# EFFECTS OF OSMOTIN OVEREXPRESSION IN TRANSGENIC COTTON ON COTTON APHID, *APHIS GOSSYPII,* REPRODUCTION AND COLONIZATION

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## Abstract

Cotton that has been transgenically modified to over express the protein osmotin was evaluated for resistance to cotton aphid, *Aphis gossypii*. Life table studies conducted in the growth chamber indicated that aphids on cotton over expressing osmotin had a slightly lower natural rate of increase and a shorter longevity than those on a non-trangenic isoline. These data suggest that antibiosis is the mechanism of resistance. Field studies were similar to the growth chamber studies. Plants with amplified osmotin expression tended to exhibit slightly fewer aphids per plant than the non-transgenically modified cultivar. Although the aphid resistance exhibited was insufficient for preventing a treatable aphid population, it did indicate that further refinement might be applicable.

## Introduction

Cotton aphid, *Aphis gossypii* is an economically damaging pest to cotton and in southern climates the population is 100% female (Figure 1). The cotton aphid reproduces asexually through parthogenesis and females are born pregnant with the ability to produce up to 60 offspring per generation. Cotton aphids feed on the terminals, abaxial leaf surfaces and the occasional square bracts of the cotton plant by ingesting phloem fluids and filtering the amino acids and sugars from those fluids. Excess water and sugar are excreted by the aphid as "honey dew" that can cover the plant depriving it of its photosynthesizing capabilities and causing the lint to become "sticky" inhibiting proper ginning. Yield loss is dependent on aphid population density, duration and the degree of secondary stresses on the cotton plant (i.e. fruit load, water deficit, etc).

Plants naturally produce defense proteins called pathogenesis-related (PR) proteins directed against fungal pathogens (Abad et al 1996, Selitrennikoff 2001). Osmotin or osmotin like proteins belong to the class of PR proteins and are made in response to fungal stress or osmotic stress (water deprivation or salt exposure). It may be possible to use the osmotin gene as a defense gene effective against biotic and/or abiotic stresses (Rajam et al 2007).

Transgenic potato and rice plants that overproduce osmotin are shown to provide resistance against fungal diseases (Liu et al 1994, Zhu et al 1996). Cotton plants engineered with tobacco osmotin were shown to confer resistance against drought (Parkhi et al 2009). Thus, the main objective of this research is to genetically engineer cottons with one of its own osmotin genes to overproduce the PR protein in transgenic cotton plants to provide natural resistance against biotic (fungal pathogens and/ or insects) and/or abiotic (drought resistance etc.) stresses. In this project we have concentrated on understanding and analyzing the interactions between Gh. OSMII engineered cotton plants and cotton aphid pest (*Aphis gossypii*).

## Methods

### Genetically Modifying

All Genetic work was done by Dr. Kent Chapman and his program at the University of North Texas by using an agrobacterium-mediated transformation to overexpress osmotin in four independent events. A modified method utilizing the cotton embryogenic cell lines and Agrobacterium-mediated transformation was used to generate four independent transgenic events. The coding sequence of Cotton OSMII under the control of the CaMV 35S promoter was engineered into the Gateway binary vector, pMDC 32 and pMDC43, for transformation. Transgenic cells and embryos were selected on hygromycin and regenerated plants were transferred to the greenhouse for maturation and

seed production. Transgenic T0 plants and T1 progeny derived from two independent fertile T0 transgenic lines were confirmed as transgenic by PCR amplification of the transgene promoter sequence.

**Laboratory Experiments**

Laboratory experiments were conducted under the direction of Dr. David Kerns at the Texas A&M AgriLife Research & Extension Center in Lubbock, Texas. A cotton aphid colony was derived from a single aphid to be used for aphid life table studies. Each OSM line and the non-transformed parent Coker 312 were infested with a single adult aphid. Each line had 8 replicates grown in fully contained cages in an environmental growth chamber operated at 21 ± 2 ° C with a 12:12 (L:D) photoperiod. Each line had 8 replicates grown in fully contained cages in an environmental growth chamber operated at 21 ± 2 ° C with a 12:12 (L:D) photoperiod. Each aphid was monitored every 24 h at which time mortality was noted, time to reproductive maturity, and number of progeny (removed upon counting). Life statistics that were recorded in this study were as follows: Days to reproductive maturity (d), Number of progeny (daily) throughout life, generation time (T), finite daily increase (λ), population doubling time (DT) (DeLoach, 1974), and Natural rate of increase ($r_m$) based on Wyatt and White (1977) $r_m = 0.738(\log_e M_d)/d$, where $M_d$ = number of progeny in d at the initiation of reproduction. All data was analyzed using ANOVA, F protected SNK ($P < 0.05$).

**Field Component**

Field validation was performed under the direction of Dr. David Kerns at the LSU AgCenter's Macon Ridge Research Station near Winnsboro, Louisiana. Transgenic OSM events were planted in 2012 and 2013 in a randomized complete block (RCB) with four replicates and plot size was 2 or 4 rows wide × 7 meters long. Aphids were monitored weekly as soon aphids were first detected. Aphid density evaluations were collected from five leaves from the 3rd node and five leaves from the mid to low canopy from each plot and then data was pooled. Cumulative aphid days were calculated at the end of each study (Ruppel 1983). Data was analyzed using ANOVA, means were separated using an F protected, Student-Newman-Keuls ($P < 0.05$).

## Summary

Dr. Chapman was successful at genetically modifying the cotton to overexpress osmotin. Life table studies suggest that there may be some slight suppression in reproductive aphid potential and longevity. Natural rate of increase ($r_m$) was not significant in 2010 or 2011but was close. Aphid longevity in 2011 was significantly lower in the 2 OSM II T3 and 2 OSM II T2 lines than in the non-modified Coker variety. The field study conducted in 2012 showed no statistical differences throughout the entire testing period. Total aphids per 10 leaves were seen to be close to differing statistically at 55 days after planting ($P = 0.08$) between the transgenic events and the non-modified Coker variety. The 2013 field trial showed that significant differences in total aphids per 10 leaves were observed between events at 83 days after planting ($P = 0.01$) and for the seasonal mean($P = 0.03$) as well.

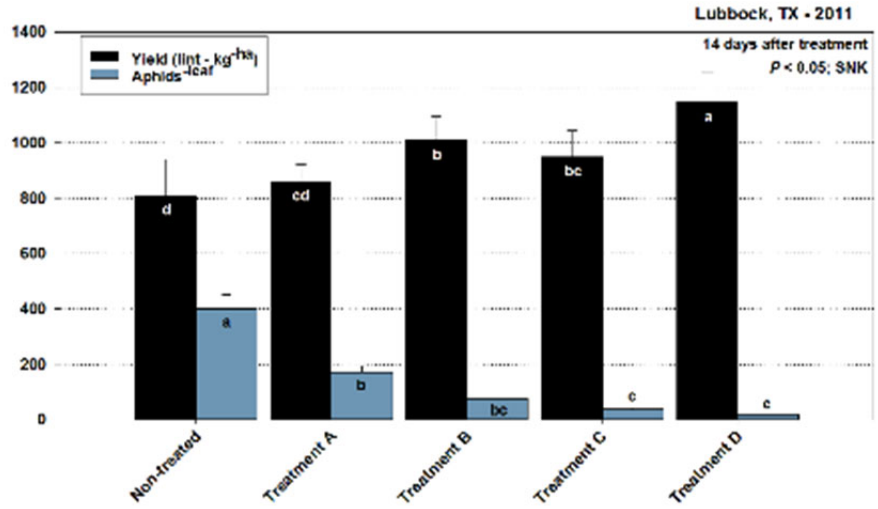


Figure 1: Cotton Aphid Impact on Yield

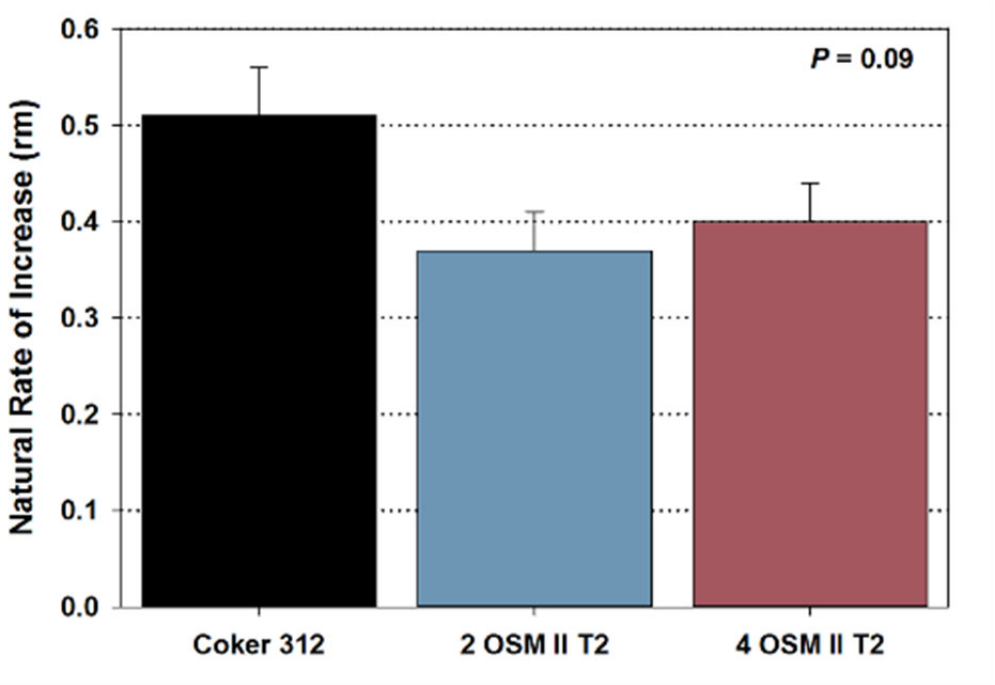


Figure 2: Natural Rate of Increase ($r_m$) – 2010

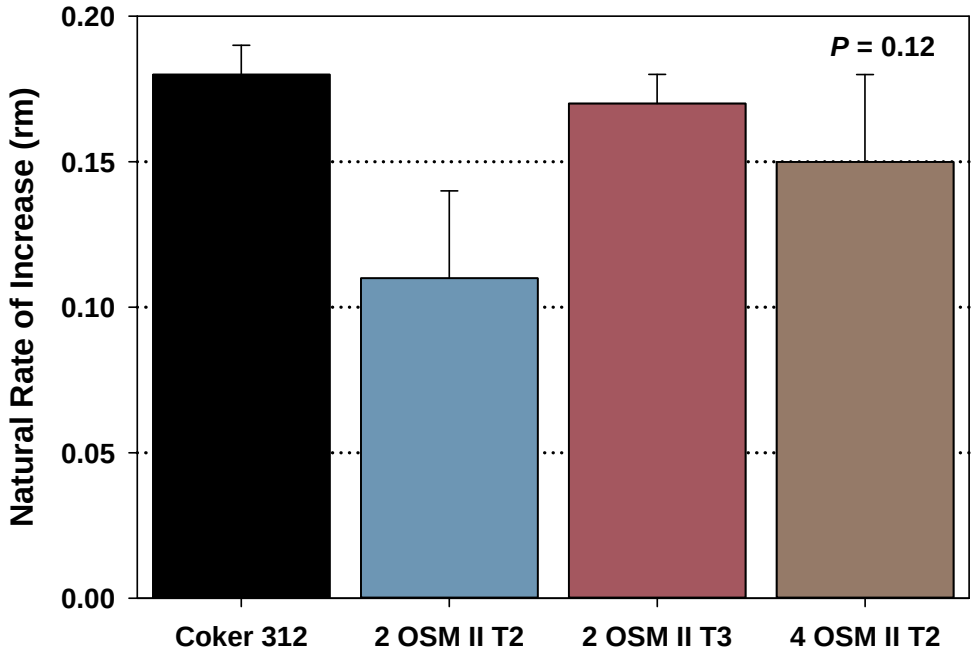


Figure 3: Natural Rate of Increase ($r_m$) – 2011

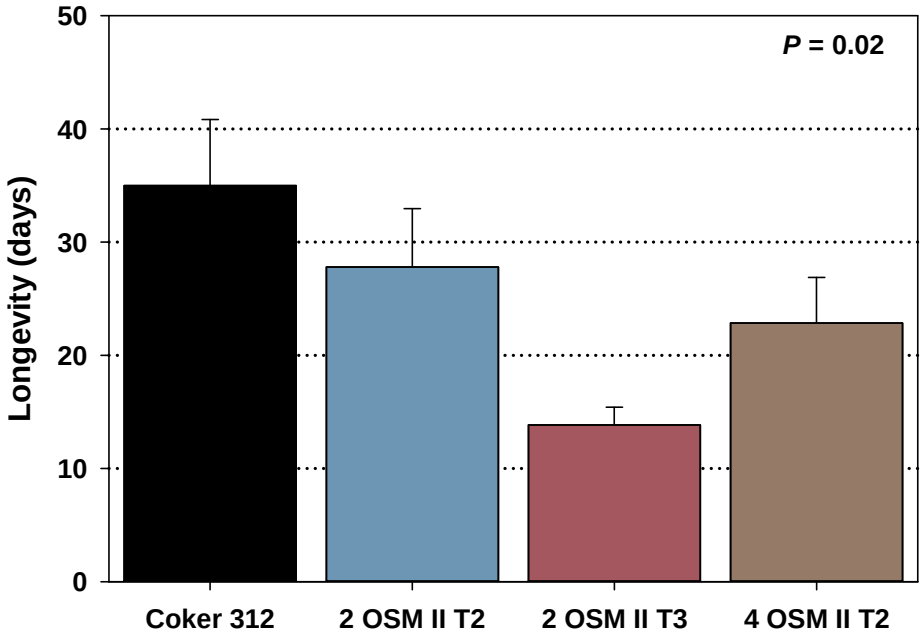


Figure 4: Longevity (L)-2011

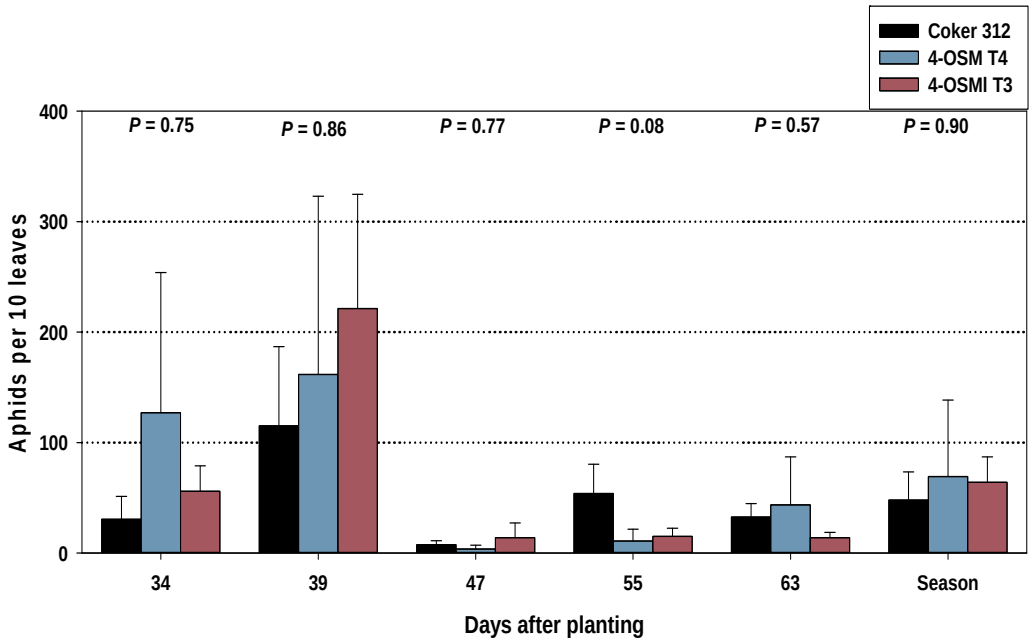


Figure 5: Field Data 2012- Macon Ridge, LA

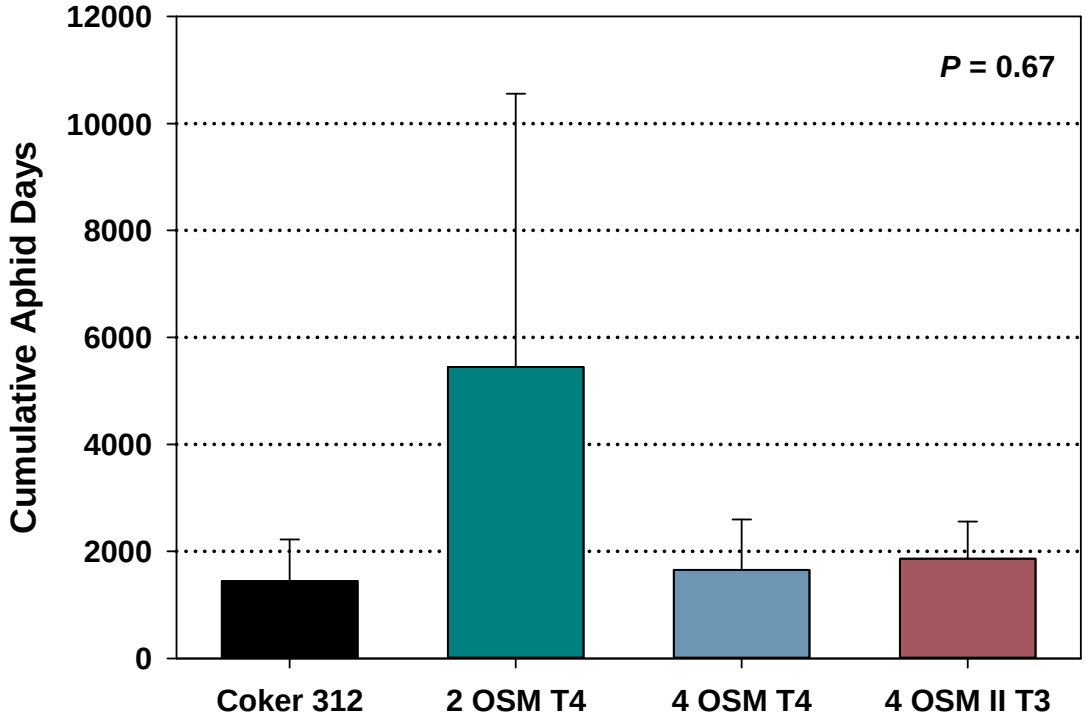


Figure 6: Aphid Days (2012) - Cumulative Number of Aphids/ 10 Leaves/ Day

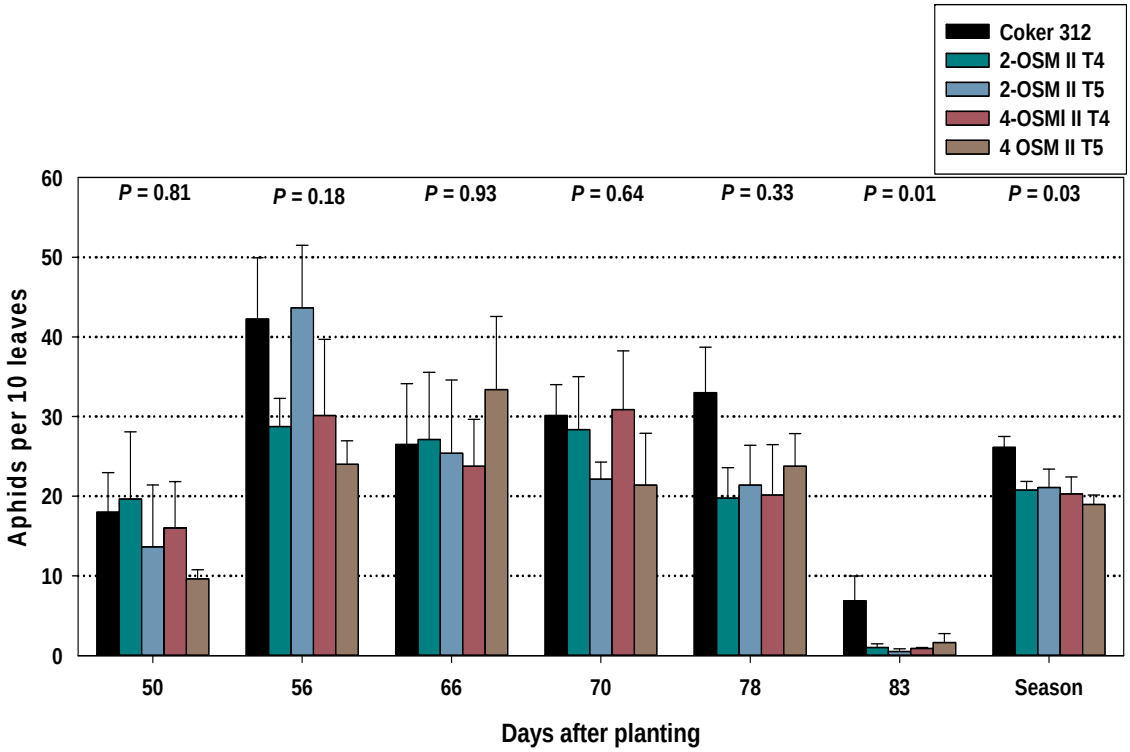


Figure 7: Field Data 2013- Macon Ridge, LA

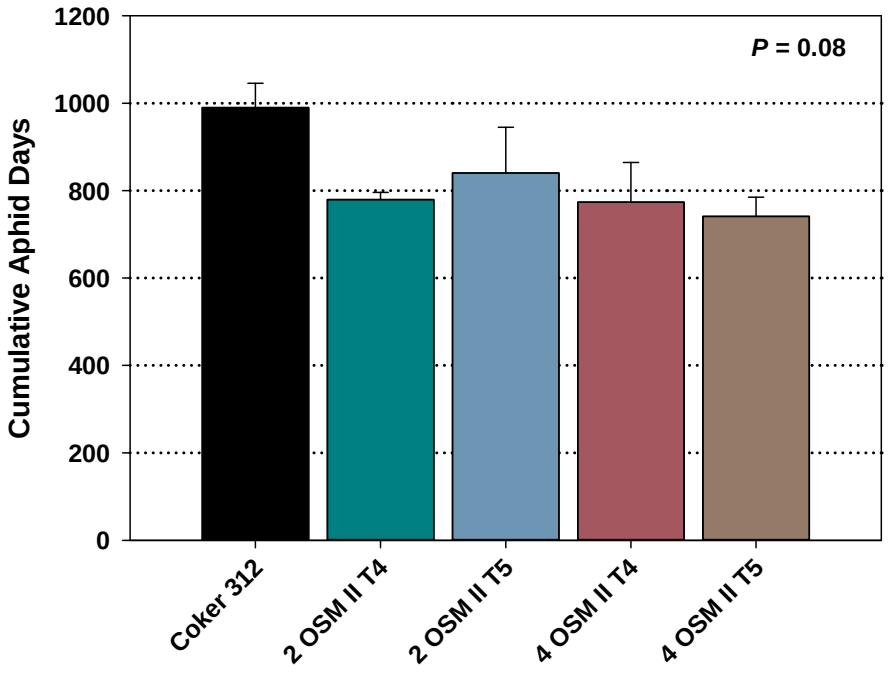


Figure 8: Aphid Days (2013) - Cumulative Number of Aphids/ 10 Leaves/ Day

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