Incidence of *Heliothis virescens*[1] on Garbanzo[2] Varieties in Northwestern Mississippi

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Abstract. The tobacco budworm, *Heliothis virescens* F., is one of the most important insect pests of cotton, *Gossypium hirsutum* L., and other crops. In recent years tobacco budworm has been successfully controlled with *Bacillus thuringiensis*-expressing cottons but might acquire resistance to this bacterium insecticide. To test its susceptibility to *B. thuringiensis*, a large number of tobacco budworms should be evaluated. However, obtaining large numbers of this pest has proven difficult in recent years. Varieties of garbanzo, *Cicer arietinum* L., one of the most preferred hosts were tested against tobacco budworm in the field. Sierra, C-104, and Annigeri varieties that can harbor $\geq$10 late-instar tobacco budworm larvae per meter of row and can withstand the biotic and abiotic conditions of northwestern Mississippi, were identified as good hosts for tobacco budworm. This information is of value for obtaining sufficient samples of tobacco budworms for multiple purposes and can serve as a baseline for evaluating varieties of garbanzo for commercial purposes.

Resumen. El gusano de la yema del tabaco, *Heliothis virescens* F., es una de las plagas más importantes del algodonero y otros cultivos, que recientemente se ha podido controlar efectivamente a través del uso de algodonero transgénico que expresa toxinas de *Bacillus thuringiensis.* Para determinar el nivel de susceptibilidad de este insecto a *B. thuringiensis*, se necesita un gran número de muestras. Sin embargo, el obtener dicho número de insectos en la actualidad ha sido uno de los mayores retos para realizar dichas pruebas. Se evaluaron en el campo varias variedades de garbanzo, *Cicer arietinum* L., uno de los hospederos más importantes de esta plaga, con el fin identificar variedades que produzcan el mayor número de larvas en el campo. Las variedades Sierra, C-104 y Annigeri tienen la capacidad de soportar $\geq$10 larvas de *H. virescens* de estadios avanzados por metro de surco, bajo las condiciones bióticas y abióticas del noroeste de Misisipi, lo cual facilita la colecta de campo de este insecto para diversos fines. Esta información puede ser también útil como línea base para seleccionar variedades de garbanzo con fines comerciales.

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Introduction

Tobacco budworm, *Heliothis virescens* F., belongs to the Heliothinae, one of the most economically important insect subfamilies in the world (Fitt 1989). Tobacco budworm, together with bollworm, *Helicoverpa zea* (Boddie), have been considered the most important pests of cotton, *Gossypium hirsutum* L., for the past 25 years in the U.S. (Williams 2008). The most recent outbreak, due to insecticide resistance, occurred in Tamaulipas, Mexico, where tobacco budworm was very resistant to pyrethroid and organophosphate insecticides (Terán-Vargas et al. 2005). One of the strategies implemented in that region to overcome lack of control was adoption of *Bacillus thuringiensis*-expressing cotton (Bt cotton), which together with other measures, successfully reestablished insecticide susceptibility in tobacco budworm.

Bt cotton has been widely adopted in all major cotton-growing areas of the world (James 2007). But the "Achilles' heel" of this agricultural biotechnology might reside in development of resistance by tobacco budworm and/or other lepidopteran pests to *B. thuringiensis* (Roush 1997). Therefore, the U.S. Environmental Protection Agency (Matten and Reynolds 2003) and other regulatory agencies around the world requested monitoring of targeted insect pests for susceptibility to *B. thuringiensis*. The level of resistance to Cry1Ac, the *B. thuringiensis* protein common to all registered Bt cottons, is very low in tobacco budworm (Gould et al. 1997, Gahan et al. 2007, Blanco et al. 2009a), necessitating evaluating a large number of these insects for reliable monitoring.

Paradoxically, although the tobacco budworm is a major pest, it has been difficult to obtain from cotton in recent years (Blanco et al. 2008, Zenner de Polanía et al. 2008). Because this pest is also known to feed on at least 18 other crops and $\geq$100 non-crop plants (Hallman 1980, Fitt 1989, Blanco et al. 2008), the possibility of finding it on other plant species is feasible. Garbanzo, *Cicer arietinum* L., is one of the preferred hosts of tobacco budworm in North America (Potter and Watson 1983, Shaver and Lopez 1996, Blanco et al. 2007). This crop is suitable for well-drained soils (Jodha 1987), but harbors large numbers of tobacco budworms in heavy soils in Mississippi (Blanco et al. 2007). Garbanzo is adversely affected by many biotic conditions such as attack by several species of the subfamily Heliothinae around the world (Reed et al. 1987) and a complex of several diseases, among the most important being *Ascochyta* sp., *Fusarium* sp., *Botrytis* sp., and *Rhyzoctonia* sp. (de Miguel-Gordillo 1991, Singh and Saxena 1999). The goal of this study was to evaluate several garbanzo varieties that can withstand the agroecosystem of northwestern Mississippi and function as areas where large numbers of tobacco budworms can be collected.

Materials and Methods

Plots were four (1.0-m center) rows 15 m long of Dundee silty clay soil, replicated four times with two blank rows between replications and 2-m alleys between plots, arranged in a randomized complete block design. Garbanzo varieties were obtained from the Grain Legume Genetics and Physiology Research Unit of the USDA, Agricultural Research Service of Pullman, WA, except ICCC-37 that was obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) of India. Seeds were coated with hummus inoculant (Urbana

Laboratories, St. Joseph, MO) and planted on 17 May 2004 and 16 May 2005. A cone-type seeder planter (Model CTS-P, Almaco, Nevada, IA) delivering 10 seeds per meter of row was used to plant the seeds 2 cm deep. Plots were treated twice with clethodim (120 g ai/ha, Valent USA Corporation, Walnut Creek, CA) for post-emergence grass control, while broadleaf weeds were removed by hand several times during the duration of the experiments. No fertilizers, insecticides, or fungicides were used on the plots.

When naturally-occurring heliothine larvae infested garbanzo foliage, plants were carefully shaken five times over a 1-m² beat cloth, and heliothine larvae were counted. Every plot was evaluated for heliothine larvae five times from randomly chosen places in the center two rows of plants on each evaluation date. All the heliothines on the beat cloth were removed, placed on insect artificial diet (Blanco et al. 2009b), and maintained at 28 ±0.4°C, 75 ± 10% relative humidity, and 14:10 light:dark hours until moths emerged and species (tobacco budworm or bollworm) were identified by using the description of Brazzel et al. (1953). The proportion of tobacco budworms to bollworms was used to determine the number of tobacco budworms in the field at each evaluation.

Plants without signs of disease were counted in two randomly chosen 3-m transects in the center two rows during the garbanzo-growing season. In 2005, plants were left to dry, and 2 m from the center of the two middle rows were harvested by hand 161 days after planting. Seeds from each plot were placed at 65°C in an oven for 72 hours and weighed.

Analysis of variance (ANOVA) was performed on larvae per meter of row, non-diseased plants per meter of row, and yield measurements. The ANOVA was for a split-plot design with plant varieties as the main unit treatment, and the main unit design was a randomized complete block. Sampling date was a repeated measure subunit effect. Means were separated based on least significant differences (LSD) at $P \leq 0.05$. Proc Mixed in SAS was used for ANOVA (SAS Institute 2001).

Results and Discussion

The average number of tobacco budworm larvae differed significantly among the 11 varieties of garbanzo in 2004 (F = 11.42; df = 11, 29; P < 0.0001), but sampling date was not a significant factor in these differences (F = 8.59; df = 1, 3; P = 0.055). Interaction between treatment and evaluation date was not significant (F = 1.18; df = 11, 29; P = 0.33). Sierra, with an average of six tobacco budworm larvae per meter, was the variety significantly most infested, followed by C-104. Fewest tobacco budworms were found on ICCC-37 (Fig. 1).

The 11 garbanzo varieties did not differ significantly in susceptibility to diseases (F = 0.62, df = 11, 29, P = 0.79), nor in interaction between variety per evaluation day (F = 1.04; df = 11, 1; P = 0.43) in 2004. Fewest plants of WR-315 remained at the end of the growing season. By the end of the season, more than half of the plants were lost because of diseases.

Five of the varieties tested in 2004 and a new one, L-550, were used in 2005. In 2005, results were similar to those in 2004. Significant differences were found among varieties (F = 8.52; df = 5, 45; P < 0.0001), sampling date (F = 21.2; df = 2, 6; P = 0.001), and interaction between plant variety and sampling day (F = 5.97; df = 10, 45; P < 0.0001), with Sierra, C-104, and Annigeri most attacked by tobacco budworm. The number of larvae did not differ significantly among the varieties on

the first two evaluation dates, but they did on the last evaluation date (F = 19.75; df = 5, 45; P < 0.0001) (Fig. 2).

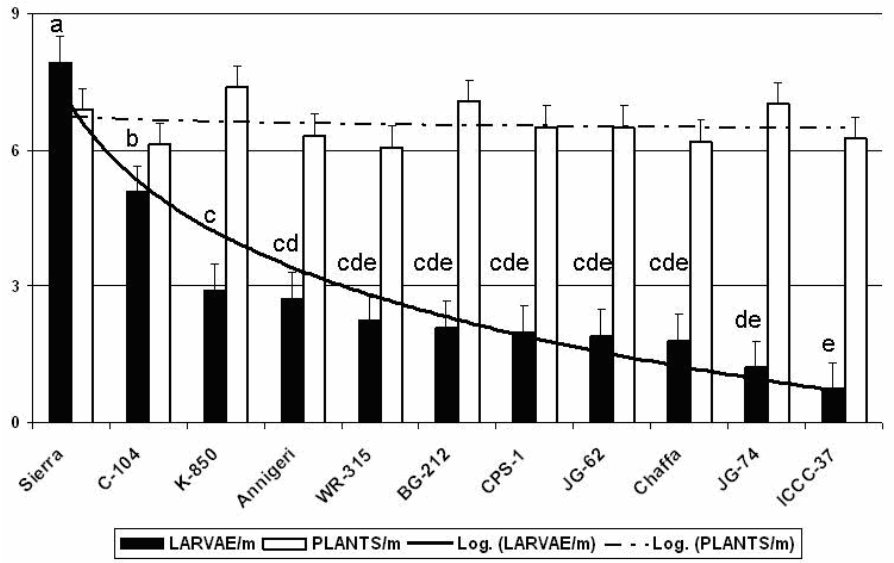

Fig. 1. Average (+ standard error) number of *Heliothis virescens* larvae (black bars) and garbanzo plants (clear bars) per meter of row per variety, combining all the evaluation dates from a field experiment at Stoneville, MS, in 2004. Bars representing larvae with a common letter are not significantly different at P < 0.05.

The number of plants per variety decreased significantly during the seven evaluation dates (F = 285.3; df = 6, 18; P < 0.0001), but did not differ significantly among the varieties when data for all dates were combined (F = 0.42; df = 5, 15; P = 0.09). Interaction between variety and evaluation date was also significant (F = 5.49; df = 30, 90; P < 0.0001). Although on the different evaluation dates certain varieties had more plants than others, variety C-104 initially had more plants, while Annigeri initially was not as abundant on the first two evaluation dates but was significantly more abundant from the third evaluation on (22 days after planting). Sierra had consistently fewer plants per meter than the rest of the varieties. Figs. 1 and 2 show that the number of tobacco budworm larvae per meter of row was not influenced by plant density, demonstrating that there are intrinsic factors and not more larvae because of more plants. This indicated that varieties such as Sierra and C-104 were more attractive to larvae while some varieties such as ICCC-37 consistently had few tobacco budworms.

In 2005, seed production differed significantly by variety (F = 14.4, df = 5, 17; P < 0.0001), with L-550, followed by Annigeri, ICCC-37, K-850, and C-104 yielding most and Sierra yielding fewest seeds. Although this experiment was not aimed at demonstrating the potential of these varieties as a commercially feasible crop for

northwestern Mississippi, their seed production indicates that seeds are produced and can be used for replanting plots the following season. Varieties least infested by tobacco budworm larvae yielded most. Sierra, one of the varieties preferred by this insect, was entirely eaten at the end of the experiment and produced only a small amount of seed.

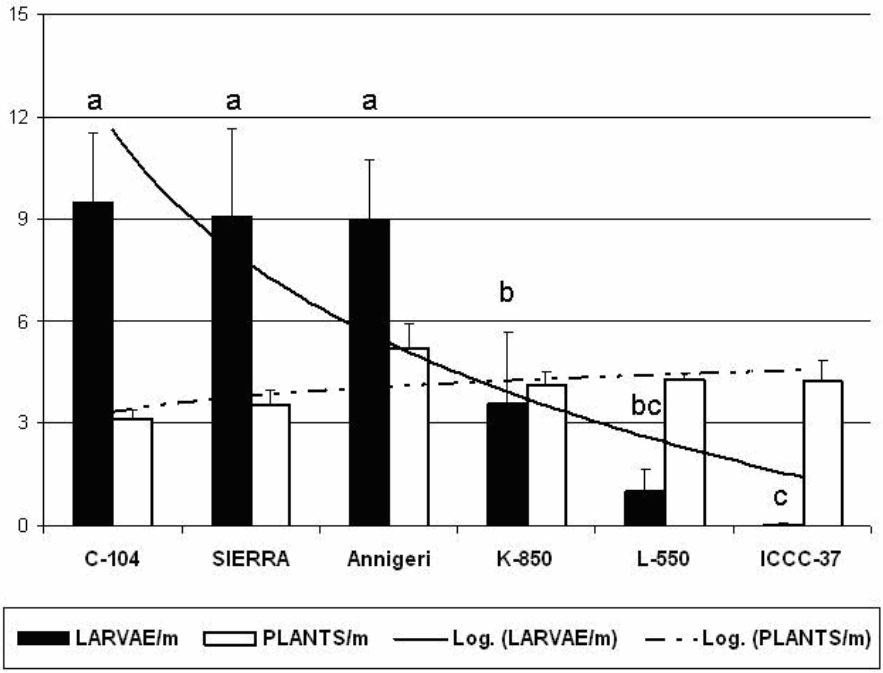

Fig. 2. Average (+ standard error) number of *Heliothis virescens* larvae (black bars) and garbanzo plants (clear bars) per meter of row per variety with all the evaluation dates combined from a field experiment at Stoneville, MS in 2005. Bars representing larvae with a common letter are not significantly different at $P < 0.05$.

Sierra has been used at Stoneville since 2003, with consistent attack year after year by tobacco budworm (Blanco et al. 2007). This insect has been present on garbanzo plants during all their four distinctive generations within a year. This variety also has been adopted at College Station, TX, where it has consistently harbored numerous tobacco budworms. Bollworm was found occasionally but at low density during these evaluations; therefore, data were not presented here. Beet armyworm, *Spodoptera exigua* Hübner, can also be very abundant on the plants. Since 2005, fall armyworm, *S. frugiperda* (J. E. Smith), larvae have occasionally been found on garbanzo plants.

The goal of this study was achieved by identifying three varieties of garbanzo that can potentially harbor large quantities of tobacco budworms. Sierra, C-104

(similar to Sierra, F. M Muehlbauer, personal communication), and Annigeri can withstand the northwestern Mississippi environment. The intrinsic value of these varieties for attracting tobacco budworms is demonstrated when compared with ICCC-37, the standard variety used for heliothine-resistance experiments at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) of India (H. C. Sharma, personal communication). Although tobacco budworm larvae were found on all varieties of garbanzo tested in these experiments, the most infested varieties provide the opportunity to gather larger numbers of larvae per unit-area. Additionally, this study provides information for garbanzo breeding programs with the aim of developing varieties less susceptible to attack by tobacco budworms.

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