ARTHROPOD MANAGEMENT

Cotton (Gossypium hirsutum) Response to Simulated Repeated Damage by Helicoverpa spp. Larvae

Tom T. Lei

INTERPRETIVE SUMMARY

The cost of controlling the cotton bollworm (Helicoverpa armigera) and the native budworm (H. punctigera) constitutes a substantial portion of cotton production cost in Australia. In addition, a concern over cotton bollworm insecticide resistance has prompted the implementation of a rigorous scheme of insecticide rotation to prevent the further development of resistance. Options for reduced insecticide use on these pests are being investigated to achieve greater sustainability and profitability in Australian cotton production. One way to achieve this goal would be by raising the present threshold for treating bollworms. If it can be demonstrated that cotton can recover from higher levels of bollworm damage, the threshold can be raised. This study examined the response of cotton to various levels of repeated damage to plant terminals and fruit. Five levels of damage equivalent to that caused by 0, 2, 4, 6 and 8 larvae per m$^{-2}$ were manually imposed. Because there are usually multiple infestations during the growing season, the trial was designed to quantify the cumulative effect of several damage events. The damage regime consisted of two bouts of terminal damage before squaring followed by two bouts of fruit loss. The results showed that repeated damage, which simulated larvae numbers of Helicoverpa spp. that are two to four times greater than the current recommended threshold (i.e., at 2 larvae per m$^{-2}$), did not have a significant effect on yield but did delay maturity by 3 to 8 days. It appears that terminal damage modified the canopy structure, which enhanced light interception, and may have contributed to the recovery process. Compensation was largely attributed to a higher retention of later developing fruit following damage rather than by increasing fruit production. The determination of the capacity of cotton to recover from these levels of damage by Helicoverpa spp. will make it possible to manage Australian cotton crops at a higher threshold up to 4 weeks after first flower. This will result in fewer insecticide sprays, and allow growers to reap the attendant benefits of reducing costs, forestalling pesticide resistance, promoting predators and parasitoids, and minimizing environmental impact.

ABSTRACT

Cotton (Gossypium hirsutum) experiences frequent episodes of insect infestation during the growing season in Australia. The most important pests are larvae of the cotton bollworm (Helicoverpa armigera) and the native budworm (H. punctigera). While cotton has some ability to recover from repeated pest damage without yield loss, the degree of damage it can tolerate is still not well defined. In a two-year field trial, the recovery of yield and maturity rate of cotton subjected to manual damage to terminals (twice) and to fruit (twice) mimicking repeated infestations by Helicoverpa spp. was examined. The terminals of 80% of the plants were removed from all but the control treatments. Following terminal damage, five levels of fruit damage simulating that caused by 0, 2, 4, 6, and 8 Helicoverpa larvae per m$^{-2}$ were imposed. Yield loss was not statistically significant for any damage level, but there was a delay of 3 to 8 days in maturity. An examination of the process of compensation revealed that early season terminal damage could affect cotton canopy structure and may increase light interception. The pattern of fruit production in damaged plants suggests that replacement of lost fruit was achieved largely through greater retention because no significant increase in fruit production occurred. Fruit development was delayed but the 10 weeks between last damage and maturity was sufficient for full yield recovery.

Tom T. Lei, CSIRO Cotton Research Unit, Locked Bag 59, Narrabri, NSW 2390 Australia. tom.lei@csiro.au
The larvae of *Helicoverpa armigera* Hübner (cotton bollworm) and *H. punctigera* Wallengren (native budworm) are among the most serious pests of cotton (*Gossypium hirsutum* L.) in Australia (Fitt, 1989, 1994). Insecticides used to control these insects amount to a significant portion of the management costs of Australian cotton crops (White et al., 1995). Studies have shown that cotton can tolerate and recover from damage caused by these pests at levels higher than that sustained at the recommended threshold (Brook et al., 1992a; Jones et al., 1996; Ungar et al., 1987; Wilson 1986). These studies have rarely included repeated damage simulating the actual feeding rate by larvae of *Helicoverpa* spp. (Brook et al., 1992a). Thus, if repeated damage at higher thresholds does not result in yield losses or significant delay in maturity, then the crop may be managed with fewer pesticide sprays thereby garnering substantial benefit by lowering pesticide costs, pest resistance, non-target effects (i.e., beneficial arthropods), and the risk of environmental contamination. Furthermore, some studies have found that the loss of terminal or fruit can, in some situations, enhance cotton growth and result in a yield gain relative to undamaged plants (e.g., Eaton, 1931; Brook et al., 1992a, b). It may be possible to establish a level of damage which confers a positive effect on crop yield.

The process of compensation following pest damage is closely associated with changes in the canopy architecture and fruiting dynamics of cotton plants (Sadras, 1995, 1996b). Early season damage to plant terminals and young fruit may stimulate branch and leaf development improving canopy light interception and carbon gain (Sadras, 1996b). An improved carbon gain could allow the plant to accelerate the replacement of lost plant parts, increase the boll load, or increase boll size. This type of response may explain the larger fruit production and yield gain in some damaged crops (Brook et al., 1992a, b). Compensation may also occur in the situation where early fruit loss leads to a delay in peak flowering, which shifts fruit development to coincide with more favorable weather conditions (Pettigrew et al., 1992; Kennedy et al., 1986). Conversely, since damage causes some delay in the fruiting process, compensation in yield may be associated with later maturity and unfavorable weather for boll maturation.

In this study, the ability of cotton to recover from repeated damage at levels up to the equivalent of 8 *Helicoverpa* larvae per m². Infestations by *Helicoverpa* spp. span the entire cotton growing season, feeding mainly on shoot terminals early in the season, and later, on flower buds (squares), flowers and bolls. To mimic realistic infestations, repeated damage to plant terminals and fruit were imposed on plants during two cropping seasons. Changes in canopy development in response to damage were assessed by measuring leaf area and light penetration during the season. Fruiting patterns were also monitored to evaluate the effect of damage levels.

**MATERIALS AND METHODS**

This two-year study was conducted at the Australian Cotton Research Institute in Narrabri, Australia (30.4oS, 149.8°E). Seeds of cotton cultivar Sicale V-2 (1998) and Sicale V-2i (transgenic cotton containing the Monsanto Co. Cry 1Ac gene called INGARD cotton, 1999) were sown in 1m spaced rows in mid-October both years. Seedlings were thinned to 10 plants per meter after establishment. Anhydrous ammonia fertilizer was applied to the field at 100 kg ha⁻¹ N (1998-99, a naturally more fertile field) and 184 kg ha⁻¹ N (1999-00). The fields were furrow irrigated when moisture deficit reached ca. 90mm. Weeds and pests (such as above-threshold *Helicoverpa* spp., aphids and spider mites) were controlled using standard management practices which were applied uniformly to all treatment plots. Five treatments with 5 replicate plots were arranged in a complete randomized block design. Each plot was 3 rows by 5 m. The five treatments were an undamaged control and four damage treatments simulating feeding on fruit by 2 to 8 larvae per m².

Prior to the start of flower bud production (sowering), terminals of all damage treatments were removed twice at 35 (4 leaf stage) and 55 (8 leaf stage) days after sowing. In both damage events, 80% of the terminals, randomly determined, were removed from all 3 rows using tweezers. This level of terminal damage simulates heavy larval infestation at the vegetative stage where a small number of plants will escape damage. By the time of the second damage event, many plants had grown multiple shoots and only the terminal of the dominant shoot (tallest) was removed. This was followed by two fruit removal events at 85 and 115 days after sowing or about one and two months after
first square, respectively. The number of fruit removed in the four damage treatments was equivalent to that affected by a cohort of 2, 4, 6, and 8 Helicoverpa larvae per m$^2$ during their two-week life span (Hassan and Wilson 1993). At the first damage event, a total of ca. 15, 30, 44 and 59 squares (of three size classes) corresponding to the four damage levels were removed. The proportion of square classes removed was 4% small (less than 0.5 cm), 28% medium (0.5 – 1.0 cm) and 68% large squares (greater than 1.0 cm). At the second damage event, a total of ca. 12, 23, 35 and 47 squares and bolls were removed for the same four damage treatments. The distribution of fruit classes removed was 4% small squares, 23% medium squares, 25% large squares, 25% flowers, 15% small bolls (less than 2.5 cm diameter), and 8% large bolls (greater than 2.5 cm). Fractional fruit numbers were rounded to the closest integers. Fruit were removed by hand where the specified number for each size class was taken haphazardly but evenly across each meter of plants. Most of the fruit were taken from the upper layer of the canopy. Each fruit removal event spanned an interval of one- (1999) or two- (1998) weeks to better mimic the feeding pattern of larvae of Helicoverpa species.

Fruit were removed evenly across the center row only. This is because the effect of fruit damage on canopy development is relatively small compared to terminal damage; therefore, treatment applied to the outside rows was considered unnecessary. Measurements of plant growth, light interception, photosynthesis, yield, and maturity date were all made in the center row, as described below.

Light interception through the canopy was measured during the peak growth phase (Jan. – Mar.) using a ceptometer (AccuPAR, Decagon Devices, Inc. Pullman, Washington). Measurements were taken within ±1.5 h of solar noon under clear skies above the crop canopy, at mid-canopy depth, and at ground level. Readings were taken with the 80 cm sensor rod perpendicular to the plant row and centered at the plant line.

All plants within a 0.5 m row of each plot were destructively sampled periodically during the season. Plants were sorted to taproot, leaves, stems and fruit (squares, green bolls and open bolls). Leaf area index (LAI) was determined by measuring the total leaf area (seedling stage). Later in the season, LAI was estimated by using the leaf area of a ca. 10 g dry weight subsample and the proportional dry mass of the sub-sample to the total. Dry mass (70°C for 48 h) of taproot, leaves (excluding petioles), stems and fruit (squares and bolls) was determined. Weekly hand picking of open bolls in 2 m of the center row began when 20% of bolls were open. Crop maturity was defined as the date on which 60% of the bolls had opened. Seed cotton was ginned to yield the dry mass of lint and cotton seed. Fiber quality was determined using the high volume instrument (HVI) system.

Data were analyzed using ANOVA, and if significant, differences among treatment means were separated using Waller-Duncan k-ratio t test (release 6.03, SAS Institute Inc., Cary, NC.).

RESULTS

None of the damage treatments resulted in a significant reduction in yield compared with the control in either year (Fig. 1). Maturity date was generally delayed with higher levels of simulated damage. The maximum delay in maturity of 8 days (significantly higher than the control, $P=0.005$) occurred in the 8 larvae m$^2$ treatment in both years.

When the terminals of cotton plants were

![Figure 1. The effect of the five damage treatments that simulate 0 to 8 Helicoverpa larvae per m$^2$ on average lint yield in 1998 and 1999. Maturity date (days after planting) of each treatment for 1998 and 1999 is shown on the bottom panel. Different letters within the bars for each year indicate significant differences ($P<0.05$) between treatments according to Waller-Duncan k-ratio t-test.](image-url)
damaged prior to flower bud production, plants responded by reducing canopy height but increasing lateral branch growth (Table 1). Comparing measurements taken at peak fruiting (110 - 113 days after sowing), plant height of all damage treatments was lower by up to 16% than the control (significant in 1999, P=0.005). Total length of vegetative branches per m\(^{-2}\) of cotton in damage treatments was also higher than the control in both years, with significant treatment differences in 1998 (Table 1). A maximum LAI of between 2.7 and 3.6 was achieved for all treatments in both years, but there were no significant differences in leaf area among treatments (P=0.59 and 0.23 for 1998 and 1999, respectively, Table 1). There were some variations in the penetration of light through the canopy to mid-canopy depth and to ground level (Fig. 2). Measurements taken in 1998 showed higher light levels beneath control plant canopy in six out of 11 cases at both canopy depths (Fig. 2). This indicates that relative to damage plants, less light was intercepted by control plants. The greater light interception at ground level for damaged over control plants was most obvious late in the season, at 140 days after sowing (P=0.002, at mid-canopy). Dry matter accumulation was not significantly different among treatments in either year based on sequential increments of stem and tap root dry weight (P>0.05, Table 1 shows only results for time of peak fruiting).

Damage treatments were not significantly different from the control in square and boll numbers for all sampled dates of the two years (Fig. 3). Only at 113 days after sowing in 1999 were the differences significant among treatments (Table 1 shows only results for time of peak fruiting).
among damage treatments significant. There was a trend for the heavy damage treatments to maintain a higher fruit production and a slower boll development (Table 2, Fig. 3). This trend was supported by the average dry mass of single bolls at 110 days after sowing (1998) where it was highest in 2 larvae m$^{-2}$ (4.59±1.36g), intermediate in the control (3.95±0.90g) and lowest in 8 larvae m$^{-2}$ (1.30±0.33g). Similar results were found in 1999 (113 days after sowing) where mean dry mass per boll of the 8 larvae m$^{-2}$ treatment was significantly lower (at 1.04±0.13g, Waller, $P<0.05$) compared with the control (at 1.76±0.22g). Other damage treatments showed intermediate boll mass values.

Fiber quality of harvest from the first year was analyzed but fiber characteristics were not significantly different among treatments (P>0.05, data not shown).

Table 2. Development (mean dry mass±SE) for squares and bolls on cotton plants subjected to five damage treatments simulating repeated fruit damage by 0 (control), 2, 4, 6, and 8 Helicoverpa larvae per m$^{-2}$

<table>
<thead>
<tr>
<th>Damage$^y$</th>
<th>110 DAP*</th>
<th>138 DAP</th>
<th>113 DAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Square Dry Weight (g m$^{-1}$)</td>
<td>Boll Dry Weight (g m$^{-1}$)</td>
<td>Square Dry Weight (g m$^{-1}$)</td>
</tr>
<tr>
<td>0</td>
<td>23.6±4.9</td>
<td>72.4±17.2</td>
<td>3.7±2.3</td>
</tr>
<tr>
<td>2</td>
<td>22.7±2.0</td>
<td>82.9±34.6</td>
<td>3.4±1.0</td>
</tr>
<tr>
<td>4</td>
<td>23.7±3.6</td>
<td>83.4±36.2</td>
<td>3.5±0.9</td>
</tr>
<tr>
<td>6</td>
<td>27.4±2.9</td>
<td>24.7±7.1</td>
<td>4.7±1.8</td>
</tr>
<tr>
<td>8</td>
<td>26.9±2.7</td>
<td>25.2±8.0</td>
<td>7.4±2.7</td>
</tr>
</tbody>
</table>

$^x$ Days after planting.

$^y$ The four damage treatments (0=no damage) were imposed by removing the number of fruit that would be fed upon by 2, 4, 6, and 8 larvae per m$^{-2}$, using the feeding model by Hassan and Wilson (1993).

$^z$ Means within a column followed by the same letter are not significantly different according to Waller-Duncan $k$-ratio t-test ($P$≤0.05). Means within a column not followed by a letter indicates no significant treatment effect.
This study shows that commercial cotton cultivars, Sicala V2 (1998) and V-2i (1999) can recover from repeated damage that simulates multiple bouts of feeding by larvae of *Helicoverpa* spp. at both the pre-squaring and the fruiting stages. The effect on yield was minimal even when damage consisted of two terminal removal events combined with two fruit damage events equivalent to 8 larvae m\(^{-2}\). The ability to recover was not strongly affected by variations in yield potential between years (i.e., large yield difference in the control). The low yield in 1998 was the result of an unusually cool and wet season compounded by persistent sub-threshold grazing by larvae of *Helicoverpa* spp. late in the season. These results agree with other reports of no significant loss of yield from a single event of fruit loss (Brook et al., 1992a,b; Jones et al., 1996; Sadras, 1996a, Gore et al., 2000), and from repeated damage of terminal damage and fruit removal equivalent to 6 *Helicoverpa* larvae m\(^{-1}\) (Brook et al., 1992a). The pattern of yield recovery among treatments was similar between conventional and transgenic cotton cultivars, consistent with findings reported by Gore et al. (2000). It is clear from this study that cotton possesses the ability to recover from repeated damage at levels three to four times higher than the threshold (2 larvae m\(^{-2}\)) set by the Australia cotton industry (ENTOpak, 1999).

For compensation to occur, it is necessary for a damaged crop to maintain carbon assimilation comparable to or higher than an undamaged crop. The compensatory carbon gain may be achieved through two processes: 1) by attaining higher canopy photosynthesis through better light interception as a result of modified canopy structure in response to terminal damage; 2) by substituting and replacing lost fruit by reducing natural fruit shedding. The second process may be accompanied by a modest delay in fruit development, which could potentially be mitigated by the first process. In crops experiencing both types of damage, recovery will likely involve contribution from both processes (Brook et al., 1992b).

Damage to plant terminals triggers the production of lateral shoots (Sadras and Fitt, 1997) and improves canopy structure for light interception and plant carbon gain (Sadras, 1996b). There is some evidence to support this. While total leaf area did not differ among treatments, there was higher light interception in some damage treatments during the season. This implies that more leaves of the damaged plants are situated in the upper part of the canopy. Given that upper canopy leaves are generally younger and photosynthetically more active (Constable and Rawson, 1980), a small increase in light interception here could potentially contribute to accelerating the recovery process.

In cotton, where 60% or more of the total fruit is shed due to intrinsic over-production, there is a substantial fruit bud reserve to substitute for those lost to pests (Hearn and Room, 1979). Since none of the fruit numbers were significantly different from the control, this study suggests that compensation was achieved through greater retention of available fruit rather than an increase in fruit production. Substituting damaged fruit with those that would otherwise be shed physiologically appears a sufficient explanation for full compensation in all damage treatments in this study. The first fruit removal event consisted only of squares, and given the length of the remaining season, should have a relatively smaller impact on recovery than the second fruit damage event. The second damage event included removing 3 to 10 bolls per meter (corresponding to simulated damage by 2 to 8 larvae m\(^{-1}\)) of boll age ranging between 1 day and approximately 3 weeks. Replacing these bolls could mean a delay in maturity of up to 3 weeks, but the actual delays in maturity were much shorter.

Full recovery was still possible from damage occurring as late as 130 days after sowing and about 75 days prior to maturity. This was similar to the 66 days Kerby et al., (1987) found for late blooms of Acala cotton in California. While the delay in maturity of up to 8 days (consistent in both years) was significantly higher than the control, it was less than the 3-week delay anticipated. Comparable delays in maturity were also reported by Gore et al. (2000) in fruit damage treatments imposed 4 weeks after first flower. Brook et al. (1992a, b) also found maturity dates ranging from 2 days earlier to 5 days later than the control following similar levels of damage.

In conclusion, cotton grown in SE Australia showed clear ability to recover from multiple events of terminal damage and fruit removal. The process of recovery appears to involve higher fruit retention and may be aided by the modified canopy structure following terminal damage. If the last fruit damage occurred 10 weeks prior to maturity, then
reduction bolls will have sufficient time to fully develop, with a delay of 8 days or less. These results suggest that it may be possible to raise the threshold for Helicoverpa spp. prior to mid-fruiting period without suffering yield loss.

**ACKNOWLEDGMENT**

This study was funded by a Cotton Research and Development Corporation (CRDC) grant (CSP82C). Technical assistance was provided by Leslie Burke, Kelly Scott, Tanya Smith, Jane Caton, Kellie Baguley, Deon Cameron, and Renee Schick. Bruce Reddan, Des Magann. The farm staff is acknowledged for their cooperation in establishing and maintaining the experimental fields and crops. Steve Milroy, Lewis Wilson, Mike Bange, Brian Hearn, Greg Constable, Victor Sadras and Martin Dillon offered valuable advice at various stages of this study.

**REFERENCES**


