SUSCEPTIBILITY OF CRY 1F RESISTANT FALL ARMYWORMS, *SPODOPTERA FRUGIPERDA*, TO COTTON EXPRESSING PYRAMIDED BT TOXINS

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Abstract

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith), (FAW) is an economically damaging pest of cotton and other agricultural crops in Louisiana. Fall armyworm is predominately a foliage feeder on most crops, but will also feed on fruiting structures of the cotton plant causing direct yield loss. Since the advent of crops genetically modified to express the bacterium *Bacillus thuringiensis* (Bt) in the late 90's, fall armyworm control has primarily been an issue in conventional cotton cultivars. Recently, two strains of FAW that are highly resistant to the Cry1F protein in corn were collected from Hendry and Collier counties in Florida. These strains have been established in our laboratory on the campus of LSU in Baton Rouge, Louisiana and have been documented to be resistant to the purified Cry1F protein as well as commercial corn producing the Cry1F toxin. The objective of this research project is to evaluate survival of Cry1F corn-susceptible and resistant FAW on various Bt cotton technologies. The Hendry colony appears to be highly resistant to WideStrike cotton leaf tissue and moderately tolerant/resistant tolerant to Bollgard II leaf tissue. The Collier colony was moderately resistant/tolerant to WideStrike leaf tissue but was susceptible to Bollgard II. The TwinLink leaf tissue entry was found to be moderately to highly toxic to all populations evaluated. The Collier population was moderately susceptible to WideStrike square tissue. Square data was not obtained for the Hendry colony due insufficient insect numbers. The Collier population appeared to be highly susceptible to Bollgard II square tissue and moderately resistant to TwinLink square tissue. WideStrike 3 and TwinLink Plus were highly toxic to all populations and 100% mortality was recorded for all populations tested in leaf and square tissue bioassays.

Introduction

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), is a sporadic pest of corn and cotton in the southern region of the United States. Recently, several strains of FAW with confirmed resistance to purified Cry1F protein and commercial Cry1F corn (e.g. Herculex I) have been established in the laboratory at LSU AgCenter in Baton Rouge, Louisiana. Previous F_2 screen indicates that resistance allele frequency to Cry1F corn in field populations of FAW in the south region is not rare. Results of an F_2 screen showed that resistance allele frequency to Cry1F corn was 0.058 with a 95% confident interval (CI) of 0.04 to 0.09 for two field populations collected in Louisiana and 0.252 with a 95% CI of 0.204 to 0.303 for a population sampled in south Florida during 2011 (Huang et al. 2014). There is a concern that Cry1F-corn resistant populations of FAW may move to the cotton fields and pose a threat to Bt cotton if the Cry1F-resistance possesses cross-resistance properties to the Bt proteins expressed in the Bt cotton technologies.

The objective of the proposed project is to address this uncertainty and evaluate the survival of Cry1F-susceptible and -resistant FAW on current and emerging Bt cotton technologies in the United States. Results obtained from this study will provide useful information for the proper use of Bt cotton technology to ensure the long-term success of the technologies for pest management in the United States.

Materials and Methods

This study was conducted at the Macon Ridge Research Station near Winnsboro, LA in 2013. Cotton seed was planted in 2 gallon pots filled with potting soil and placed in a greenhouse to grow and produce leaf tissue and squares to be assayed. This study contained six different cotton cultivars tested with three different FAW populations. Cotton cultivars were FM 966LL (Non-Bt), TwinLink™ (Cry1Ab, Cry2Ae), TwinLink™ Plus™ (Cry1Ab, Cry2Ae, Vip3A), DP 0912 B2RF (Bollgard II®-Cry1Ac, Cry2Ab), PHY 375 WRF (WideStrike®-Cry1Ac, Cry1F), and WideStrike® 3 (Cry1Ac, Cry1F, Vip3A). Two FAW populations with confirmed Cry1F resistance (Hendry and Collier) and Cry1F

susceptible colony (LSU-susceptible) were utilized in this study. The resistant colonies were both collected in 2011 in south Florida in Hendry and Collier counties off of field corn containing the Cry1F toxin.

Leaf Tissue Bioassays

Leaf tissue was clipped off of plants and individually placed abaxial side down into 8 well trays with the bottom of each tray covered with agar for moisture. One neonate (<24 hr.) was placed on an individual leaf and then sealed and left to feed on the leaf tissue. Fall army worm populations were infested on leaf tissue of each cultivar totaling 20 leaves per treatment and replicated 12 times. Trays were kept under 12:12 L:D photoperiod and ~27°C environment. Mortality was recorded at two, four, six and eight days after infestation (DAI).

Square Bioassays

Individual squares were removed from cotton cultivars and placed in 2 ounce plastic cups with the base filled with agar for moisture. One first instar larva was placed in each cup, sealed and left to feed for on square tissue. Cups were kept under 12:12 L:D photoperiod and ~27°C environment. Mortality was recorded at two, four, six and eight days after infestation (DAI).

Results and Discussion

This research shows that the LSU-susceptible colony was highly susceptible to all Bt technologies which was to be expected. The Hendry colony appears to be highly resistant to WideStrike leaf tissue (Figure 3). Square data was not obtained for the Hendry colony due insufficient insect numbers. The Collier population was moderately susceptible to WideStrike leaf and square tissue (Figures 3 and 4).

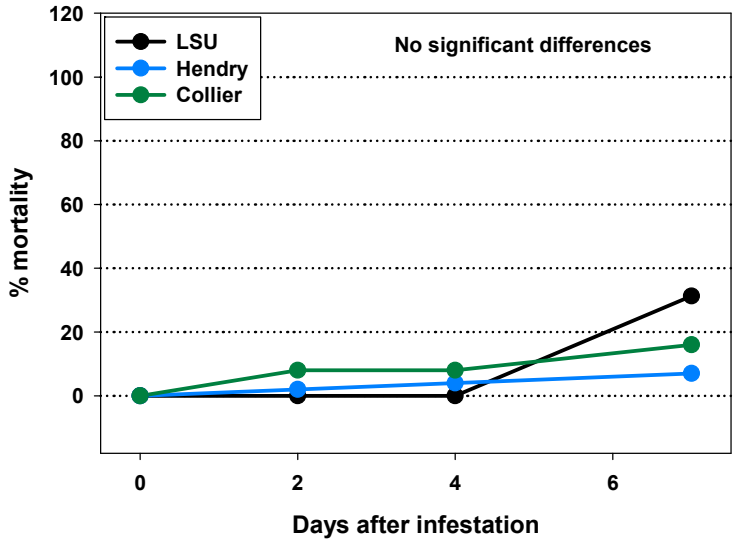

Figure 1. Non-*Bt* leaf tissue mortality.

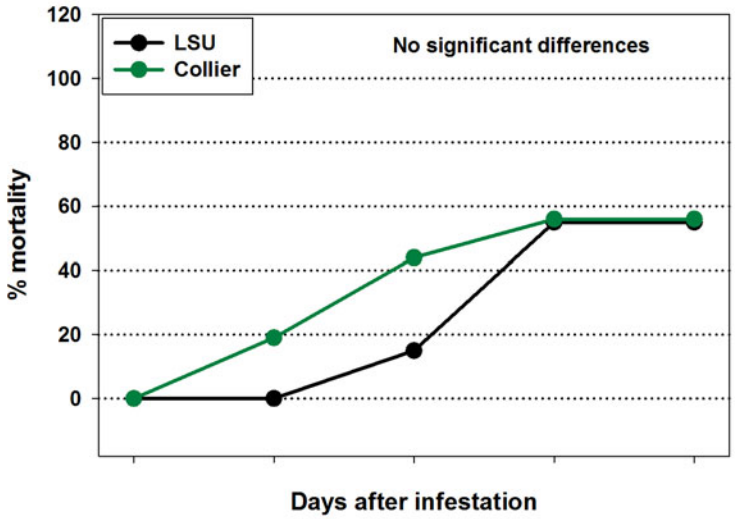

Figure 2. Non-*Bt* square tissue mortality.

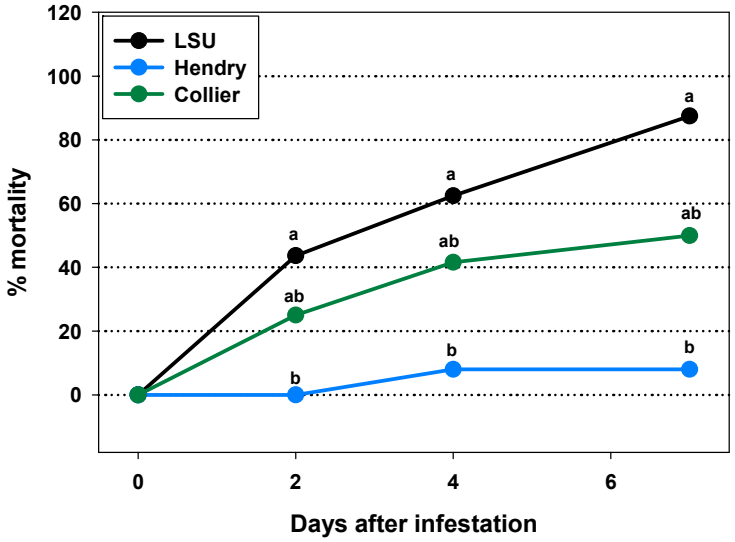

Figure 3. WideStrike leaf mortality.

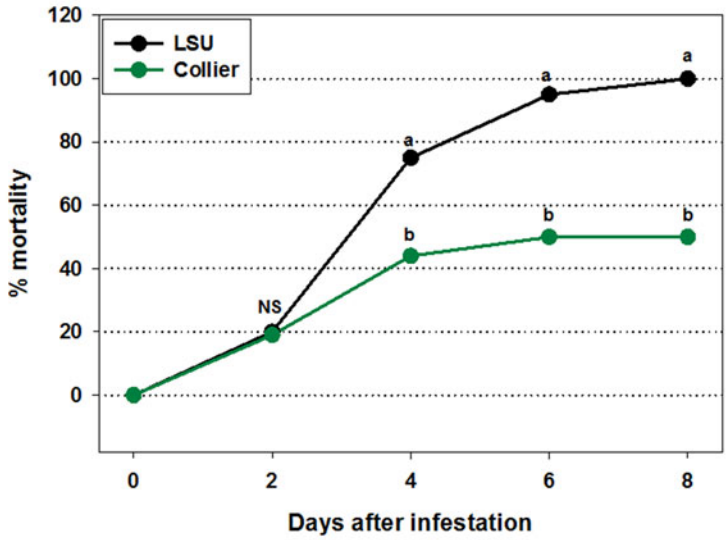

Figure 4. WideStrike square mortality.

The Collier population appeared to be moderately susceptible to Bollgard II leaf tissue and highly susceptible to square tissue (Figure 5 and 6). Mortality timing in the Collier population was significantly different from the susceptible population until 6 DAI (Figure 6). The Hendry population appears to moderately susceptible to Bollgard II leaf tissue (Figure 5).

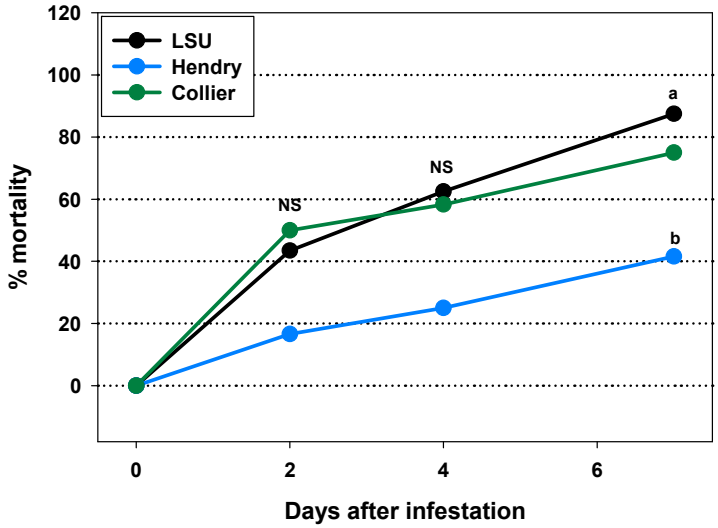

Figure 5. Bollgard II leaf mortality.

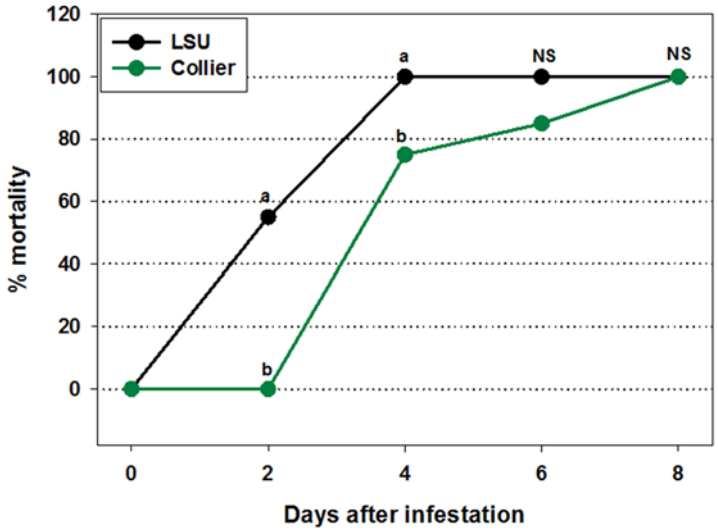

Figure 6. Bollgard II square mortality.

The Hendry and Collier populations appeared to be highly susceptible to TwinLink leaf tissue with no significant differences in mortality time compared to the LSU susceptible population (Figure 7). The Collier population was moderately resistant to TwinLink square tissue (Figure 8). This may be due *Bt* toxin expression levels, in square tissue, not investigated in this study. Square data was not obtained for the Hendry colony due insufficient insect numbers.

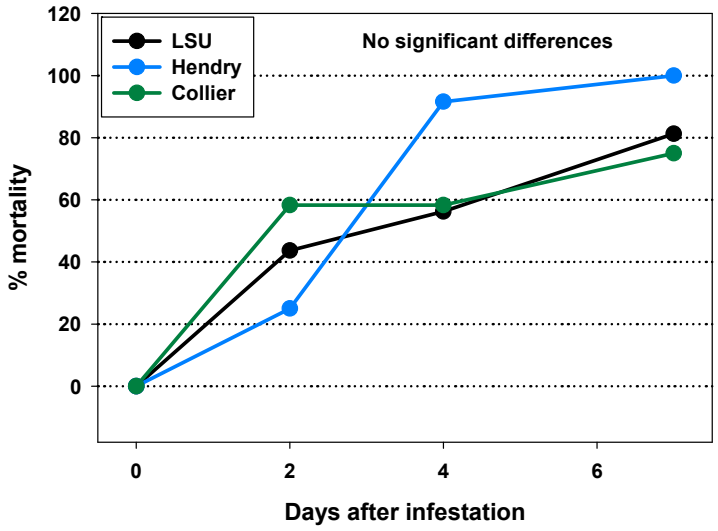

Figure 7. TwinLink leaf mortality.

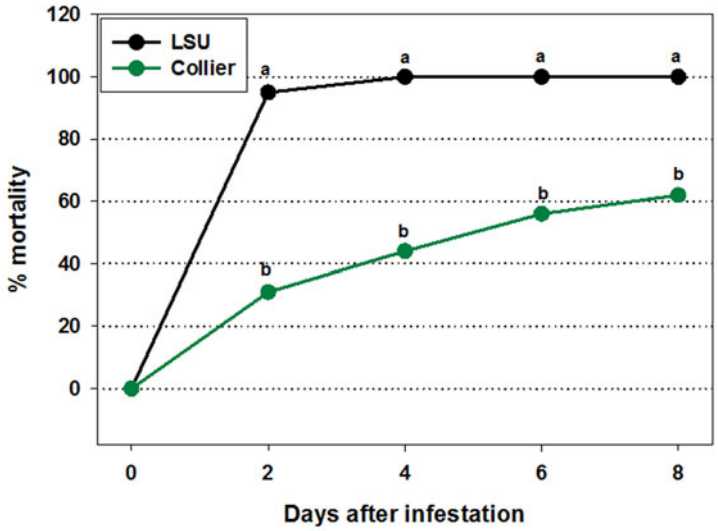

Figure 8. TwinLink square mortality.

The greater than expected survival of Cry1F resistant FAW on Bollgard II leaf tissue but not on squares, and their survival on TwinLink squares but not leaf tissue is perplexing. These data suggest that there is a possibility of cross or multiple resistance between Cry1F and Cry2 toxins, or may simply be a response to variation in toxin within cotton tissues.

WideStrike 3 and TwinLink Plus leaf and square tissue was determined to be highly toxic to all populations tested with 100% mortality observed in all bioassays (Figures 9, 10, 11, 12).

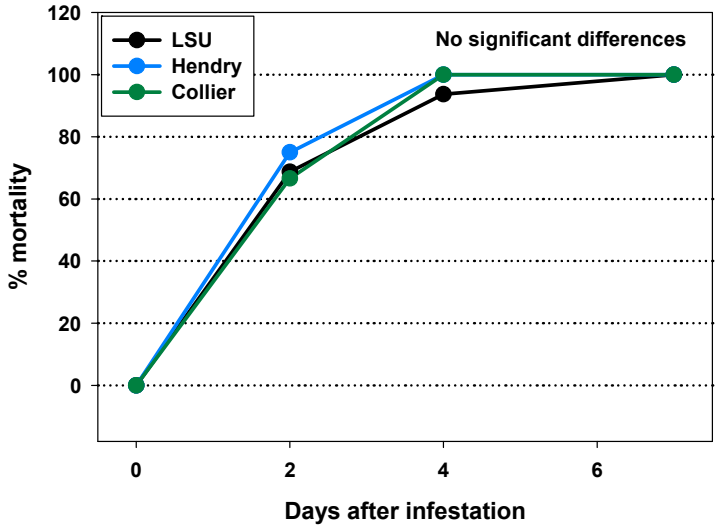

Figure 9. WideStrike 3 leaf mortality.

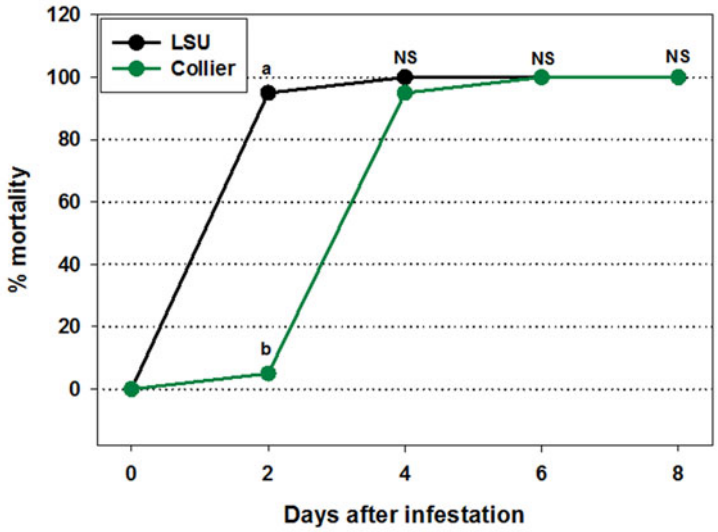

Figure 10. WideStrike 3 square mortality.

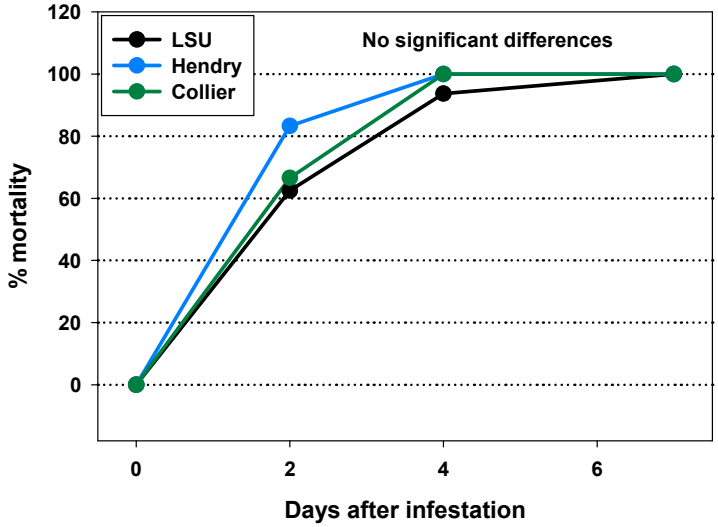

Figure 11. TwinLink Plus leaf mortality.

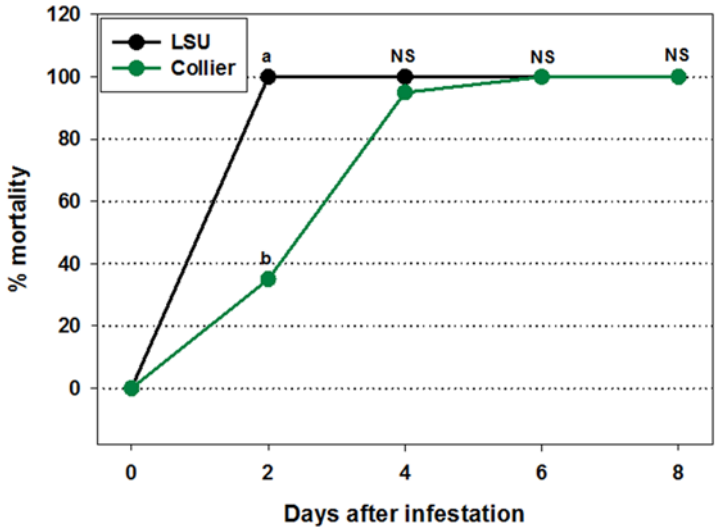

Figure 12. TwinLink Plus square mortality.

References

Huang. F, Qureshi J.A., Meagher R.L. Jr, Reisig D.D., Head G.P., et al. (2014). Cry1F Resistance in Fall Armyworm *Spodoptera frugiperda*: Single Gene versus Pyramided Bt Maize. PLoS ONE 9(11): e112958. doi:10.1371/journal.pone.0112958