ROYAL PAULOWNIA: A POTENTIAL REFUGE FOR LEPIDOPTERA C.D. Parker, Jr. Mississippi State University Central Mississippi Research and Extension Center Raymond, MS

<u>Abstract</u>

The adoption of transgenic cotton plants expressing delta endotoxin of *Bacillus thuringiensis* has greatly aided the ability to control major lepidopteran pests of cotton, particularly *Heliothis virescens* caterpillars that are resistant to conventional insecticide treatments. Resistance management plans have been implemented to provide a refuge for susceptible caterpillar larvae in hopes to delay the development of resistance to the transgenic cotton plants. Current resistance management plans use non-transgenic cotton as the refuge. This study demonstrates that a tree belonging to the genus *Paulownia* serves as a host to several of the major Lepidopteran pests of cotton, particularly *Heliothis virescens* and *Helicoverpa zea*. Preliminary data show that larval production per acre in dense plantings of *Paulownia* trees (38 in grids) is at least 2.7 times that of untreated cotton offering a new potential refuge for production of susceptible larvae.

Introduction

The introduction of transgenic cotton plants expressing endotoxin derived from *Bacillus thuringiensis* Berliner (BT cotton) has greatly aided producers in maintaining control of *Heliothis virescens* larvae (tobacco budworm) and *Helicoverpa zea* larvae (bollworm), although controlled to a lesser degree. BT cotton plants provide season long expression of the insecticidal toxins which has raised concerns about resistance development. Several strategies have been discussed (Fischoff 1992, McGaughey and Whalon 1992, Mallet and Porter 1992, Caprio 1994), but the current strategy recommends the use of plants with high dose expression and the provision of an external refuge in close proximity to the transgenic plants (ILSI HESI 1998). Producers using the BT cotton technology are allowed two possible options (S. Matten 2000). 1) For every 100 Acres, Non-BT cotton must be planted on 20% of the acreage within one mile of the transgenic field, and the non-BT may be treated with insecticides except for BT insecticide products. 2) For every 100 Acres, Non-BT cotton must be planted on 3.8% of the acreage within one mile from BT fields, but the refuge cannot be treated with any insecticide having activity on lepidopteran larvae. NonBT cotton has been adopted as the refuge because it will be temporally similar to the BT cotton and can serve as a refuge for the entire cotton production season. Option 1) was revised in 2001 to provide a 5% unsprayed refuge instead of 3.8% (IRM Guide 2002).

Other hosts for *H. virescens* and *H. zea* have been reported (Quaintance and Brues 1905, Snow and Brazzel 1965, Snow et al. 1966, Stadelbacher et al. 1986), but many of these hosts are short lived. Other studies have explored the possibility of planting identified hosts to serve as a refuge for BT cotton, but these plantings were comprised mainly of plants considered weeds in the cotton production system (Craig et al. 1999). Although the concept of using these plants as a refuge is valid, it would be easier to promote plants that are not considered weed species. Paulownia was reported as a host of *H. virescens* in a graduate student dissertation (Parker 2000), but the production of larvae per acre was not considered to be sufficient for refuge use.

Paulownia is a rapid growing tree introduced to the US from China over 150 years ago (Kays et al. 1997). There are several species but *P. tomentosa* is probably the most widespread. Others include *P. elongata*, *P. fortunei*, and *P. catalpafolia*. Paulownia has a sparse distribution across the cotton belt, but in recent years has become an interest of some tree production farms. Parker (2000) reported that the plant spacing required for the tree production limited the per acre production of larvae although larvae were utilizing the paulownia for the entire cotton production season.

If it were possible for paulownia to produce more heliothine larvae (*H. virescens* and *H. zea*) than cotton, then producers would not have to sacrifice portions of their yield and valuable land for cotton refuge. The following studies were designed to provide preliminary data as to the possibility of utilizing paulownia as a refuge by modifying the plant density. It was hypothesized that paulownia planted at high densities per acre would provide more larvae per acre than untreated cotton planted at normal plant density.

Material and Methods

Studies were conducted at the Mississippi State University Brown Loam Branch Experiment Station in Raymond, MS during 2001 to compare untreated nonBT cotton to untreated paulownia with regards to production of lepidopteran larvae.

Paulownia elongata seedlings in 2 X 2 X 3 plastic cell trays were purchased from Carolina Pacific International, Inc. (Lenox, GA). Due to a delay in shipping of the paulownia seedlings, the planting date was later than would be ideal for cotton or paulownia. The ideal planting date for both plants in Mississippi is early May (Kays et al. 1997). On July 10, 4 replicates of the two treatments (paulownia and cotton) were planted. Prior to planting, all plots were disked equally to prepare the seedbed. The paulownia plots were 50 ft in length X 16 rows wide (38 in centers), and plants were spaced 38 in apart within rows. Paulownia seedlings were hand planted and one fertilizer tablet (20N-10P-5K) purchased from Carolina Pacific International was placed approximately 2 inches to the side of the seedling. Cotton plants ('Suregrow 521R') were planted the same day using a vacuum planter. The cotton plants were planted at normal field spacing (3 to 4 plants per linear foot of row).

Paulownia plots were flood irrigated for one week to prevent seedlings from dying which is a normal practice for paulownia transplants. Plants were monitored on a weekly basis for heliothine eggs and/or larvae, and other lepidopteran larvae. Initial data included number of eggs and/or larvae visually observed per 25 plants. However, it became obvious that the size of the larvae observed was of importance. Therefore the visual observations were modified to include number of small (less than $\frac{1}{4}$ in.), medium ($\frac{1}{4} - \frac{1}{2}$ in.), and large (greater than $\frac{1}{2}$ in.) heliothine larvae, number of loopers (*Trichoplusia ni* and *Pseudoplusia includens*), number of armyworms (*Pseudaletia* and *Spodoptera spp.*), and number of other lepidopteran larvae. On September 7, a measurement of height (in inches) was obtained from 25 plants in each plot of paulownia and cotton.

The data were recorded and analyzed for the visual observations of 25 plants per plot. The data were analyzed by ANOVA with means separated by Fisher's Protected LSD. Resulting means and standard errors were converted to a per acre basis for both the cotton and Paulownia treatments.

Results and Discussion

The first sample date (August 23) showed statistically similar numbers of heliothine (*Heliothis virescens* and *Helicoverpa zea*) larvae per acre in cotton and paulownia (Figure 1). However, the paulownia soon began to grow rapidly and it is believed that the paulownia required an adjustment period for the transplanted root system. By August 29, the paulownia had more heliothine larvae than cotton (Figure 1), a trend that remained for sample dates through September 14. On September 20, larval populations on a per acre basis were low and were equivalent between paulownia and cotton. Cotton had more larvae per acre on September 26 although the total population density was relatively low. It is worth noting that most of the cotton planted on normal planting dates had reached maturity by September 26, but the cotton in this study had not reached maturity due to the late planting date. However, paulownia continued to attract heliothine larvae into October, which would be after defoliation of cotton in Mississippi. On October 16, no larvae were observed on cotton although larvae were still observed on paulownia. The paulownia trees continued to grow and stay green until the first frost which could mean an extra generation of larval production in the fall for the paulownia trees. During the peak larval number (Aug. 29, Sept. 7, and Sept. 14) the paulownia had 7.5, 5.6, and 10.84 times more larvae than the cotton, respectively.

Larvae were collected on two dates (August 30 and September 21) for species identification. On August 30, the mandibles were removed from 10 larvae and examined under a stereoscope for species separation. On September 21, the mandibles were removed from 25 larvae and examined for species separation. Data show that 40% of the larvae on August 30 were *H. virescens* and 60% were *H. zea*. Data for September 21 show that 96% of the larvae were *H. virescens* and 4% were *H. zea*.

The mean number of total eggs and total heliothine larvae are shown in Figure 2. More eggs were recorded on cotton than on paulownia, however, more larvae were recorded on paulownia. This could indicate that paulownia is a better food source than cotton (resulting in higher survival on paulownia). Paulownia did have significantly more heliothine larvae (approximately 2.7 times more larvae) when analyzed across all sample dates (Figure 2). Once the heliothine larvae data were separated into small, medium, and large heliothine larvae, the data show most heliothine larvae observed on cotton were small larvae (Figure 3). For all sample dates, paulownia and cotton did not differ in terms of small heliothine larvae per acre, but paulownia had 6 times more medium size heliothine larvae, and large larvae were only observed in the paulownia plots. In addition, paulownia had significantly more looper larvae and other lepidopteran larvae than the cotton, but cotton did have more armyworm larvae (Figure 3). There were some other lepidopteran larvae recorded on paulownia, but not on cotton. Among these other lepidopteran larvae, sphinx larvae were the predominant group.

Although the cotton and paulownia were planted at the same time, the paulownia had outgrown the cotton by September 7 (Figure 4). The paulownia did have an adjustment period after transplanting but soon made the adjustment and began its rapid growth. It could be argued that the height advantage of the paulownia contributed to its higher larval numbers as compared to the cotton. However, if we consider the larval size, the data still suggest that paulownia has a potential to produce more medium and large larvae as compared to cotton. It seems reasonable to believe this would result in higher numbers of adult moths emerging from paulownia as well, but such data has not been obtained to verify this assumption.

By first frost the paulownia had exceeded 5 feet in height. Therefore the rapid growth of the plants may require more management. The purpose of paulownia refuge plantings should not be to produce timber but only to serve as a refuge. Therefore considerations for management of such a refuge include routinely cutting the plants off at ground level and allowing the plants to produce regrowth. This technique may require a planting large enough to alternate cutting half the planting and allowing regrowth before cutting the remaining half. However, this would prevent flowering and insure that seed are not dispersed allowing the escape of the plant species. It should be noted that paulownia has been introduced into Mississippi for over 100 years and has not become a weed problem (Dr. Stephen Dicke, personal communication).

The data presented here show that *Paulownia elongata* does have potential as a refuge for heliothine larvae. In addition, it serves as a refuge for other lepidopteran larvae as well. This refuge would exist beyond the cotton-growing season and would require a one-time planting. Future research in this area will be directed at reducing plant spacing to increase larval production, establishment and management of refuge, and herbicide sensitivity.

References

Caprio, M. A. 1994. *Bacillus thuringiensis* gene deployment and resistance management in single- and multi-tactic environments. Biocontrol Sci. and Technol., 4: 487-497.

Craig, C., S. Stewart, R. Luttrell, J. Robbins, and G. Snodgrass. 1999. Use of alternate hosts as a trap for tarnished plant bugs and a refuge for BT-susceptible tobacco budworms, Pp.1056-1061. *In*, P. Dugger and D. Richter (eds.), Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Dicke, Stephen. Forestry Extension Specialist, Central Mississippi Res. and Ext. Center, Raymond, MS.

Fischoff, D. A. 1992. Management of Lepidoptera pests with insect resistant cotton: Recommended approaches, Pp 751-753. *In*, J. M. Brown and D. A. Richter (eds.), Proc. Beltwide Cotton Prod. and Res. Conf., National Cotton Council, Memphis, TN.

Kays, J., D. Johnson, and J. Stringer. 1997. How to produce and market paulownia. Univ. of Maryland, Coop. Ext. Ser. Bull. 319.

ILSI Health and Environmental Sciences Institute (ILSI HESI). 1998. An evaluation of insect resistance management in BT field corn: A science-based framework for risk assessment and risk management. Report of an expert panel. ILSI Press, Washington DC.

IRM Guide 2002. Insect resistance management: The cotton grower's guide to preserving Technology by protecting against insect resistance. Monsanto Company, St. Louis, MO.

Mallet, J. and P. Porter. 1992. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? Proc. R. Soc. Lond. 250: 165-169.

Matten, S. 2000. EPA Regulation of transgenic pesticidal crops and insect resistance management for B.T. cotton, Pp71-78. *In*, P. Dugger and D. Richter (eds.), Proceedings Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

McGaughey, W. H. and M. E. Whalon. 1992. Managing insect resistance to *Bacillus thuringiensis* toxins. Science 258: 1451-1455.

Parker, Jr., C. D. 2000. Temporal distribution of heliothines in corn-cotton cropping systems of the Mississippi Delta and relationships to yield and population growth. Dissertation, Mississippi State University, 116 pp.

Snow, J. W. and J. R. Brazzel. 1965. Seasonal host activity of the bollworm and tobacco budworm during 1963 in Northeast Mississippi. Miss. State Univ., Ag. Exp. Sta. Bull. 712.

Snow , J. W., J. H. Hamm and J. R. Brazzel. 1966. *Geranium carolinianum* as an early host for *Heliothis zea* and *H. virescens* (Lepidoptera: Noctuidae) in the southeastern United States, with notes on associated parasites. Ann. Entomol. Soc. Am. 59: 506-509.

Stadelbacher, E. A., H. M. Graham, V. E. Harris, J. D. Lopez, J. R. Phillips and S. H. Roach. 1986. *Heliothis* populations and wild host plants in the southern U.S. *In*, Theory and Tactics Oof Heliothis Population Management. Southern Coop. Series Bull. No. 316, Agr. Exp. Sta., Oklahoma State Univ., Stillwater, OK.



Sample Date

Figure 1. Mean (SEM) number of heliothine (*Heliothis virescens* and *Helicoverpa zea*) larvae per acre observed on paulownia and cotton for each sample date during 2001.



Figure 2. Mean (SEM) number of heliothine (*Heliothis virescens* and *Helicoverpa zea*) eggs and larvae per acre of paulownia and cotton for all sample dates during 2001.



Figure 3. Mean (SEM) number of small (H-small), medium (H-med), and large (H-lar) heliothine larvae (*Heliothis virescens* and *Helicoverpa zea*), number of loopers, armyworms, and other lepidopteran larvae across all sample dates for cotton and paulownia during 2001.



September 7

Figure 4. Mean (SEM) height in inches for paulownia and cotton September 7, 2001.