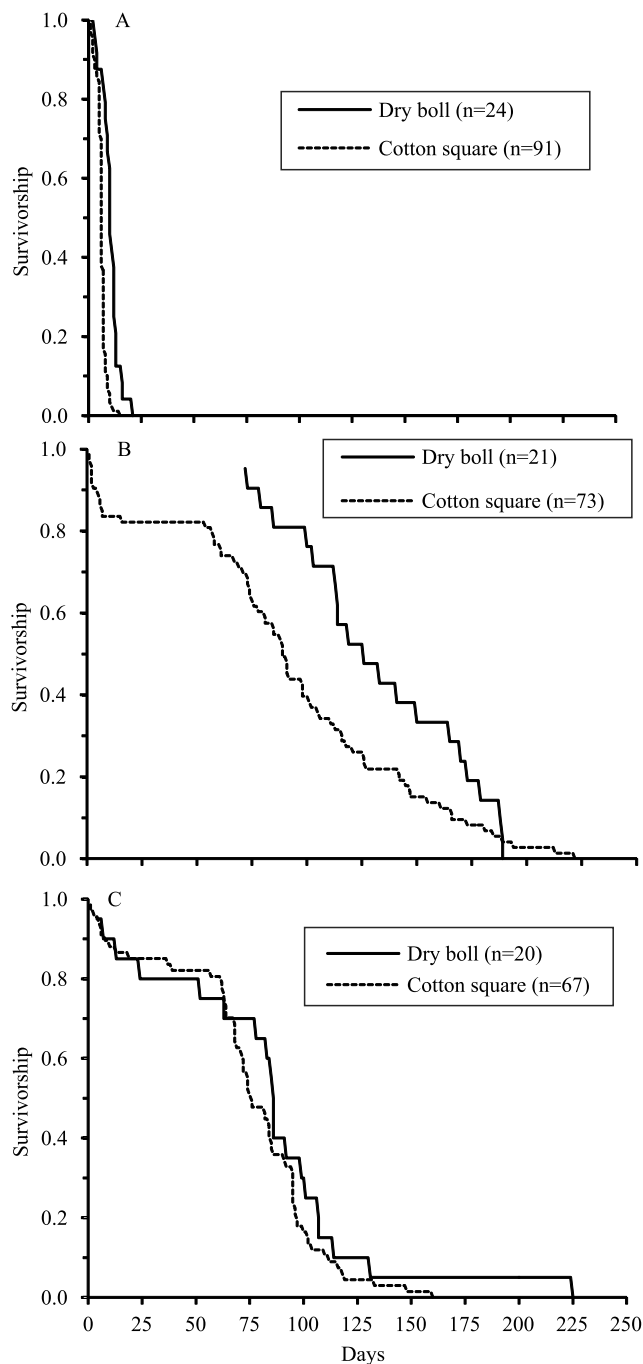


Figure 4. Survival curves for boll weevil (*Anthonomus grandis*) adults that emerged from cotton (*Gossypium hirsutum* var. *latifolium*) squares and bolls, when fed on: A, only water (control); B, cotton squares; and C, pollen and nectar from Spanish needle (*Bidens pilosa*) and hibiscus (*Hibiscus rosa-sinensis*) under laboratory conditions ($24.56 \pm 0.51^\circ\text{C}$ and $55.21 \pm 13.00\%$ relative humidity) during cotton fallow in Midwestern Brazil. The curves were constructed using the Kaplan-Meier test (Kaplan & Meier, 1958).

50 days for males and females, respectively). Pimenta et al. (2016) also assessed the nutritional suitability of alternative plants for adult boll weevils, which showed a preference for flowering okra and hibiscus over cotton in its vegetative stage. According to Ribeiro et al. (2010), pollen from different plant species found in remnants of Cerrado vegetation is used by adult boll weevils during the intercrop period. Therefore, areas of natural vegetation, such as the Cerrado, may be sources of alternative food due to the diversity of plants, as can be the case with any environment, even a cultivated field, which provides alternative food, as found by Spurgeon & Raulston (2006), Showler (2006), and Greenberg et al. (2007).

The results obtained in the present study are indicative that, in Midwestern Brazil, the majority of adult boll weevils do not use old cotton plant structures systematically as shelter during the legal fallow period of 80 days and also that, in the absence of the host plant, this insect needs alternative food to survive until the beginning of the next crop. However, a small part of the population could use cotton plant structures as shelter during the cotton fallow period, considering the confirmed percentage (99.97%) of live adults from different generations during the cotton cycle that left the reproductive structures in search of new squares and bolls for oviposition and food. At the end of the crop cycle, the adults search for alternative food sources and shelter in order to survive the fallow period. This mass egress, both during and after the harvesting procedures, has been reported in the state of São Paulo, in the Distrito Federal, and in the state of Bahia by Campanhola et al. (1988), Ribeiro et al. (2010), and Macêdo et al. (2015), respectively, and has been confirmed in monitoring studies using traps baited with pheromone, carried out in cotton-producing areas in the state of Goiás (Lima Jr. et al., 2013).

During the fallow period, when cotton plant residues are completely removed, according to legislation, in the Distrito Federal and in the state of Goiás, boll weevils are found around the cotton fields, mainly in facultative reproductive dormancy, especially the females (Paula et al., 2013). However, the physiological reduction in reproduction does not keep boll weevils from moving and searching for food to survive until the cotton fields have been planted again and squares begin to form (Jones et al., 1992; Hardee et al., 1999; Showler & Abrigo, 2007; Ribeiro et al., 2010; Macêdo et al., 2015). In addition, these areas may possibly provide

shelter for adult boll weevils to protect themselves against adverse conditions, such as low humidity and high temperatures, both typical of this region in the cotton fallow period. However, other studies need to be performed to better understand the dispersal patterns of boll weevil populations, as well as to identify and characterize the places where adults find refuge and food when cotton is not grown.

Conclusions

1. Most adult boll weevils (*Anthonomus grandis*) do not use cotton (*Gossypium hirsutum* var. *latifolium*) plant structures as shelter during the legal cotton fallow period of 80 days in Midwestern Brazil.

2. Adult boll weevils survive during the fallow season by feeding on nectar and pollen from alternative plants, such as Spanish needle (*Bidens pilosa*) and hibiscus (*Hibiscus rosa-sinensis*).

Acknowledgments

To Programa Nacional de Pós-Doutorado (PNPD) from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), for postdoctoral grants to the second and third authors; and to Gisele Chagas, Lorena Bravo, Diogo Claudino, and Renata Timbó, for their help in carrying out the experiments.

References

- AZAMBUJA, R.; DEGRANDE, P.E. Trinta anos do bicudo-do-algodoeiro no Brasil. *Arquivos do Instituto Biológico*, v.81, p.377-410, 2014. DOI: 10.1590/1808-1657000012013.
- BRAGA SOBRINHO, R.; LUKEFAHR, M.J. **Bicudo (*Anthonomus grandis*, Boheman): nova ameaça à cotonicultura brasileira: biologia e controle**. Campina Grande: EMBRAPA-CNPA, 1983. 32p. (EMBRAPA-CNPA. Documentos, 22).
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 44, de 29 de julho de 2008. [Institui o Programa Nacional de Controle do Bicudo do Algodoeiro – PNCB, no Ministério da Agricultura, Pecuária e Abastecimento, visando à prevenção e ao controle do bicudo *Anthonomus grandis* em cultivos de algodão nas Unidades da Federação]. **Diário Oficial da União**, 30 jul. 2008. Seção 1, p.4.
- CAMPANHOLA, C.; GABRIEL, D.; MARTIN, D.F.; CALCAGNOLO, G. Levantamento de adultos do bicudo *Anthonomus grandis* Boheman, 1843 (Coleoptera: Curculionidae) utilizando armadilhas com feromônio, em alguns municípios do estado de São Paulo. **Anais da Sociedade Entomológica do Brasil**, v.17, p.135-156, 1988.

- CONAB. Companhia Nacional de Abastecimento. **Custos de produção – Culturas de verão – Série histórica:** algodão 1998 a 2015. Available at: <<http://www.conab.gov.br/conteudos.php?a=1555&t=2>>. Accessed on: Mar. 3 2017.
- GABRIEL, D.; CALCAGNOLO, G.; TANCINI, R.S.; DIAS NETTO, N. Estudos de biologia do *Anthonomus grandis* Boheman, 1843 (Coleoptera, Curculionidae), em condições de laboratório. **O Biológico**, v.52, p.83-90, 1986.
- GABRIEL, D. Longevidade do bicudo do algodoeiro *Anthonomus grandis* Boh., criado em hospedeiras alternativas no laboratório. **Arquivos do Instituto Biológico**, v.69, p.123-126, 2002.
- GOIÁS. Agência Goiana de Defesa Agropecuária. Instrução Normativa nº 4/2014. **Diário Oficial [do] Estado de Goiás**, 10 jun. 2014. p.6.
- GREENBERG, S.M.; JONES, G.D.; EISCHEN, F.; COLEMAN, R.J.; ADAMCZYK JR., J.J.; LIU, T.-X.; SETAMOU, M. Survival of boll weevil (Coleoptera: Curculionidae) adults after feeding on pollens from various sources. **Insect Science**, v.14, p.503-510, 2007. DOI: 10.1111/j.1744-7917.2007.00179.x.
- GREENBERG, S.M.; SAPPINGTON, T.W.; ELZEN, G.W.; NORMAN, J.W.; SPARKS, A.N. Effects of insecticides and defoliant applied alone and in combination for control of overwintering boll weevil (*Anthonomus grandis*; Coleoptera: Curculionidae) – laboratory and field studies. **Pest Management Science**, v.60, p.849-858, 2004a. DOI: 10.1002/ps.878.
- GREENBERG, S.M.; SAPPINGTON, T.W.; SPURGEON, D.W.; SÉTAMOU, M. Boll weevil (Coleoptera: Curculionidae) feeding and reproduction as functions of cotton square availability. **Environmental Entomology**, v.32, p.698-704, 2003. DOI: 10.1603/0046-225X-32.3.698.
- GREENBERG, S.M.; SHOWLER, A.T.; SAPPINGTON, T.W.; BRADFORD, J.M. Effects of burial and soil condition on postharvest mortality of boll weevils (Coleoptera: Curculionidae) in fallen cotton fruit. **Journal of Economic Entomology**, v.97, p.409-413, 2004b. DOI: 10.1093/jee/97.2.409.
- GUERRA, A.A.; GARCIA, R.D.; TAMAYO, J.A. Physiological activity of the boll weevil during the fall and winter in subtropical areas of the Rio Grande Valley of Texas. **Journal of Economic Entomology**, v.75, p.11-15, 1982. DOI: 10.1093/jee/75.1.11.
- HARDEE, D.D.; JONES, G.D.; ADAMS, L.C. Emergence, movement, and host plants of boll weevils (Coleoptera: Curculionidae) in the Delta of Mississippi. **Journal of Economic Entomology**, v.92, p.130-139, 1999. DOI: 10.1093/jee/92.1.130.
- JONES, R.W.; CATE, J.R.; HERNANDEZ, E.M.; NAVARRO, R.T. Hosts and seasonal activity of the boll weevil (Coleoptera: Curculionidae) in tropical and subtropical habitats of northeastern Mexico. **Journal of Economic Entomology**, v.85, p.74-82, 1992. DOI: 10.1093/jee/85.1.74.
- KAPLAN, E.L.; MEIER, P. Nonparametric estimation from incomplete observations. **Journal of the American Statistical Association**, v.53, p.457-481, 1958. DOI: 10.1080/01621459.1958.10501452.
- LIMA JR., I.S. de; DEGRANDE, P.E.; MIRANDA, J.E.; SANTOS, W.J. dos. Evaluation of the boll weevil *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) suppression program in the state of Goiás, Brazil. **Neotropical Entomology**, v.42, p.82-88, 2013. DOI: 10.1007/s13744-012-0083-3.
- MACÊDO, J.A. de; CASTELLANI, M.A.; SANTOS, F. de A.R. dos; OLIVEIRA, P.P.; MALUF, R.P. Fontes alternativas de pólen utilizadas pelo bicudo-do-algodoeiro em duas regiões produtoras de algodão na Bahia. **Revista Caatinga**, v.28, p.255-262, 2015. DOI: 10.1590/1983-21252015v28n329rc.
- MACÊDO, J.A. **Fontes alternativas de alimento e sobrevivência do bicudo *Anthonomus grandis* Boheman, 1843 (Coleoptera, Curculionidae) em carimã na entressafra.** 2014. 87p. Dissertação (Mestrado) – Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista.
- NAKANO, O. Bicudo: a praga mais importante do algodão. **Agroquímica**, v.21, p.10-14, 1983.
- PAULA, D.P.; CLAUDINO, D.; TIMBÓ, R.V.; MIRANDA, J.E.; BEMQUERER, M.P.; RIBEIRO, A.C.J.; SUJII, E.R.; FONTES, E.M.G.; PIRES, C.S.S. Reproductive dormancy in boll-weevil from populations of the Midwest of Brazil. **Journal of Economic Entomology**, v.106, p.86-96, 2013. DOI: 10.1603/EC12092.
- PIMENTA, M.; MATA, R.A.; VENZON, M.; CUNHA, D.N.C.; FONTES, E.M.G.; PIRES, C.S.S.; SUJII, E.R. Survival and preference of cotton boll weevil adults for alternative food sources. **Brazilian Journal of Biology**, v.76, p.387-395, 2016. DOI: 10.1590/1519-6984.16214.
- R CORE TEAM. **R: a language and environment for statistical computing.** Vienna: R Foundation for Statistical Computing, 2011. Available at: <<http://www.R-project.org/>>. Accessed on: June 15 2011.
- RIBEIRO, E.B.; CASTELLANI, M.A.; SILVA, C.A.D. da; MELO, T.L.; SILVA, G. dos S.; VALE, W.S. do; SANTOS, A.S. Métodos de destruição de restos de cultura do algodoeiro e sobrevivência do bicudo. **Pesquisa Agropecuária Brasileira**, v.50, p.993-998, 2015. DOI: 10.1590/S0100-204X2015001100001.
- RIBEIRO, P. de A.; SUJII, E.R.; DINIZ, I.R.; MEDEIROS, M.A. de; SALGADO-LABORIAU, M.L.; BRANCO, M.C.; PIRES, C.S.S.; FONTES, E.M.G. Alternative food sources and overwintering feeding behavior of the boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) under the tropical conditions of Central Brazil. **Neotropical Entomology**, v.39, p.28-34, 2010. DOI: 10.1590/S1519-566X2010000100005.
- ROLIM, G.G. **Desenvolvimento, reprodução e sobrevivência do bicudo-do-algodoeiro em função do tipo de alimento.** 2014. 40p. Dissertação (Mestrado) – Universidade Federal Rural de Pernambuco, Recife.
- ROSOLEM, C.A. Ecofisiologia e manejo da cultura do algodoeiro. **Informações Agrônomicas**, n.95, p.1-9, 2001.
- SHOWLER, A.T. Effects of routine late-season field operations on numbers of boll weevils (Coleoptera: Curculionidae) captured in large-capacity pheromone traps. **Journal of Economic Entomology**, v.96, p.680-689, 2003. DOI: 10.1093/jee/96.3.680.
- SHOWLER, A.T. Short-range dispersal and overwintering habitats of boll weevils (Coleoptera: Curculionidae) during and

after harvest in the subtropics. **Journal of Economic Entomology**, v.99, p.1152-1160, 2006. DOI: 10.1093/jee/99.4.1152.

SHOWLER, A.T.; ABRIGO, V. Common subtropical and tropical nonpollen food sources of the boll weevil (Coleoptera: Curculionidae). **Environmental Entomology**, v.36, p.99-104, 2007. DOI: 10.1093/ee/36.1.99.

SPURGEON, D.W.; RAULSTON, J.R. Boll weevil (Coleoptera: Curculionidae) adult diapause responses to selected environmental and dietary conditions. **Annals of the Entomological Society of America**, v.99, p.1085-1100, 2006. DOI: 10.1603/0013-8746(2006)99[1085:BWCCAD]2.0.CO; 2.

Received on July 18, 2016 and accepted on November 17, 2016