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# Evaluation for Antibiosis Resistance in Cotton to *Helicoverpa armigera* Larvae

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## ABSTRACT

The antibiotic effect of the new cotton lines, NR1, NR2, NR3, NR4 and NR5 and the control, SR3, on selected developmental parameters of the 2nd instar *Helicoverpa armigera* larvae was investigated. The larvae were fed on four diet types of each variety/line: fresh leaf, fresh square, artificial diet mixed with lyophilized powder of leaf and artificial diet mixed with lyophilized powder of square. RCB design was employed with four replicates. Each replicate had 10 diet cups of each variety/line with one larva. The results showed that the larval and pupal weights and the adult longevity of larvae reared on all diet types from NR1 were significantly lower and the larval period was significantly longer than those from SR3. The larvae produced on 4 diets from the other tested lines were found to have only some parameters significantly differed from the control. Fresh leaves/squares had better antibiotic effects on the bollworm development than the mixed diet with dried parts.

**Key words:** antibiosis, cotton, *Helicoverpa armigera*, bollworm

## INTRODUCTION

Cotton cultivation was abandoned in many areas in Thailand due to the increase of pest damage which severely reduces the yields and fiber qualities. One of the major insect pests of cotton is the american bollworm, *Helicoverpa armigera*. The primary reason for the importance of this insect is the difficulty the cotton growers have in controlling it once an economic level is reached plus no effective insecticides are available. Plant breeders and entomologists have devoted attention to find source of resistance as the control measure for this bollworm in the past several years.

In the cotton plants, small though conspicuous, pigment glands are distributed throughout all portions of the plants. The principal constituent of these glands is gossypol (Lukefahr *et al.*, 1966). Gossypol has been shown to confer some resistance to insects in cotton (Bottger *et al.*, 1964). The glandless cotton was developed by plant breeders and was proved to be susceptible to the insect pest, *Heliothis* spp. It has also been suggested that certain related substances also found in and around the pigment glands with high activity against cotton pest as well. The biochemical variation in plants may differentially affect the biology of insect feeding on them including insect growth and survival. The purpose of the study was, therefore, to determine the effect of several promising cotton lines for source of antibiosis on larval growth and developmental times of *H. armigera*.

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## MATERIALS AND METHODS

### Laboratory test with *H. armigera* larvae

Five cotton lines namely NR1, NR2, NR3, NR4 and NR5 derived from The Cotton Breeding Program of Dr. Ngarmchuen Ratanadilok, Department of Agronomy, Faculty of Agriculture, Kampaengsan Campus, Kasetsart University were tested for antibiosis against the 2nd instar bollworm larvae. SR3, one commercially grown variety was used for comparison in the following experiments. All variety/lines were glanded cotton.

### 1) Feeding with fresh young leaves and squares (flower buds)

Each cotton variety/line was planted in pot kept in the greenhouse. Young leaves and squares of 3-4 cm and 6 mm in diameter, respectively, from each line and the control were placed in 1 oz plastic cup (replicated 10 times per variety/line). They were washed in diluted sodium hypochlorite for 1 min to remove pathogen contamination. Then the leaves/squares were rinsed with tap water, spreaded on paper towels and allowed to dry. Moistened paper towel with distilled water was placed in the bottom of each cup to maintain approximately 70% RH. After that, one 2nd instar bollworm larva was placed in each cup. The cups were kept at room temperature and were arranged in RCB design with 4 replicates. Seven days after feeding, the larval were individually weighed and were kept on feeding with fresh leaves/squares until they pupated and emerged into adult stage. The following data were recorded for each larva: larval weight after feeding on leaves/squares for 7 days, larval period, pupal weight and adult longevity.

### 2) Feeding with lyophilized powders of leaf and square incorporated in artificial diets

Artificial diet used in mixing with lyophilized powder of leaf/square was the modified formula prepared for the bollworm by The Insect Mass Rearing of Insect Pathology Lab, Department of Entomology, Ministry of Agriculture. Leaves/squares, of the same sizes as used in the preceding experiment, from the control and 5 tested lines were collected, washed and lyophilized, and reconstituted in the media using 40 cc of distilled water to 10 g of powder. A small piece of 1 $cm^3$ mixed diet with leaves/squares of each variety/line was placed in a small cup with one 2nd instar bollworm larva. The same procedure and data recording as the preceding test were employed.

## RESULTS

### 1. Feeding with fresh young leaves and squares (flower buds)

Table 1 shows the mean larval and pupal weights, the mean larval period and the mean adult longevity of the 2nd instar *H. armigera* larvae feeding on fresh leaves of SR3 and the tested lines, NR1, NR2, NR3, NR4 and NR5 for 7 days. It was found that both mean weights of the larvae and pupae on the control were significantly greater than those of the larvae reared on all new lines. The mean adult longevity of larvae on the tested lines were significantly shorter than that of the larvae feeding on SR3. Significant difference between NR1 and SR3 was also observed in the larval period.

The mean weights of larva and pupa on fresh squares of the 5 lines were found to be less than those of the control (Table 2). Adults reared on squares of SR3 also lived significantly longer than those on the squares of the tested lines. Both results were similar to those on fresh leaves. As for the larval period of the larvae reared on NR3 and NR5, they were found not to be significantly different from that of the larvae feeding on the control (Table 2).

### 2. Feeding with lyophilized powders of leaf and square incorporated in artificial diets

The effects of mixed diet with dried

leaves of SR3 and the tested lines on the development of bollworm larvae are shown in Table 3. The mean larval weight of the insects reared on NR3 was not significantly different from that on SR3 while only pupal weight of *H. armigera* produced on NR1 was significantly lower than that on the control. No significant difference was found between the adult longevity of the larvae reared on NR1 and NR3 but was significantly different from those of the larvae on the rest tested lines including SR3. Only the larval period of larvae produced on NR1 was significantly shorter than the control.

Table 4 presents the effects of the control and tested lines on the developmental parameters of the 2nd instar *H. armigera* larvae produced on artificial diet mixed with lyophilized square powder. It was found that only mean larval weight of larvae reared on NR3 did not significantly differ from that of larvae on SR3. The pupae of insects feeding on NR1 and NR5 were significantly smaller than those of the control and the tested lines.

With the exception of mean larval period exhibited on NR3, those reared on all tested lines were significantly longer than that on SR3. The mean adult longevities of the bollworm on NR1, NR2 and NR3 were also observed to be significantly shorter than that of NR3 whereas only that of larvae produced on NR1 was significantly shorter than all.

NR1 as made in all diet types was found to give the least development in all parameters to *H. armigera*.

**Table 1** Antibiotic effects of the control and tested cotton lines on development of second instar *Helicoverpa armigera* larvae reared on fresh leaves.

| Var/Line | Means[1/] | | | |
|---|---|---|---|---|
| | Larval wt. (g) | Pupal wt. (g) | Larval period (day) | Adult longevity (day) |
| NR1 | 0.0200 a | 0.1521 a | 25.2500 c | 11.2825 ab |
| NR2 | 0.0215 a | 0.1530 a | 24.3125 bc | 9.8250 a |
| NR3 | 0.0202 a | 0.1525 a | 23.5200 ab | 11.2325 ab |
| NR4 | 0.0340 a | 0.1526 a | 23.9500 ab | 13.8200 c |
| NR5 | 0.0407 ab | 0.1685 ab | 24.4250 bc | 12.4200 bc |
| SR3 (control) | 0.0793 c | 0.2025 c | 21.9325 a | 16.0900 d |

[1/] Means within column not followed by a common letter are significantly different at 0.05% level by DMRT.

**Table 2** Antibiotic effects of the control and tested cotton lines on development of second instar *Helicoverpa armigera* larvae reared on fresh squares.

| Var/Line | Means[1/] | | | |
|---|---|---|---|---|
| | Larval wt. (g) | Pupal wt. (g) | Larval period (day) | Adult longevity (day) |
| NR1 | 0.0440 a | 0.1649 ab | 23.4975 bc | 11.8450 ab |
| NR2 | 0.0759 abc | 0.1461 a | 22.1125 bc | 11.2325 a |
| NR3 | 0.0896 bc | 0.1886 bc | 21.4750 ab | 14.5750 bc |
| NR4 | 0.0734 ab | 0.1924 c | 22.2700 bc | 13.4600 b |
| NR5 | 0.0865 bc | 0.1868 bc | 21.4225 ab | 15.6450 c |
| SR3 (control) | 0.1185 d | 0.2288 d | 19.7850 a | 17.4375 d |

[1/] Means within column not followed by a common letter are significantly different at 0.05% level by DMRT.

**Table 3** Antibiotic effects of the control and tested cotton lines on development of second instar *Helicoverpa armigera* larvae reared on artificial diet mixed with lyophilized powder of leaf.

| Var/Line | Means[1/] | | | |
|---|---|---|---|---|
| | Larval wt. (g) | Pupal wt. (g) | Larval period (day) | Adult longevity (day) |
| NR1 | 0.0466 a | 0.2244 a | 20.225 a | 10.2700 a |
| NR2 | 0.0663 ab | 0.2487 b | 17.2150 b | 14.2575 b |
| NR3 | 0.1249 c | 0.2311 ab | 16.5800 ab | 13.1500 b |
| NR4 | 0.1956 d | 0.2729 c | 14.3600 c | 11.7900 a |
| NR5 | 0.1716 d | 0.2466 b | 14.7300 b | 15.5075 bc |
| SR3 (control) | 0.1381 c | 0.2644 bc | 14.6650 bc | 14.4900 b |

[1/] Means within column not followed by a common letter are significantly different at 0.05% level by DMRT.

**Table 4** Antibiotic effects of the control and tested cotton lines on development of second instar *Helicoverpa armigera* larvae reared on artificial diet mixed with lyophilized powder of square.

| Var/Line | Means[1/] | | | |
|---|---|---|---|---|
| | Larval wt. (g) | Pupal wt. (g) | Larval period (day) | Adult longevity (day) |
| NR1 | 0.1404 a | 0.2433 a | 17.0375 c | 11.2150 a |
| NR2 | 0.1490 a | 0.2694 b | 16.1175 bc | 13.1187 b |
| NR3 | 0.1934 c | 0.2636 b | 14.6550 ab | 13.3975 b |
| NR4 | 0.1694 b | 0.2600 b | 15.4862 bc | 14.8825 bc |
| NR5 | 0.2323 d | 0.2403 a | 15.2075 bc | 16.6450 c |
| SR3 (control) | 0.1977 c | 0.2659 b | 13.3650 a | 17.9025 c |

[1/] Means within column not followed by a common letter are significantly different at 0.05% level by DMRT.

## DISCUSSION

Extensive studies have been made on antibiosis as a source of resistance to *Heliothis* spp. One practical way to identify antibiosis resistance in cotton population was through estimates of the insect development . Because of the poor survival normally exhibited by the first instar larvae, studies on the effects of cotton line on *Helicoverpa* larval development were executed using the 2nd instar larvae previously fed on artificial diet. Comparison of the experimental results revealed substantial differences in most parameters measured from the tested cotton lines, NR1, NR2, NR3, NR4 and NR5 with SR3, the control. However, it was found that only the larval and pupal weights and the adult longevity of the 2nd instar *H.armigera* larvae fed on both fresh leaves/squares and lyophilized leaf/square powders of NR1 incorporated in artificial diets were less and the larval period was longer than those observed in SR3. Larvae produced on some diets from the other tested lines had only some developmental characters better than the control. This should be because there were higher amounts of harmful chemicals in NR1 than in the rest as supported by Lukefahr *et al.* (1966) who indicated the difference in % gossypol in cotton lines to have different effects on tobacco budworm and bollworm.

The fresh young leaves of all variety/ lines were observed to be more toxic to the larvae than the other feeds according to the development data acquired in this study. Also, the lyophilized

powders in artificial diets were found to have less effect to the insects than the fresh ones. The results were actually uncomparable in terms of effects to the insects because young leaves and squares, as for fresh feed as well as for lyophilization, were collected at different times of the day. This was supported by McKay (1974) who stated that the concentration of secondary compounds in most plants varied diurnally. Furthermore, the quantities of the compounds are likely to be altered by climatic and edaphic factors. However, it could be that young leaves had chemical activity greater than the other plant parts since they lack the tough tissues used as the defense against the insects.

Maxwell *et al.* (1965 ) and Lukefahr *et al.* (1966) reported that the glandless cotton was more susceptible to attack by several insect species than their glanded counterparts which should be retained and utilized as a natural protective mechanism against cotton insects. The tested lines also had pigment glands in every part which should be good in insect protection as indicated by those authors. Lukefahr *et al.* (1966) demonstrated that the larval growth of the cotton bollworm, *Heliothis zea* and *Heliothis virescens* was related to the content of gossypol. Shaver and Parrot (1970) showed inhibited growth, larval development period and high mortality of *Heliothis* spp. on high gossypol diet. It was reported by Chan *et al.* (1978) that condense tannins were toxic to the bollworm by laboratory feeding tests, yet, the inheritance of condense tannins in cotton was not documented. Gossypol and tannin showed negatively correlated with weight gains of tobacco budworm larvae fed in the field on plant terminals ( Hedin *et al*., 1983). He also reported the contents of chrysanthemin and gossypol showing negatively correlated with tobacco budworm larval growth in the field while tannins were slightly positively correlated. It was observed that the flower buds from certain wild and primitive cottons showed more insecticidal activity than could be accounted for gossypol. The additional activity was ascribed for "X" factors which were identified as sesquiterpenoid quinone, hemigossypol and heliocides (Gray *et al.*, 1976; Bell and Stipanovic, 1977). In accordance, it appeared that chemical resistance in cotton to the bollworm insect was due to multiple chemicals.

According to the results, it was not known about the real nature of resistance in the tested lines, but some apparently possessed certain level of antibiosis as shown by the reduced growth for bollworm larvae as compared to the control, especially NR1. Yet, which chemicals produced such effects, further analysis must be pursued. Or other resistance mechanisms may be further studied. If the plant has antibiotic characteristics, the probability of finding a resistant factor will be greater if the plant part on which the insect feeds during the early life stage is investigated (Parrot *et al.*, 1978).

## CONCLUSION

Of all lines tested for antibiosis resistance, NR1 was the only one that had all parameters tested, the mean larval and pupal weights, the larval period and the adult longevity, significantly better than those of SR3, the control. Therefore, further investigation on the chemicals responsible for the cause of antibiosis should be pursued.

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