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PROJECT TITLE: Development of a rapid mass screening technique for the selection of cotton plant antibiotic resistance to *Helicoverpa* spp.

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ABSTRACT: When searching for an ideal 'mass screening technique' for cotton plant antibiotic resistance to Helicoverpa spp., a '48 hour feeding on squares using final instar larvae' bioassay was found to be the most suitable. It correlated better, than the other techniques trialed, with the 'Standard' larval bioassay traditionally used as an antibiotic resistance measuring tool and was more energy efficient than the 'Standard'.
An ideal mass screening technique using the two spotted mite (Tetranychus *urticae*) on cotton seedlings failed to correlate with the 'Standard' rendering it unsuitable for measuring antibiotic resistance to Helicoverpa spp..
The widely reported finding that gossypols are very important to the antibiotic activity against the cotton bollworm (*Helicoverpa armigera*) was supported with highly significant correlations betwen gossypol content of plant material and percent larval weight gains. In contrast, condensed tannin content of 'fresh plant' diet incurred insignificant antibiotic activity against the cotton bollworm but important antibiotic activity against the two spotted mite (Tetranychus urticae).

INTRODUCTION: The aim of this project is to develop a mass screening technique for the selection of cotton plant antibiosis resistance to Helicoverpa spp.. Ideally, for the purpose of mass screening, this technique needs to be accurate, simple, economic, dependable and fast. Such a method could be used as a tool to select for Helicoverpa spp. resistant material resulting in a reduced dependence on insecticides hence slowing the development of insecticide resistance.

The legacy of the cotton industries complete dependance on chemicals for control of its insect pests is now very apparent. As well as being both economically and environmentally taxing, insecticides are losing their effectiveness through the development of insecticide resistance. This has led to a serious consideration of alternative control measures of which 'host plant resistance' (H.P.R.) is one.
Antibiosis is a phenomenon in which one organism is in opposition to another. It is one of the three recognised plant resistance mechanisms associated with H.P.R. along with morphological and tolerance characteristics, yet, ignored in current cotton breeding programmes. Antibiotic resistance is the focus of present research being carried out here at the Biloela Research Station. To introduce this facet of plant resistance into the breeding programmes could only strengthen the efficacy of H.P.R..

The chemical - pest relationship in plant antibiotic resistance. is highly complex and, to this day, is still largely misunderstood. Frankel (1959) suggests that all primary nutrients for insects are found in all higher plants, but the attractancy and repellancy are governed by elaborate 'secondary plant products'. These secondry products are known as allelochemicals, defined by Reese (1979) as being non nutritional chemicals produced by one organism that effects the growth, health. behaviour. or population biology of members of other species.
Because of the multiplicity in chemical ecology and the diversity in the biological systems in the tropics where cotton evolved, it became a plant with many of these secondry products. It has a greater array of allelo chemical elements than any other plant group in the temperate zone. But typical of most modern agronomic plants, selective breeding has led to a narrower genetic base. Selection pressure bias for higher yields has been at the expense of the plants defencive mechanisms rendering the plant more susceptable to its insect pests.
Several groups of chemicals have been isolated and identified as having an antibiotic or deterrant effect on the major Lepidopteran pests of the cotton plant. Gossypols (and other terpene aldehydes), cyclopropenoid fatty acids, polymeric condensed tannins (and other flavonoids) are all recognised secondry products of the cotton plant with the gossypols and condensed tannins considered most important.
Both have been found to cause an inhibition in growth rate and even mortality of the larvae through either an antibiotic or feeding deterrant action. Gossypols are stored in black pigment glands that are present in large numbers on the roots, stems, leaves and fruit of most cotton plants and are known to involve both actions but the effect of tannins is less clear. Condensed tannins are a water based compound occurring systemically throughout the plant and have been noted to cause a reduction in growth gains in young larvae when introduced via an artificial diet media in the laboratory. This seems to be due to a reduction in food intake and not to internal food conversion efficacy suggesting a deterrant action. Whether this action is actively expressed in the field is still vague.

A great deal of emphasis has been placed on breeding for antibiotic resistance to Helicoverpa spp. both here and in the U.S.A.. During 1972 - 79, Mungomery, Twine and Noble put a large research effort into developing resistant cultivars. They were relatively unsuccessful in developing a resistant cultivar with suitable agronomic characteristics. The suggested reason for this failure inferred testing cultivars was too time consuming restricting the number of genotypes possibly tested to 300 per season. This small sample size reduced the chances of identifying a high yielding Helicoverpa spp. resistant recombinant.
To develop a more efficient testing technique, a number of old and new 'antibiotic resistance' measuring techniques were trialled using both commercial and test cotton cultivars. These included larval bioassays, a mite trial and chemical assays. The chemical analyses for gossypols and condensed tannins was to determine the importance of either to antibiosis resistance and, if so, whether this assaying could be used successfully as a mass screening tool. The accuracy of these techniques was tested by correlating their results with resultant larval weight gains from a traditional '8 day feeding on squares using 2nd instar larvae' bioassay as a standard. Their ability to satisfy the required criterias for mass screening were also tested.
Condensed tannins were analysed using a standard butanol/HCl extraction (Land and Schuster, 1981) while gossypols were analysed using a spectrophotometric method with analine (R.M.Noble, 1980) technique. All results were expressed as a percentage of chemical per dry matter.
All chemical analyses were performed by R. M. Noble (Senior Chemist, Agricultural Chemistry Branch).
Field trials were carried out to demonstrate the importance of antibiosis resistance in the field.

LARVAL BIOASSAYS

Biological assays have traditionally been considered the most effective way of measuring active relationships between two organisms. Assays that allow all mechanisms of the relationship to be expressed then measured by observing responses are considered best to measure one as complex and unchartered as the interaction cotton has with its major insect pests, Helicoverpa spp.. An 8 day larval bioassay is one such assay traditionally adopted. However, when developing a mass screening programme, this technique is laborious and energy inefficient. A more suitable technique satisfying the necessary criteria for a successful mass screening technique is required.

Methods and Materials:

During the 1985/86 season, various larval weight gain bioassays were trialled for workability. Heliothis larvae were initially fed on cotton plant terminals and weight gains measured. Problems occurred with neonate larvae being lost or drowning in the water provided to keep the terminals fresh. Consequently squares were trialled and found to be very suitable for assaying larval weight gains.

The following season, '10 day' and '24 hour' larval bioassays were carried out on both leaves and squares. Leaves were being studied as an alternative food source to squares as they are easier to harvest and the larvae easier to retrieve when changing diet and weighing. Results indicated a differing in response by Helicoverpa spp. to the two different food sources. Further collecting of data was necessary.

The 1987/88 season was used to develop the bioassays further and to collect data. In all, 8 different larval bioassays were trialled including the '8 day feeding on squares' used as a traditional standard. The trials differed in length of feeding time, larval stage and plant material used (Appendix 1). Within each of the trials, larvae used were reared from laboratory collected Helicoverpa armigera eggs (Bob Teakle, Brisbane) and raised on an artificial diet until reaching a required level of development. The larvae, selected at a particular instar and within a specific weight range to restrict variability (2nd instar / 4-10mg, 3rd instar / 20-50mg, final instar / 150-200mg), were then placed on to cotton plant material in individual 50 ml pomade jars. The cultivars were arranged in a tri replicated randomized block design with 12 samples of each cultivar per replicate. The jars were then placed in a constant temperature cabinet at 27 degrees centigrade for the duration of the technique. Artificial light was set at 12:12 N/D.
With the 48 and 24 hour techniques, the grubs were reweighed after the specific period and recorded. With the 8 day assays, fresh plant material was introduced and jars cleaned out every two days with weight gain measurements taken at day 6 and day 8. Fresh food every 48 hours was important to maintain representative levels of allelochemicals.
All plant material used was field grown but unexposed to any pesticide treatment. Squares used were at the stage of developement where approximately 1/3 of the square bud was exposed petal. Leaves selected were the first fully opened at the terminal.
The seven cultivars trialled were selected for their similarity in morphological characteristics but diversity in levels of allelochemicals.

The following and final season was used to repeat the main bioassays ('8 day feeding on squares using final instar larvae, and the '48 hour feeding on squares using final instar larvae') and to trial a new technique using glasshouse seedlings. If seedlings could be used in the larval bioassay, a much more time efficient technique would result without waiting for the cotton plants to mature before assaying.
Cotton seedlings were reared in a fertilized soil mix within polystyrene crates in the glasshouse. The seedlings were harvested from above the cotyledons so the first 3.5 to 4 true leaves, and the terminal, were bioassayed.
Trial conditions were identical to the previous season except for the '8 day' technique which, due to unfortunate circumstances, was run at 24-25 degrees centigrade (i.e. 2-3 degrees centigrade cooler than the previous year).

Weight gains, through all seasons, were measured and expressed as 'percentage weight gains'(initial weight/final weight*100) and were treated as expressions of resistance. All data was sublect to analysis of variance. Correlations and lineal relationships were observed with the standard 8 day larval bioassay.

Chemical analyses for both condensed tannins and gossypols were performed similtaneously with the square and seedling bioassays. Replicated sampling of squares, from unsprayed field grown cotton, was carried out with the fresh food sampling for the 8 day larval bioassays. The squares were of similar size and maturity as of those used in the larval bioassays. The bracks were removed and the square buds freezed dried before the were subjected to the analyses. These chemical analyses were carried out through both larval bioassay seasons with 7 cultivars being tested in the first season and 11 cultivars in the second.

During the Larval Bioassays, larvae were exposed to the fresh food diet for a maximum period of 48 hours. To be certain the fresh food was maintaining naturally representative levels of both gossypols and condensed tannins, squares were analysed at the beginning and end of this 48 hour period. Replicated samples were taken from the field. Four cultivars (CS8310, Deltapine 90, Sicala, HT35-14-3) were analysed for tannin and gossypols. some immediately after picking and others 48 hours latter after being kept in the same environment as the larval bioassays.

Results:

Results of all the larval bioassays from the 1987/88 season and their comparisons with the 'standard technique' (8 day feeding on squares using 2nd instar larvae) are shown in Table 2(a). During this season there were significant variations in larval weight gains recorded from feeding on the 7 cultivars. With the 'Standard' technique an experimental line HT35-14-3 significantly produced the least weight gains, demonstrating greatest resistance, while a glandless variety Deltapine 16gl produced the largest weight gains. All bioassays correlated significantely with the 'Standard' with the '48 hour feeding on squares using final instar larvae' assay correlating best (r=0.97**). All the techniques were more energy efficient than the standard requiring less time and man hours to run (Figure 7).

The bioassays using leaves as food source were more time efficient than the squares as sampling could begin before peak squaring and handling of grubs easier. However, the larval weight gains resulting from the leaves demonstrated significantly different results. in some cultivars. to the squares (Figure 1). CS8310 proved more resistant in the leaves than the squares while Sicala was significantly more resistant than Deltapine 90 in the leaves than squares. Because squares are more the natural diet of the Helicoverpa spp. larvae, using leaves as a measure of antibiosis resistance to Helicoverpa spp. may not be as accurate as using squares.

Results of the accompanying gossypol and condensed tannin analyses, and their correlations with the 'Standard', are shown in Table 2(b). Of the cultivars trialled, HT35-14-3 had the highest gossypol content (1.07%) while Deltapine 16gl (glandless) understandably had the lowest (0.04%). When correlated with the 'standard', a highly significant negative correlation (r=-0.91**) resulted suggesting these gossypols play a very important role in the antibiosis process. The higher the gossypol percent content of the plant the smaller the larval weight gained or the more resistant the plants were. The gossypol analyses themselves were faster than the larval bioassay but much more expensive to operate. However, a refinement of analysis equipment and proceedure would improve the efficiency of this technique.

Condensed tannin content of the squares varied from 11.1% (HT35-14-3) and 10.5% (Deltapine 16gl) down to 7.8% (Sicala) and 7.4% (Deltapine 90). An insignificant correlation (r=0.13) occurred when these results were compared with the 'Standard'. Deltapine 16(gl), a cultivar low in gossypol but high in condensed tannin content demonstrated least resistance suggesting condensed tannins aren't important in antibiosis resistance to Helicoverpa armigera.

The following seasons data, from 11 different cultivars, significantly supported the above findings in both the larval and chemical assays (Figures 2 and 3). Results of the 1988/89 larval and chemical assays are shown in Table 2(c). Because the '8 day' larval bioassay was run at a 3 degree centigrade lower temperature, larval percent weight gains were significantly down on the previous seasons. Gossypol and condensed tannin content of plant materials were significantly lower this season with gossypol levels ranging from a high of 0.81% (HG P-9-13) to 0.05% (Deltapine 16gl) and tannins from a high of 7.6% (Deltapine 16gl) to 5.5% (Siokra) supporting previous findings that these chemical concentrations in the plants can fluctuate from season to season. However these levels were still consistant between the relative cultivars. Again the gossypol levels correlated highly significantly with the 'Standard' while the condensed tannin levels did not.

The '48 hour feeding using final instar larvae' bioassay, using glasshouse grown seedlings, produced variable results. Results in Table 2(c) show an insignificant correlation with the 'Standard' suggesting cotton seedlings would be unsuitable for measuring the true antbiotic capabilities of the mature plant. Gossypol levels in the seedlings were much lower than in the squares and were difficult to measure with the analysis technique adopted. There was as slight correlation (r=0.67*) between the gossypol levels in the squares and seedlings suggesting levels at the seedling stage may be indicative of those in the mature plant.

Resultant analyses of chemical levels in the squares throughout the duration of the 48 hour feeding period are shown in Table 6. The gossypol levels didn't vary as there was no significant difference between levels at the beginning and those after the 48 hours. Condensed tannin levels, however, significantly increased in 3 of the 4 cultivars trialled after 48 hours. These results assured there were minimal representative levels of these chemicals throughout the time the larvae were exposed to the fresh plant diets.

FIELD TRIAL

To demonstrate the importance of antibiosis resistance in the field, we ran a field trial using percent yield losses under varying levels of Helicoverpa spp. pressure as an expression of total resistance. All resistance mechanisms, antibiosis, tollerance and morphological characteristics were allowed to act and yield losses were then correlated with the 'Standard' larval bioassay (measure of antibiosis resistance) to get an expression of antibiosis resistance in the field.

Methods and Materials:

This programme was designed to measure the lint yield potential of different cotton cultivars under different levels of insect pressure. These levels of insect pressure were achieved by adopting varying spray regimes to naturally occuring Helicoverpa spp. populations. Each season, one trial, which was fully sprayed to allow the full lint yield potential of each cultivar to be expressed, would be accompanied by another trial subjected to reduced or maximum insect pressure. Lint yields would be recorded and percent lint yield losses calculated.

The first two seasons of field trialling (1985/86 and 1986/87) were plagued

with problems and were very much a learning process for adapting this trialling technique to our environment. During the first season, none of the cultivars demonstrated any significant resistance with yield losses ranging from 80 to 89 percent. There was no significant differences between the cultivars. It appeared that under excessive insect pressure, natural resistance mechanisms were overpowered. To prevent this occurring the following season, insect pressure was moderated by introducing a 'semi-spray' spray regime. However, unfavourable growing conditions led to poor plant stands and poor drainage in some parts of the trial resulted in patchy nitrogen stressing rendering results unreliable. There was however a better seperation of lint yields between the cultivars this season, supporting the use of the 'semi-spray regime.
During the 1985/86 season, analyis for condensed tannins and gossypols were carried out at three stages of the plants developement (early, mid and late squaring) to observe suspected level changes throughout the season. Squares and terminals from two cultivars were tested.

In 1987/88, this trial was repeated. Nineteen different cultivars, consisting of both test and commercial lines were grown in three different trial plots. To similate different levels of insect pressure per trial, natural Helicoverpa spp. infestations were tailored by adopting three different spray regimes:

1. Maximum spray - to similate minimum Helicoverpa pressure to allow the cultivars to express their true yield potential.
2. Semi-spray - to similate moderate Helicoverpa pressure. Helicoverpa numbers were allowed to reach three times the SIRATAC recommended level for economic injury before controlling sprays were applied.
3. Minimum spray - to similate maximum Helicoverpa pressure. No Helicoverpa control sprays were applied.

The latter two spray regime trials were grown side by side in a seperate paddock to the maximum spray trial to minimise chemical drift effects. These spray regimes were introduced after the plants started squaring. Prior to this, all trials were controlled for all insect pests. Normal irrigation, weed and soil management practises were adopted. Each trial was of 19 cultivars by 3 replications in a randomised block design with a standard (Deltapine 90) grown in 4 plots per replicate. Each plot consisted of 4 rows (2 guard and 2 data) by 18 meters in the two reduced Helicoverpa control trials and by 10 meters in the fully controlled trial. In each of the trials, insect infestations were monitored twice weekly and spray decisions based on SIRATAC recommendations.

At the end of the season, the trials were harvested by an 'experimental plot' cotton picker and yields recorded. Samples were ginned to give a lint yield record and sent away to Geelong for quality analysis to observe fibre quality differences between the cultivars and to monitor any quality changes that may occur under the different levels of insect pressure. Yield measurements from the two reduced control trials were compared to the full control trial to give a measure of percent yield loss under varying Helicoverpa pressure. This percent yield loss was treated as a measure of overall resistance.
Both lint yields per cultivar and lint yield losses between the spray regimes were subject to analysis of variance.

In the 1988/89 season, the trial was expanded to include 27 cultivars. Unfortunately the 'full control' plot was exposed to 2-4D herbicide drift from neighbouring paddocks. This caused severe disruption to the normal growth pattern of the cotton plants leading to some lines producing no cotton at all. Only yield data from the 'reduced spray' plots was recorded.

Results and Discussion:

Chemical levels of squares and terminals through the 1985/86 season are shown in Table 7. This data confirms the variability of percent condensed tannin content of the plants throughout the season. The level of tannins in the squares and particularly the terminals, increased markedly in late squaring. The gossypol levels were less variable between cultivars and sampling dates. Gossypol content was higher in the squares than the terminals.
Results of the 1987/88 season are as follows. Prior to the spray regime, all plots were sprayed twice for thrips alone (Rogor @ 800 mls/hectare) and once for thrip, Helicoverpa and tipworm (Thiodan @ 2.1 l/h, Rogor @ 800mls/h). Through the trial, the 'full spray' trial was sprayed 8 times for Helicoverpa while 3 sprays were applied for moderate control and 0 sprays on the minimal spray trial.
Resultant lint yields and lint yield losses are shown in Table 3. Lint yield losses were substantial ranging from 79% (Stoneville 213) to 46% (HT 35-14-3) under moderate Helicoverpa pressure and 93% (NM 838 gl) to 57% (Tam 8-1) under maximum pressure. The glandless cultivars suffered greater yield losses then the glanded varieties. Some of the high tannin lines also performed poorly.
Differences in quality measurements, shown in Table 5, (50% span, 2.5% span, Uniformity Ratio, strength and Strength:Weight ratio) changed significantly little between the different spray regimes.

Results of the correlations with the 'Standard' larval bioassay, are demonstrated in Figure 4. There was a significant correlation with percent yield loss under moderate Helicoverpa pressure suggesting antibiosis resistance is being expressed in the field under moderate pressure. However, there was no such correlation with yield loss under maximum insect pressure. Antibiosis resistance seems to break down altogether under heavy Helicoverpa spp. infestations.

MITE TRIAL

A suitable technique for the purpose of mass screening cotton cultivars for resistance to the two spotted mite (Tetranychus urticae) was developed by Schuster, Maxwell et. al.(1972). If Helicoverpa spp. were found to respond to the same resistance mechanisms as the mites, then this technque would be very suitable to mass screen new cotton lines for antibiosis resistance to Helicoverpa.
Condensed Tannin analyses of cotton plant material was carried out in association with the mite trials to observe any relationship that may occur between mite damage and condensed tannin content.

Methods and Materials:

During the 1985/86 season the mite 'mass screening' technique was trialled for the first time. Resultant mite damage to the 20 cultivars, after 33 days of exposure, was moderate with all the cultivars recording a damage rating between 3 and 4. There was very poor seperation of the cultivars with insignificant differences between the mite damage indexes. It was

latter noticed that the mites used in this trial weren.t T. urticae but T. ludini.
The following season the trial was repeated, this time with T. urticae. Twenty cultivars were assessed after 28 days of mite infestation. Again (although inproved on the previous season) poor seperation of the cultivars resulted, possibly due to low numbers of mites initially introduced to the trial. Accompanying condensed tannin analyses of cotton plant terminals within 5 cultivars demonstrated a highly significant correlation with the 'Mite Damage Index'. This relationship was further studied in the following seasons.

During the 1987/88 season, two mite trials were performed. The following methodology is the same as adopted the previous seasons. For each trial, a colony of two spotted mites was nurtured and maintained on barlotti beans until required.
Within the first trial (beginning 15/10/87), 20 different cotton cultivars were grown in polystyrene trays (445mm x 285mm x 100mm deep) using a sand, peat moss, perlite mixture (2:1:1). Water was supplied by trickle irrigation and nutrients were provided by osmocote slow release fertilizer to keep physical disturbance of the seedlings to a minimum.
One hundred and twenty seeds of each cultivar were planted per tray and the trays were arranged in a randomized block design replicated 4 times. Seeds were precoated with Thiram to prevent root rot.
Five days after emmergence, the cotton seedlings were infested with the mites by placing a single layer of infected bean leaves (10 leaves with approximately 25 adult mites per leaf) upside down on to the cotton seedlings. It was assumed an even distribution of mites over the whole trial area would result.
The trial was run inside a glasshouse at temperatures ranging from 30 to 35 degrees centergrade.
After 17 days, leaf damage on each seedling was visually assessed and rated using the following damage ratings:

1. no visible damage.
2. light feeding on true leaves evident in any degree in the form of strippling, but no evident damage to the plant such as reduced leaf growth or leaf curling.
3. feeding very evident, leaves mostly silvered by feeding and individual sripples occurred, leaf size reduced.
4. ragging and/or curling of leaves, stunting of plants, defoliation evident, 70% of older leaf surface showing desicated condition resulting from heavy feeding.
5. defoliation complete to terminal or death of terminal.

The Mite Damage Index (M.D.I.) was calculated by summing (the products of injury score multiplied by the number of plants in each rating category) and dividing by the total number of plants scored.

Beginning 28/4/88, this trial was repeated. Mite numbers were less (approximately 15 adults per leaf) causing the seedlings to take longer to show clear measurable responses. Consequently the seedlings were rated after 25 days of mite infestation. A fifth replicate was incorporated and kept mite free to be analysed for condensed tannins.
All replicates were re-randomised every 7 days and the glasshouse temperature was set at between 30 to 35 degrees centigrade.

Results were subject to analysis of variance and correlations with the

'Standard'.

To investigate any relationship between mite population developement and condensed tannin content of seedlings, analyses for tannins was carried out on a fifth 'unmited' replicate. Levels were measured at two stages of the seedlings development. Firstly at day 5 after emmergence (when mites were introduced to the other 4 replicates) and at day 25 (when mite damage was assessed. Leaves and stems from above the soil were analysed.

In association with this work. four of these cultivars were trialled in the field to demonstrate the effect of these genotypes on developement of the mite populations in the field. Due to the lack of two spotted mite activity in the Biloela area, this work was carried at the CSIRO Cotton Research Unit at Narrabri by L.J.Wilson and L.H.Bauer.
The four genotypes trialled were CS8310, HT35-14-3, Tam 8-1 and Deltapine 90. The experimental design was randomised complete blocks design replicated three times. Size of plots was limited by seed quantity, with each plot 3 rows wide by 20 meters. No insecticide was applied. Mite populations were monitored by counting the number of female mites on leaves from the third and sixth nodes below the plant terminal for 15 randomly selected plants per plot. Because mite numbers were low, the experimental plots were artificially infected from glasshouse reared infected plants. Infected leaves were placed on to plants at about the sixth node. Deltapine 90 was used as a test standard and where significant differences in mite counts occurred, means were compared with those of Deltapine 90 using Fishers protected LSD at P=0.05 and P=0.01.

Results and Discussion:

The results of both 1987/88 mite trials and accompanying tannin analysis are shown in Table 1.
The levels of susceptability to mites, in the 1987 season. varied considerably from a mite damage index of 2.40 (HT 35-14-3) to 4.99 (Deltapine 90). There was good seperation of the cultivars with Deltapine 90 showing significantly poorer resistance to the mites than some of the high tannin test lines.
The following season the mite damage index varied from 2.07 (HT 35-14-3) to 4.58 (Deltapine 90). No cultivar demonstrated total resistance to the mites but there were some cultivars that were significantly more resistant then others.
When compared with the 'Standard' assay (Figure 5(b)), there was an insignificant correlation (r=0.05). While Deltapine 90 and Tam 8-1 demonstrated very little resistance against the mites. they did show resistance to the Helicoverpa larvae. Contry to this, Deltapine 16(gl), while showing moderate resistance to the mites, was least resistant to the larvae. HT 35-14-3 was resistant to both. These findings were supported in the following season (Figure 5(a)). It seems that mites react differently to the plants antibiosis mechanisms then do Helicoverpa armigera making this technique unsuitable for measuring cotton plant antibiosis resistance to Helicoverpa spp..
There was a highly significant correlation (r=0.88**) between the data of the two seasons indicating the technique itself is consistant.

Condensed Tannin content of the seedlings at day 5 were difficult to measure. Levels were low with very little significant difference between the cultivars. Tannin levels in the 25 day emmerged plants were higher and while only approximately half of the levels experienced in the field grown squares, were relatively consistant with the squares. This suggests condensed tannin content of cotton seedlings can be indicative of relative tannin levels in the mature plants.

When comparing condensed tannin levels with the M.D.I. (Figure 6), a significant correlation resulted (r=0.60*). These tannins seem to be an important antibiotic agent against the two spotted mite. Generally the higher the tannin content, the lesser the mite damage. There were, however, a few exceptions. Stoneville 213(gl), Sicala, NM838(gl) and HG P-9-13 demonstrated good resistance the mites while possessing low tannin levels. Other factors seem to be also important requiring further investigation in this area.

Results of the field trial are shown in Table 4. At 1 week post infestation, CS8310 had significantly more mites at node 3 than Deltapine 90. This was probably due to to chance differences in infestation levels. At 3 weeks post infestation, CS8310 had significantly fewer mites at the 6th node and in total than the Deltapine 90 plots. At 4 weeks post infestation, CS8310 had significantly fewer mites than Deltapine 90 and a similar trend was emerging for HT35-14-3. Thereafter all three of the test genotypes had fewer mites than Deltapine 90, in descending order, Tam 8-1, HT35-14-3, CS8310.

These results express a similar general pattern of resistance to the two spotted mites between genotypes that the seedling screening technique did suggesting effects in the glasshouse using seedlings can be indicative of what is happening in the field.

SUMMARY

- The most suitable mass screening technique to measure antibiosis resistance to Helicoverpa was a '48 hour feeding on squares using final instar larvae' bioassay. It correlated best with the standard and was more energy efficient.

- The widely reported findings that gossypols are very important to antibiotic resistance to Helicoverpa was supported here with a highly significant negative correlation between larval weight gains and gossypol content of fresh plant material.

- No correlation between condensed tannin content of squares and larval weight gains developed suggesting condensed tannins aren't important to antibiosis resistance to Helicoverpa spp.

- The mite damage index failed to correlate with larval weight gains rendering this efficient mass screening technique unsuitable for measuring cotton plant antibiosis resistance to Helicoverpa spp.

- Mites in the field demonstrated a similar response between genotypes as was shown in the seedling screening technique suggesting the seedling screening technique may be useful in evaluating the potential stability of new genotypes for the developement of two spotted mites.

- Antibiotic resistance was expressed in the field with a significant correlation between percent yield loss under moderate insect pressure and larval weight gains.

- Significant differences were noted between larval weight gains an leaves of some cultivars and larval weight gains on squares (eg. CS8310 was more resistant than Deltapine 16gl in the leaves than squares and Sicala was more resistant than Deltapine 90 in the leaves than squares.

- A significant correlation between the M.D.I. and condensed tannin content of seedlings resulted suggesting condensed tannins are an important antibiotic agent against mites.

CONCLUSION

In these studies we found the cotton plants existing antibiotic resistance mechanisms to be important against Helicoverpa spp. To increase the quantity and quality of these mechanism through a breeding programme would, one could summise, only increase the efficiency of antibiotic resistance hence the whole of H.P.R.

However, it is important to note that gaining an understanding of phytochemical factors is futile without a knowledge of insect feeding behaviour. Although these studies weren't carried out here, it has been observed by Chan et al (1981) in America that young larvae of Helicoverpa spp. over there have developed, to an extent, behavioural resistance to some of these plant allelochemicals. First instar larvae were recorded avoiding the gossypol glands while feeding. At there most susceptable age direct exposure to these toxic chemicals wasn't evident hence mortality, due to these glanded allelochemicals, was low. This avoidance was not recorded in older larvae but, from 2nd instar upwards, larvae demonstrated severly retarded weight gains largely due to gossypols. This leads to a weakened larvae and an extended generation period rendering them more susceptable to environmental factors and natural predators.

Gossypols aren't the whole antibiosis resistance story. The plant - pest interaction is complex and there are many factors involved so, to select for gossypols alone by using the gossypol analysis as a mass screening tool, would prejudice against these other important antibiotic factors. Only a larval bioassay can do this and the most efficient of these trialled here was the '48 hour feeding on squares using final instar larvae' bioassay (Figure 7). The practicallity of adopting such a technique into current cotton breeding practises is good. In the normal workings of a cotton breeding programme, newly developed cotton lines are yield tested in the field and selected upon there performance. A larval bioassay could be incorporated at this stage using squares of the mature plants. The antibiotic potential of the plant could be assessed along with the yield potential. The selection of favoured lines could then involve the consideration of both measurements culminating in a plant with greater antibiotic capabilities and good yielding potential.

Antibiosis isn't a panocia to the insect problems of the cotton industry. A more practical aim is to develope a plant that is less susceptable to its insecty pests depending less on chemicals for protection. To reduce the seasonal sprays by 2 or 3 would be of significant economic and environmental benifit. Developing antibiosis resistance in the cotton plant can only enhance the efficacy of 'host plant resistance' and, along with other biological control agents such mass releasing of parasitic and microbial enemies of Helicoverpa spp., could lead to an industry far less dependant on chemical protection.

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Table ... }sultant data and correlations of the mite and tannin assays.

Cultivar	Mite Damage Index 1987	1988	Tannin % sdl(25days)
Deltapine 90	4.990 a<	4.580 a	2.37 de
HT 35-5-6-13	4.404 b	3.440 b	3.43 abcd
Tam 8-1	4.286 bc	3.473 b	2.93 bcde
Siokra	3.959 cd	3.655 b	3.00 bcde
Deltapine 16(gl)	3.784 de	4.148 a	2.97 bcde
HG DD SN-3	3.540 ef	2.882 c	3.37 abcd
HT 3	3.312 fg	2.739 cd	3.97 ab
Sicala	3.304 fg	2.955 c	2.40 de
Stoneville 213(gl)	3.088 g	3.214 bc	2.13 e
CS 8316	3.039 g	2.808 cd	3.63 abc
HG P-9-13	3.019 g	2.845 cd	2.83 cde
NM 838(gl)	2.950 g	2.845 cd	2.77 cde
CS 8310	2.420 h	2.394 de	4.27 a
HT 35-14-3	2.401 h	2.071 e	4.07 ab
"r values	0.88**	1.00	-0.60*
S.D.	0.275	0.337	0.660

(gl) glandless.
sdl glasshouse reared seedlings.
< means in the same column followed by a common letter don't differ significantly (P<0.05).
" correlations with the 1988 Mite Trial.
* significant at a 5% level
** significant at a 1% level

Table 2(a). The 1986/87 results of all the larval bioassays trialled and correlations with the `Standard'.

Cultivars	+#8D%sq	#8D%le	=24H%sq	=24H%le	^48H%sq	^48H%le	@48H%3sq	@48H%3le	^48Hsdl
Deltapine 16gl	51394 a<	52253 a	153 ab	162 a	195 a	225 a	421 a	500 a	204 a
CS 8310	45844 a	21029 bc	160 a	134 b	190 a	188 bc	406 ab	312 b	198 a
Sicala	32537 b	16915 cd	141 cd	122 cd	174 b	170 c	346 bc	242 c	182 b
CS 8316	33071 b	24323 b	144 bcd	130 bc	173 b	183 bc	364 ab	235 c	202 a
Deltapine 90	31868 b	24366 b	135 de	137 b	170 b	204 b	380 ab	315 b	204 a
Tam 8-1	28217 bc	11761 d	147 bc	127 bc	175 b	178 c	370 ab	260 c	208 a
HT35-14-3	18167 c	11985 d	127 e	114 d	162 b	146 d	283 c	184 d	201 a
"r values	1.00	0.79*	0.86*	0.86*	0.97**	0.83*	0.91**	0.86*	0.23
S.D.	20981.1	12130.5	23.77	24.03	28.15	45.66	137.72	100.56	24.85

Table 2 Results of the 1987/88 larval and chemical assays and their correlations with the `Standard'.

CULTIVAR	+#8D% sq	~Tan% sq	~Gos% sq
Deltapine 16 (gl)	51393.5 a<	10.5 a	0.04 a
CS 8310	45843.6 a	10.2 a	0.53 b
Sicala	32536.8 b	7.8 bc	0.95 cd
CS 8316	33071.2 b	9.1 d	0.91 cd
Deltapine 90	31868.2 b	7.4 b	0.98 c
Tam 8-1	28217.1 bc	8.8 cd	0.89 d
HT 35-14-3	18166.7 c	11.1 a	1.07 e
"r values	1.00	0.13	-0.91**
S.D.	20981.1		

Table 2(c). Results of the 1989 larval and chemical assays and their correlations with the standard.

CULTIVAR	+#8D% sq	^48H% sq	^48H% sdl	~Gos% sq	~Gos% sdl	~Tan% sq
Deltapine 16(gl)	24944.8 a<	211.9 a	205.0 ab	0.05 g	0.01 e	7.60 ab
CS 8310	25097.3 a	205.3 ab	198.6 ab	0.30 f	0.13 cd	7.40 bc
Deltapine 16	22995.7 a	207.7 ab	204.5 ab	0.35 ef	0.16 abc	7.00 bcd
Siokra	17719.7 b	178.2 e	208.3 a	0.53 cd	0.10 d	5.50 g
CS 8316	16986.9 b	191.2 cd	203.2 ab	0.57 bcd	0.14 bcd	6.76 bcd
Tam 8-1	15709.4 bc	187.0 cde	209.8 a	0.59 bc	0.20 ab	6.43 def
Sicot 3	16040.1 bcd	187.3 cde	208.1 a	0.39 e	0.18 abc	6.60 cde
Deltapine 90	14868.3 bcd	197.4 bc	206.2 ab	0.63 b	0.22 a	5.83 efg
Sicala	12709.7 cde	184.9 cde	182.3 c	0.57 bcd	0.15 bcd	5.66 fg
HT 35-14-3	11467.3 de	179.5 de	201.2 ab	0.49 d	0.17 abc	8.40 a
HG P-9-13	9623.1 e	156.9 f	188.4 bc	0.81 a	0.16 abc	5.80 efg
"r values	1.00	0.84**	0.42	-0.84**	-0.57	0.37
S.D.	7606.13	21.50	24.85	0.354	0.035	0.521

< means in the same column not followed by a common letter differ significantely (P<0.05)
+ `Standard' technique
" correlations with the `Standard'
8 day percent weight gain using 2nd instar larvae
^ 48 hour percent weight gain using final instar larvae
@ 48 hour percent weight gain using 3rd instar larvae
= 24 hour percent weight gain using final instar larvae
~ Gossypol and Condensed Tannin % dry matter
sdl - glasshouse reared seedlings (~30 days).
sq - squares from field grown cotton.
* significant at a 5% level.
** significant at a 1% level.

Table 3. sultant lint yield losses in the field under moderate and heavy insect pressure.

Cultivars	Yield (kg/ha)				
	Fullsp	Semisp	%loss	Minsp.	%loss
NM 838 (gl)	1817.65	475.68	73	126.17	93
Stoneville 213 (gl) . .	1960.65	415.62	79	217.69	89
Deltapine 16 (gl). . .	1876.65	757.75	59	278.65	85
M 35-14-3*EGL (gl) . .	1829.30	454.51	74	253.02	85
HT 35-5-6-13	1269.34	496.48	61	250.74	80
Sicala	2103.70	919.87	54	416.23	79
HT 3	1377.05	443.82	66	280.53	78
L*571*1124-4-3 . . .	1505.91	532.37	68	311.66	78
HG DD SN-3	1751.79	616.87	65	395.07	77
Deltapine 90	2142.42	916.80	57	479.89	77
MHR-1	1359.51	669.30	51	320.72	76
TX-LEBOCDS-3-81 . . .	1669.24	695.03	59	434.00	74
CS8316	1064.38	335.10	67	300.45	71
PD695	1839.54	733.82	60	550.00	70
HT 35-14-3	1084.07	571.12	46	338.03	68
HG P-9-13	1675.91	796.02	53	544.84	67
CS8310	1369.38	470.45	65	520.69	62
Siokra	2267.08	918.98	60	908.18	60
TAM 8-1	1093.47	466.35	55	470.20	55
S.D.	126.92	150.74	8.94	72.71	5.07
L.S.D.(5%)	210.18	248.39	14.70	119.99	8.34

Fullsp - lint yields under minimum Heliothis spp. pressure (7 sprays).
Semisp - " " " moderate " " " (2 ").
Minsp - " " " maximum " " " (0 ").
< means in the same column not followed by a common letter differ significantly (P<0.05).

Table 4. Mean number of adult T.urticae per leaf for leaves from both the 3rd and 6th node below the plant terminal.

Cultivar	Date					Season Total
	7/2	14/2	21/2	27/2	7/3	
CS 8310	2.41	2.58	6.02*	10.82*	18.33**	40.16**
HT 35-14-3	2.49	3.35	12.61	20.74	20.82**	61.01**
Tam 8-1	2.29	1.78	9.12	23.17	34.64**	71.00**
Deltapine 90	1.46	3.37	11.40	24.18	50.09	90.50

* significant at a 5% level
** significant at a 1% level

Table Demonstration of Significant Differences between the three Field Trial treatments (full-spray, semi-spray, minimum-spray) within the five fibre quality measurements.

Cultivars	50% span(cm)			2.5% span(cm)			U.R.			Strength (gm)			Strength/Wt.		
	f-s	f-m	s-m	f-s	f-m	s-m	f-s	f-m	s-m	f-s	f-m	s-m	f-s	f-m	s-m
NM 838 (gl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S'ville 213 (gl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deltapine 16(GL)	-	-	-	-	-	-	*	-	*	-	-	-	-	-	-
M 35-14-3*EGL(gl)	-	-	-	-	-	-	-	-	-	-	-	-	**	**	-
HT 35-5-6-13	*	-	-	**	**	-	-	-	-	**	**	-	**	*	-
Sicala	-	-	-	-	-	-	-	-	-	-	-	-	**	*	-
HT 3	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-
L*571*1124-4-3	-	-	-	*	-	-	-	-	*	-	-	-	-	-	-
HG DD SN-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deltapine 90	*	-	-	-	-	-	**	-	**	-	-	-	**	-	**
NHR-1	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-
TX-LEBOCDS-3-81	-	-	-	-	-	-	-	-	-	-	-	-	**	**	-
CS 8316	-	-	-	*	-	-	**	-	**	-	-	-	**	-	**
DP 695	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-
HT 35-14-3	-	-	-	-	-	-	-	-	**	-	-	-	-	-	-
HG P-9-13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CS 8310	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Siokra	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-
Tam 8-1	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-

* significant at a 5% level
** significant at a 1% level
f-s comparison between full-spray and semi-spray
f-m " " full-spray and minimum-spray
s-m " " semi-spray and minimum-spray
U.R. Uniformity Ratio.

Table 6. Levels of Condensed Tannins and Gossypols in squares over a 48 hour period after being picked.

Time	HT 35-14-3		CS8310		Sicala		Deltapine 90	
	Tan%	Gos%	Tan%	Gos%	Tan%	Gos%	Tan%	Gos%
0 hours	8.40 a<	0.50 a	7.40 a	0.30 a	5.67 a	0.57 a	5.83 a	0.63 a
48 hours	10.53 b	0.56 a	9.37 b	0.31 a	8.63 b	0.59 a	6.17 a	0.59 a

< means in the same column not followed by a common letter differ significantly (P<0.05).

Table . . Condensed Tannin content (percent dry matter) of Squares and Terminals throughout the season.

Cultivar	Sampling Stages		
	Early squaring	Mid squaring	Late squaring
		Squares	
Deltapine 90	7.31	7.47	10.00
HT 35-14-3	10.57	10.88	13.05
		Terminals	
Deltapine 90	8.03	8.79	16.35
HT 35-14-3	10.43	12.77	21.02

Table 7 (b). Gossypol content (percent dry matter) of Squares and Terminals throughout the season.

Cultivar	Sampling Stages		
	Early squaring	Mid squaring	Late squaring
		Squares	
Deltapine 90	0.96	0.95	0.99
HT 35-14-3	0.96	1.01	0.98
		Terminals	
Deltapine 90	0.58	0.44	0.49
HT 35-14-3	0.71	0.61	0.62

Appendi List of all larval bioassays trialled.

+*8 day	feeding	on	squares	using	2nd	instar	larvae
8 day	"	"	leaves	"	"	"	"
24 hour	"	"	squares	"	final	"	"
24 hour	"	"	leaves	"	"	"	"
*48 hour	"	"	squares	"	"	"	"
48 hour	"	"	leaves	"	"	"	"
48 hour	"	"	squares	"	3rd	"	"
48 hour	"	"	leaves	"	"	"	"
48 hour	"	"	seedlings	"	final	"	"

\+ a traditional antibiosis resistance measuring technique used here as the 'Standard'.

* techniques repeated for 2nd set of data.

Figure 1. ʼorrelations and Lineal Regressions of all Larval .ioassays trialled with the 'Standard' in 1988 using both squares (a) and leaves (b).
(*) significant at 5% level
(**) significant at 1% level

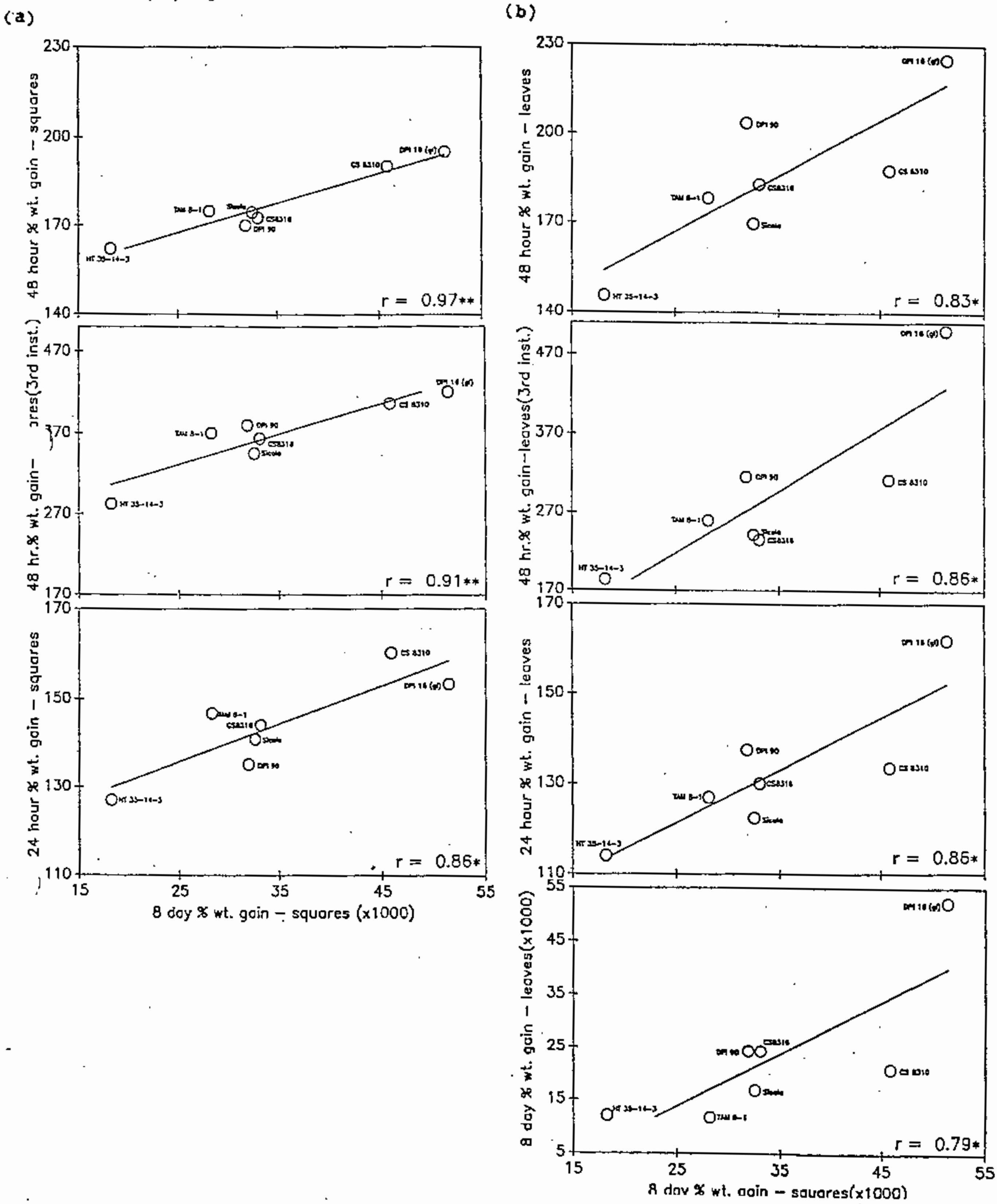


Figure 2. Correlations and Lineal Regressions of Gossypol percent content of Squares with the 'Standard' Larval Bioassay in 1989 (a) and 1988 (b). (**) significant at 1% level

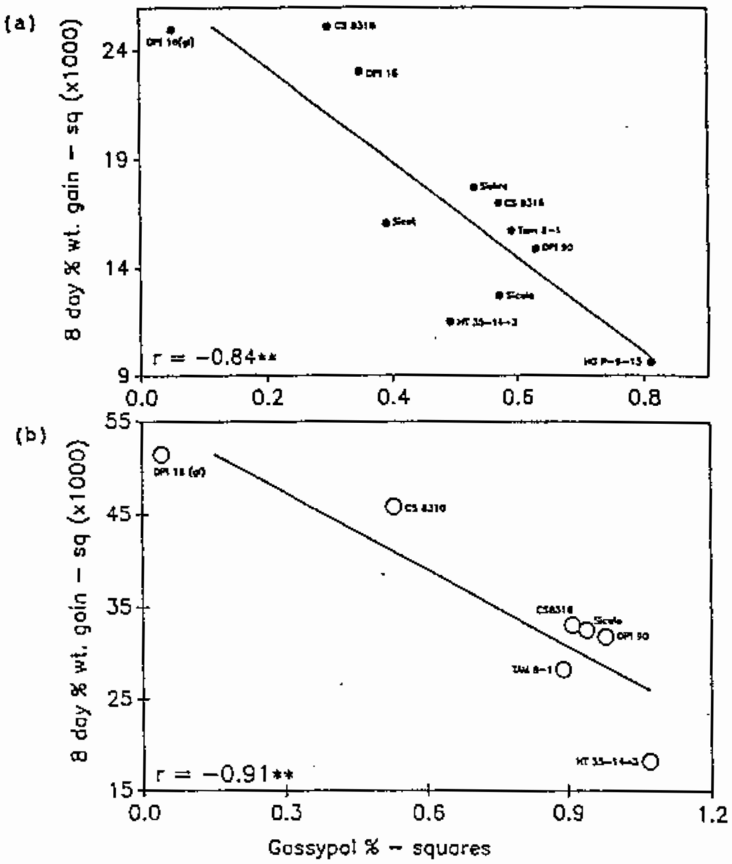


Figure 3. Correlations and Lineal Regressions of Condensed Tannin percent content of Squares used in the 'Standard' Larval Bioassays in both 1989 (a) and 1988 (b).

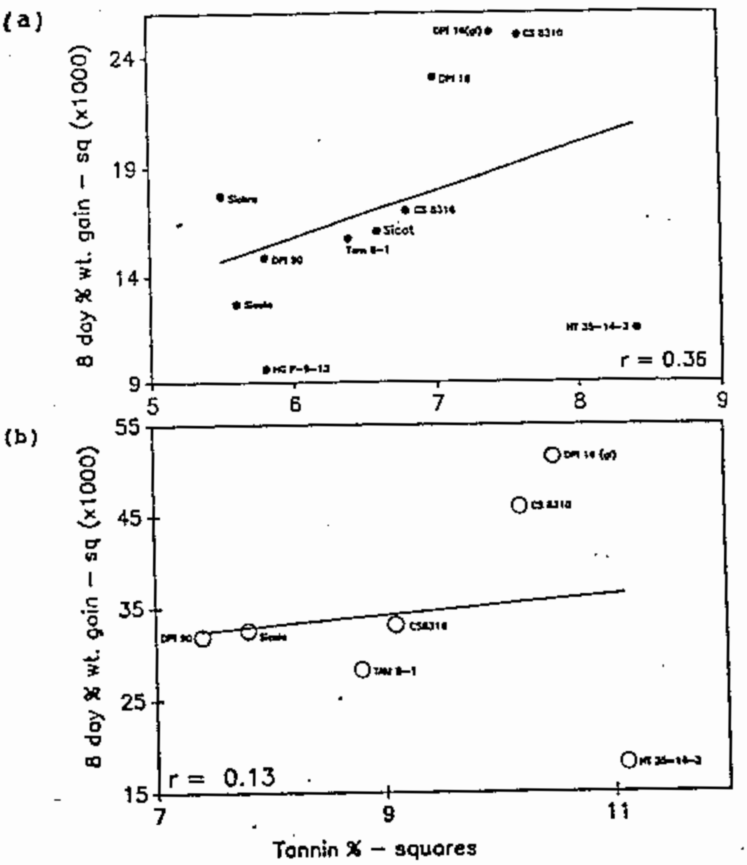


Figure 4. Correlations and Lineal Regressions of Percent Lint Yield Losses in the Field, under different levels of insect pressure, with the 'Standard'. (*) significant at 5% level

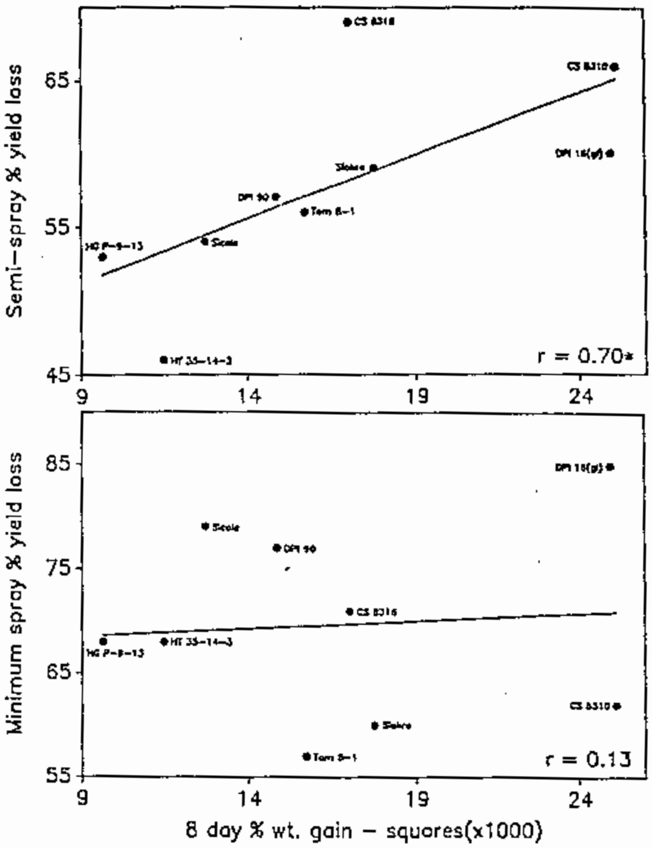


Figure 5. Correlations and Lineal Regressions of the Mite Damage Index with the 'Standard' both in 1989 (a) and 1988 (b).

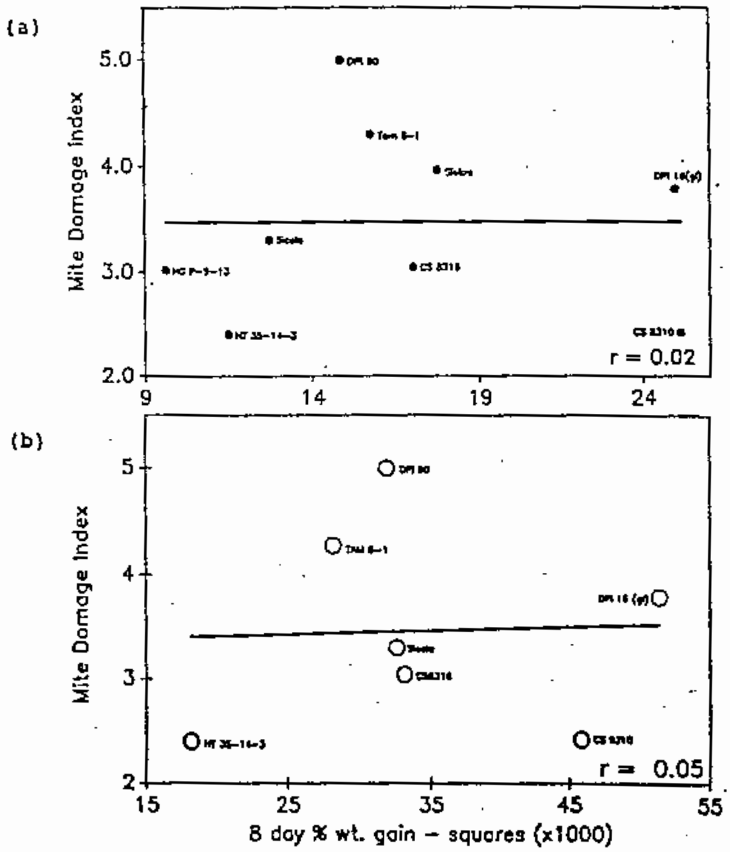


Figure 6. Correlations and Lineal Regressions of Condensed Tannin percent of Seedlings with the Mite Damage Index.
(*) significant at 5% level

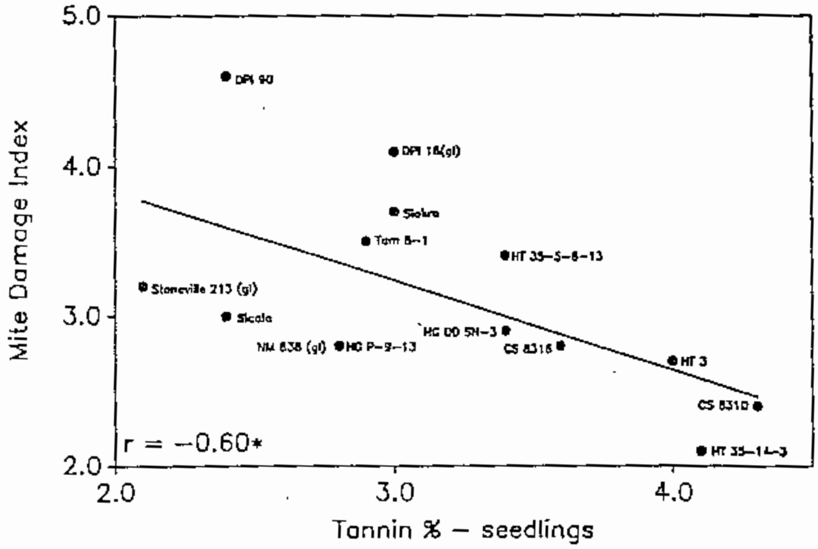
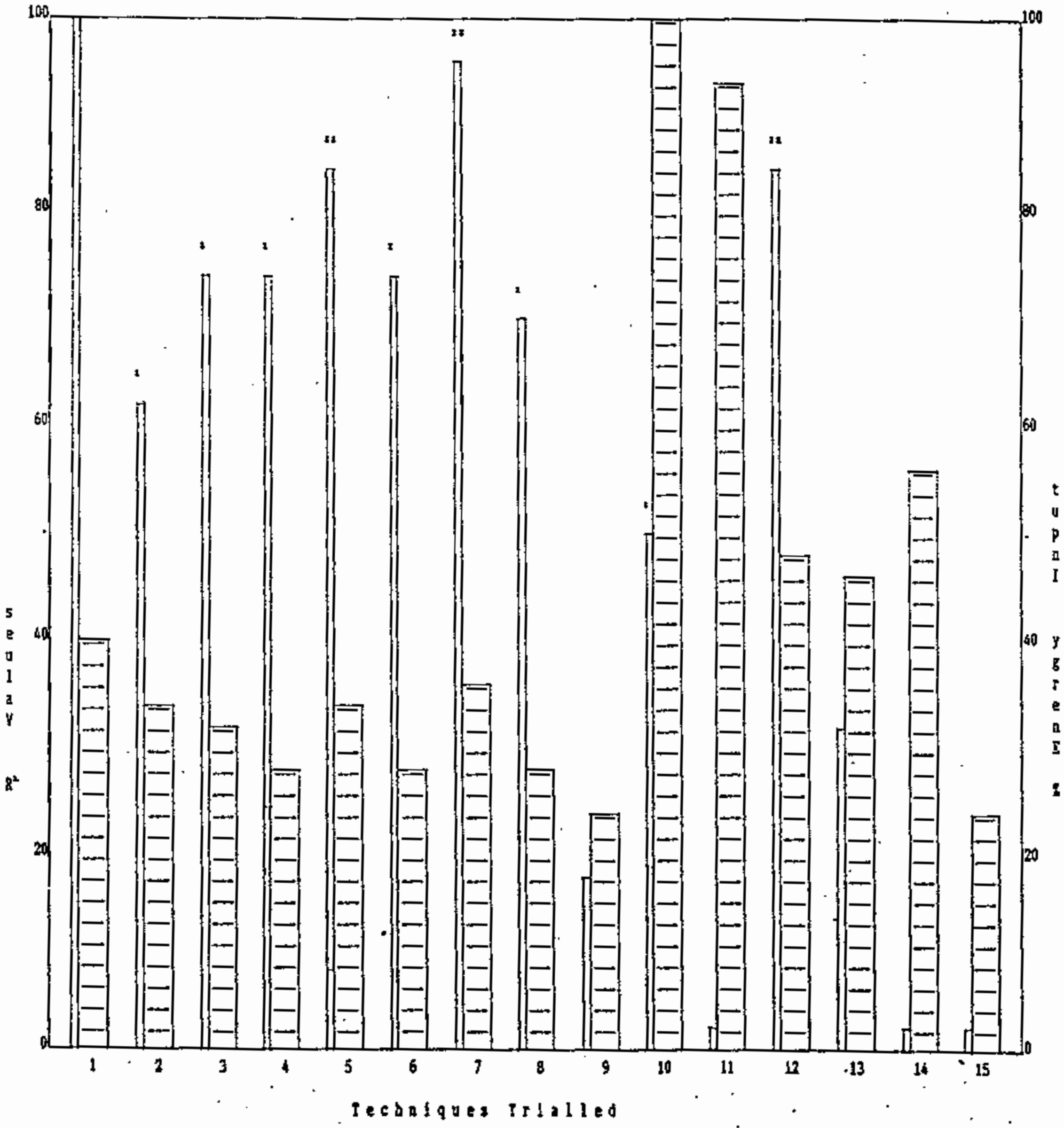


Figure 7. Graphic Summation of the Accuracy and Energy Efficiency of all techniques trialled compared with the 'Standard'.

(*) significant at 5% level
(**) significant at 1% level

Accuracy
Energy Input (Material costs, Labour hours, Length of trial)



1. 8 day feeding on squares using 2nd instar larvae
2. 8 day " " leaves " " " "
3. 24 hour " " squares " final " "
4. 24 hour " " leaves " " " "
5. 48 hour " " squares " 3rd " "
6. 48 hour " " leaves " " " "
7. 48 hour " " squares " final " "
8. 48 hour " " leaves " " " "
9. 48 hour " " seedlings " " " "
10. Lint yield losses under moderate insect pressure
11. Lint yield losses under maximum insect pressure
12. Gossypol % dry matter squares
13. Gossypol % dry matter of seedlings
14. Tannin % dry matter of squares
15. Mite Damage Index on glasshouse seedlings