NOTAS CIENTÍFICAS

Mechanical damage in cotton buds caused by the boll weevil[1]

Roseane Cavalcanti Santos[2], Lucília Helena Marcellino[3], Rose Gomes Monnerat[3] and Eugen Silvano Gander[3]

Abstract – The boll weevil (*Anthonomus grandis* Boheman) causes high levels of bud abscission in cotton plants due to feeding or oviposition punctures. It has been reported that abscission is mainly due to enzymes present in the insect's saliva, but mechanical damage could also contribute to square abscission. The objective of this paper was to undertake an analysis of the morphological damages caused by the insect in cotton squares using microscopy. Anthers and ovules are the main target of boll weevil feeding. The process initiates by perforation of young sepal and petal tissues and proceeds with subsequent alimentation on stamen and ovary leading to abscission of floral structures.

Index terms: *Gossypium hirsutum*, *Anthonomus grandis*, abscission, animal feeding.

Danos mecânicos em botões florais de algodão causados pelo bicudo-do-algodoeiro

Resumo – O bicudo (*Anthonomus grandis* Boheman) provoca alto porcentual de abscisão de botões florais no algodoeiro causada pelos orifícios de alimentação e oviposição. Embora a abscisão ocorra em virtude principalmente da ação de enzimas presentes na saliva do inseto, o dano mecânico também poderia contribuir para a queda dos frutos. O objetivo desse trabalho foi avaliar danos morfológicos causados pelo inseto nos botões florais com auxílio da microscopia. O principal alvo de alimentação do bicudo são as anteras e os óvulos. Esse processo, preferencialmente iniciado nas sépalas e pétalas mais novas, inclui a perfuração dos tecidos e posterior mastigação dos estames e ovário levando a abscisão da estrutura floral.

Termos para indexação**:** *Gossypium hirsutum*, *Anthonomus grandis*, abscisão, alimentação animal.

(1)Accepted for publication on September 4, 2003.

(2)Embrapa-Centro Nacional de Pesquisa de Algodão, Caixa Postal 174, CEP 58100-000 Campina Grande, PB, Brasil. E-mail: roseane@cnpa.embrapa.br

(3)Embrapa-Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia, Parque Estação Biológica Final W3 Norte, CEP 70770-900 Brasília, DF, Brasil. E-mail: lucilia@cenargen.embrapa.br, rose@cenargen.embrapa.br, egander@cenargen.embrapa.br

The boll weevil (*Anthonomus grandis*, Boheman 1843) is, since its first occurrence in Brazil in the mid 80s, one of the major insect pests responsible for substantial overall losses in cotton production and for the reduction in fiber quality. The economic importance of cotton industry in Brazil and abroad leads many institutions involved with research to obtain cotton varieties resistant or tolerant to boll weevil attacks through classical breeding methods (Freire & Beltrão,1997; Almeida, 2000).

The Empresa Brasileira de Pesquisa Agropecuária (Embrapa) developed the earliness cultivars 'CNPA Precoce 1', 'CNPA Precoce 2' and 'EMBRAPA BRS 186' which are characterized by concentrated and massif fructification, small stature and a premature maturation cycle of 110 to 120 days. This time of maturation is 20 days shorter than in other varieties, thus producing good yield before boll weevil population attains dimensions big enough to cause economically significant damages (Carvalho & Moreira, 1990; Carvalho, 1999). However, this resulted in limited success, as effective control of the boll weevil population is only possible through application of massif doses of chemical insecticides because of the extraordinary growth rate of the insect population. During one generation of cotton, which lasts between 110 and 170 days, five to six boll weevil cycles occur. Thus every ten adults that enter diapause, produce, at the end of the harvest season, 100,000 adults (Barbosa et al., 1986), a fact that makes this insect one of the most effective and dynamic plagues.

Phenological studies in cotton show that under physiological conditions the plant aborts between 40 and 60% of its buds (Tanskiy, 1969; Rosolem, 1999). The abscission rate in plants occupied by the boll weevil is significantly higher since the insect establishes itself on its host at the beginning of flower development (Vieira & Lima, 1999). The reproductive structures are the main targets of attack and young buds (4 to 9 mm diameter) are preferentially used for feeding as well as for oviposition. Buds usually show at least three feeding and one oviposition perforation and shedding or rotting occurs 5 to 10 days after oviposition (Leigh & Lincoln, 1964).

Though some authors believe that the insects feed on all flower structures, including the bracteoles, anthers and ovules are the prime target since, even when young, they contain essential nutrients, e.g. starch and sterols, necessary to the development of larvae and adults (Earle et al., 1967; Barbosa et al., 1986; Carvalho & Moreira, 1990; Carvalho, 1999).

Bud abscission is mainly due to enzymes present in the insect's saliva and subsequent necrosis of the tissue surrounding the perforation site (Coakley et al., 1969, King & Lane, 1969). However, the mechanical damage resulting from the weevils' attacks could also contribute to bud abscission.

The objective of this paper was to undertake an analysis of the morphological damages caused by the boll weevil in young cotton buds using light and scanning microscopy.

Young floral buds (∅: 5 mm) from the EMBRAPA BRS 186 cultivar presenting one feeding penetration were collected in the cotton field and kept on ice. The bud tissues (sepal, petals, anthers and exocarp) were dissected and fixed in glutaraldehyde-paraformaldehyde (2 and 4%, respectively) in 0.05 M sodium cacodylate buffer (pH 7.3) containing 5 mM $CaCl_2$. Postfixation was done in 2% OsO_4 plus 1.6% $K_3Fe(CN)_6$. After dehydration in an acetone gradient and mounting, the samples were gold-coated for scanning microscopy. Boll weevil rostra were prepared identically, with the exception that 0.1 M cacodylate buffer was used during fixation.

Alimentary residues in the insect's midgut were studied by preparing midgut sections of imagoes and second instar larvae, which were fixed and processed as described for the rostra and then embedded in Spur. Semi-thin 500 nm sections were prepared on a Leica Ultracut microtome and stained with toluidine blue for light microscopy. Alternatively larval intestines were dissected and immediately stained with acid carmine (0.4%) and analyzed in the light microscope using differential interferential contrast.

The feeding act of the weevil itself proceeds in two steps. First, the insect has to perforate the protective layers of the flower bud and only then actual alimentation can start. In young buds such as the one shown in Figure 1A, penetration usually starts in the middle or close to floral receptacle of the bud where the tissue is tender. In older buds, however, the attack is always in the middle part of the bud since in the basal region the tissue is more rigid. The opening caused by the insect can measure up to 1 mm^2 and the middle part of the calyx is the first target (Figure 1B), followed by the corolla whose soft petals (Figure 1C) represent no great problem for the weevil's rostrum.

Most probably if only these parts were attacked, bud abscission would be less severe. In buds of this size, however, the rostrum penetrates these layers easily and leaves a mixture of debris and saliva at the borders of the wound. The rigid and thick exocarp of the ovary (Figure 1D) is perforated next and finally the rostrum, now completely buried (Figure 1E), reaches the anthers and the ovules. Figure 1F shows a transverse section through a perforated flower bud and it is obvious that both, anthers and ovules, are severely damaged by the insect. A detail of the damaged anthers is shown in Figure 1G; pollen production is reduced and probably completely inhibited.

The mechanical damage caused by the boll weevil's snout cannot be underestimated. This organ is usually 3 to 4 mm long, which amounts to half of the insects body length (Pfadt, 1978), and shows characteristic “elbowed” antennae (Figure 2A). Along its length the rostrum is equipped with chitineous bristles (Figure 2B) which, during perforation, help to open up the plant tissue. At the distal end of the rostrum the mandibles form a claw-like structure, designed to open the cotton square (Figures 2C and 2D).

Each developmental stage during the life-cycle of the boll weevil can cause mechanical harm to the cotton fruits. In particular the larvae, which feed on the anthers and ingest pollen (Figure 1H), cause severe damage to the buds which putrefy and ultimately abscise.

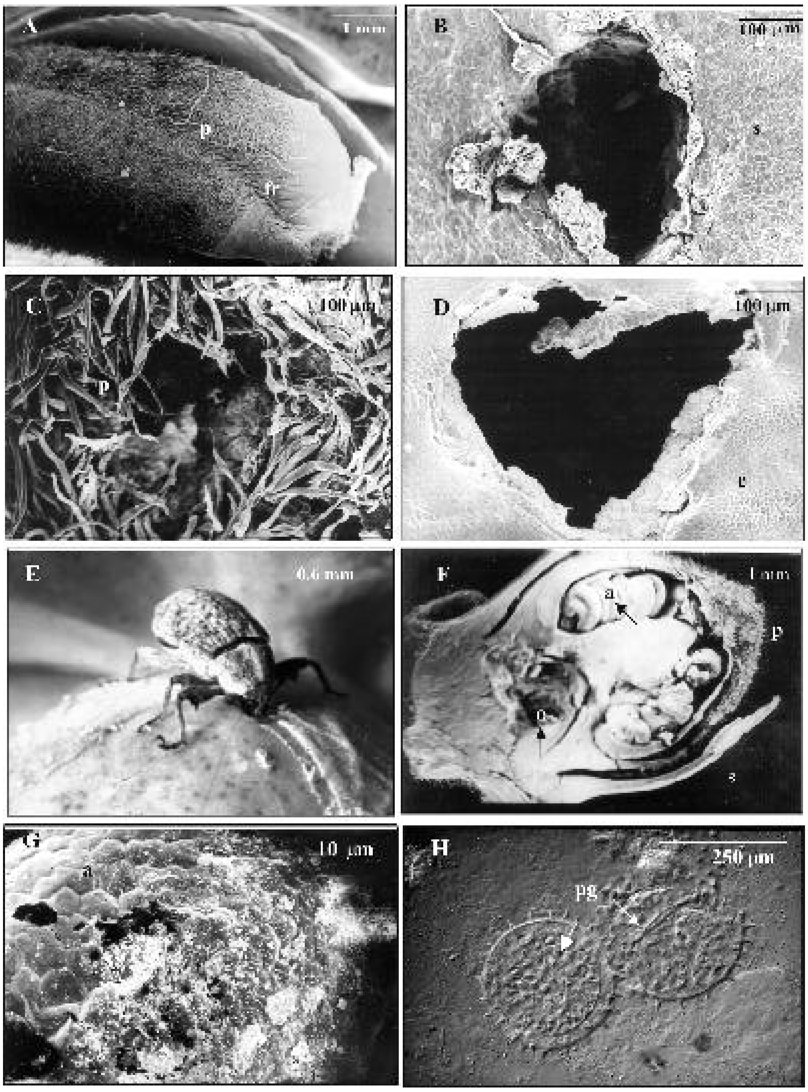

Figure 1. Petals (p), floral receptacle (fr), ovary (o), sepal (s), exocarp (e), anther (a) and pollen grain (pg) from cotton buds attacked by boll weevil.

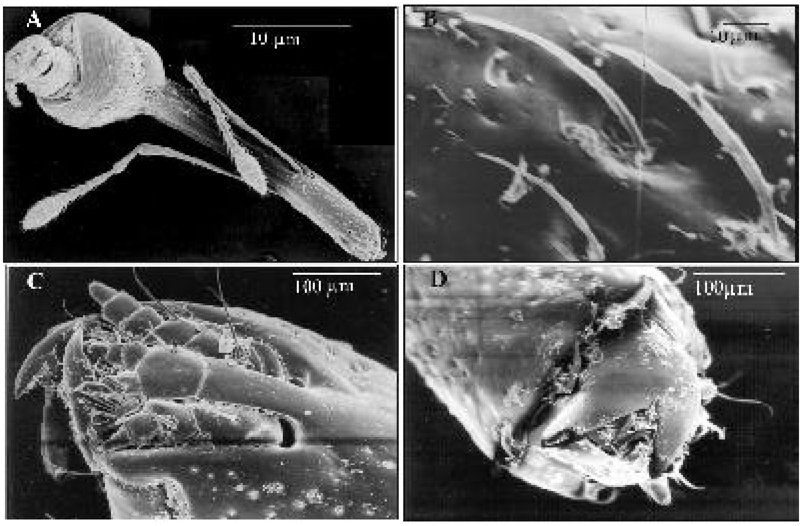

Figure 2. Scanning micrographs of a boll weevil snout (A), bristles on snout (B) and mandibles (C, D).

The abscission of cotton squares caused by boll weevil is often related to proteic compounds released by 2nd and 3rd larvae into the buds (King & Lane, 1969; Coakley et al., 1969), but this work showed the influence of morphological damage in bud abscission.

Acknowledgement

To Dr. Colin Berry from Cardiff University for critical reading, suggestions and corrections of the manuscript.

References

ALMEIDA, R. P. Economic threshold for the cotton boll weevil (*Anthonomus grandis* Boheman) control. **Proceedings of Experimental and Applied Entomolology**, Amsterdam, v. 11, p. 97-102, 2000.

BARBOSA, S.; BRAGA SOBRINHO, R.; CAMPANOLA, C. O bicudo do algodoeiro no Brasil: ocorrência, distribuição geográfica e medidas de erradicação propostas. In: BARBOSA, S.; LUKEFHAR, M. J.; BRAGA SOBRINHO, R. (Ed.). **O bicudo do algodoeiro**. Campina Grande: Embrapa-DDT, 1986. p. 7-29.

CARVALHO, L. P. Contribuição do melhoramento ao cultivo do algodão no Brasil. In: BELTRÃO, N. E. M. (Org.). **O agronegócio do algodão no Brasil**. Brasília: Embrapa-SCT, 1999. v. 1, p. 255-269.

CARVALHO, L. P.; MOREIRA, A. A. N. Correlações fenotípicas envolvendo períodos de floração e rendimento em diferentes linhagens de algodoeiro herbáceo. **Pesquisa Agropecuária Brasileira**, Brasília, v. 25, n. 7, p. 983-990, 1990.

COAKLEY, J. M.; MAXWELL, F. G.; JENKINS, J. N. Influence of feeding, oviposition and egg and larval development of the boll weevil on abscission of cotton squares. **Journal of Economic Entomology**, Lanham, v. 62, p. 244-248, 1969.

EARLE, N. W.; WALKER, A. B.; BURKS, M. L.; SLATTEN, B. H. Sparing of cholesterol by cholestanol in the diet of the boll weevil, *Anthonomus grandis* (Coleoptera: Curculionidae). **Annals of the Entomological Society of America**, Lanham, v. 60, n. 3, p. 599-603, 1967.

FREIRE, E. C.; BELTRÃO, N. E. M. **Custos de produção e rentabilidade do algodão no Brasil**: safra 1996/97. Campina Grande: Embrapa-CNPA, 1997. 6 p. (Comunicado Técnico, 69).

KING, E. E.; LANE, H. C. Abscission of cotton flower buds and petioles caused by protein from boll weevil larvae. **Plant Physiology**, Rockville, v. 44, p. 903-906, 1969.

LEIGH, T. F.; LINCOLN, C. **Feeding and development of the boll weevil, *Anthonomus grandis*, Boh., on several cotton types**. Fayetteville: University of Arkansas, 1964. 18 p. (Arkansas Agricultural Experiment Station Bulletin, 692).

PFADT, R. E. **Fundamentals of applied entomology**. 4th ed. New York: Macmillan, 1978. 742 p.

ROSOLEM, C. A. Ecofisiologia e manejo cultural do algodoeiro. In: FUNDAÇÃO MT. **Mato Grosso**: liderança e competitividade. Rondonópolis: Fundação MT/Embrapa-CNPA, 1999. 182 p. (Fundação MT. Boletim, 3).

TANSKIY, V. I. The harmfulness of the cotton bollworm *Heliotis obsolets* (Lepidoptera, Noctuidae), in Southern Tadzekistan. **Entomology Review**, Washington, v. 48, p. 23-29, 1969.

VIEIRA, R. M.; LIMA, E. F. Resistência às pragas do algodoeiro. In: BELTRÃO, N. E. M. (Org.). **O agronegócio do algodão no Brasil**. Brasília: Embrapa-SCT, 1999. v. 1, p. 317-360.