QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES

DIVISION OF PLANT INDUSTRY BULLETIN NO. 790

EXPERIMENTAL PROCEDURES USED IN INSECTICIDE SCREENING TRIALS AGAINST HELIOTHIS SPP. (LEPIDOPTERA: NOCTUIDAE) LARVAE ON TOBACCO IN NORTH QUEENSLAND

By R. H. BROADLEY, B.Agr.Sc.

SUMMARY

The single-spray trial employing the randomized complete block experimental design has been found to be suitable for insecticide screening studies against *Heliothis* spp. larvae on tobacco in north Queensland. The procedures involved in the selection and preparation of the trial sites are given, and an outline of the conduct of the trials is presented. In addition, the advantages of the single spray trial are listed.

I. INTRODUCTION

Tobacco budworms (*Heliothis armigera* (Hübner) and *Heliothis punctigera* Wallengren) are considered to be the most serious insect pests of tobacco in the field in north Queensland (Broadley 1974a).

The chief type of economic damage occurs when larger larvae (figure 1) feed on the developing buds of the tobacco plant (Broadley 1974a, Cunningham 1975). This ingestion damage (figure 2) is magnified by the development of the injured leaves (Girardeau 1968). In severe cases the growing tip may be destroyed (Smith and Saunders 1961), resulting in loss of apical dominance and hence prolific lateral growth of the axillary suckers. This growth is incompatible with one of the primary objectives of tobacco growers—the production of a uniform crop. Sucker removal is expensive and time consuming. In addition any increase in the number of suckers may lead to greater budworm infestation as has been shown with *Heliothis virescens* (F.), which attacks tobacco in north Carolina (Reagan, Rabb and Collins 1974).

Control of the tobacco budworm in north Queensland is based essentially on the use of insecticides, which are applied in conjunction with the recommendations of an insect activity forecasting service (Broadley 1974b). Other techniques such as the prompt destruction of crop remains after harvesting are also utilized (McNee 1967, Currie 1974, Broadley 1974c).

Screening of new insecticides is therefore a necessary aspect of budworm control, especially since the recent occurrence of DDT resistance in *H. armigera* (Twine and Kay 1973, Wilson 1974). The general methodology of screening trials on tobacco has been discussed previously by Smith, Champ and Saunders (1961) and Burnett and Inglis (1971). This report refers specifically to budworm screening trial procedures used in field tobacco in north Queensland.

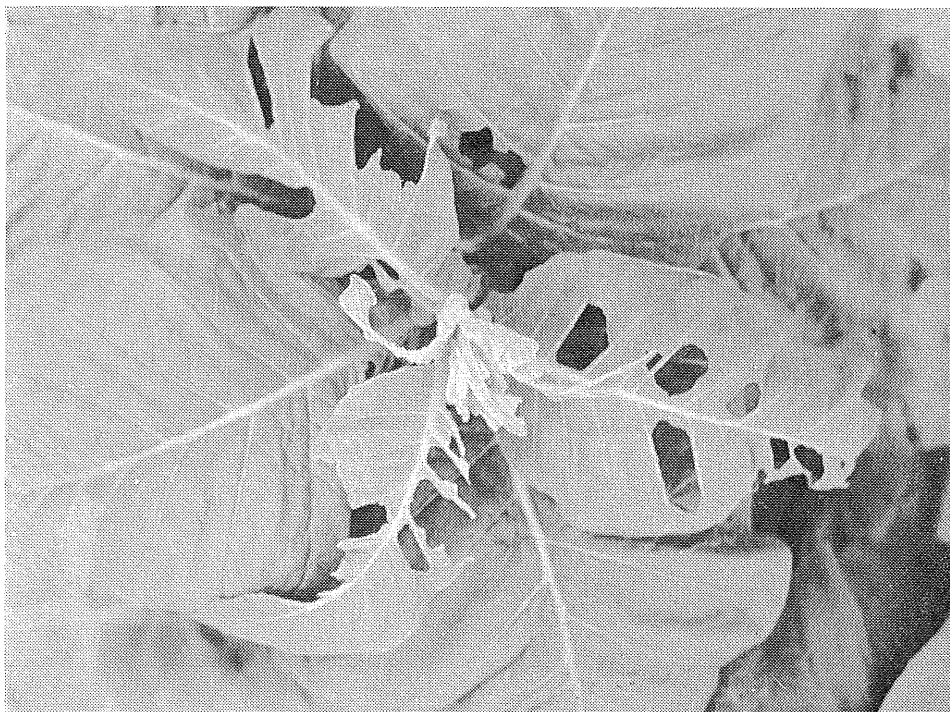Figure 1.—A Heliothis *sp. larva is shown feeding on the upper surface of a tobacco leaf.*

Figure 2.—A young tobacco plant showing characteristic Heliothis *spp. damage at the apex of the plant.*

II. MATERIALS AND METHODS

Experimental procedures are discussed under the following headings: A. growth stage of the crop, B. choice of trial site, C. experimental design, D. methodology, E. insecticide application, F. meteorology and G. General.

A. Growth Stage of the Crop

The growth stage of the crop influences its suitability for insecticide screening purposes. There are two main reasons for this.

1. The members of the genus *Heliothis* have been defined as a group of flower and/or fruit feeders (Hardwick 1965). In fact a flowering tobacco plant normally has many more eggs laid upon it than a vegetative one (Parsons 1940). Consequently larval populations on flowering plants tend to be high and it is common to find 10 or more budworm larvae per inflorescence. Thus large numbers of larvae, necessary for insecticide screening purposes, are readily found on flowering plants in the Mareeba-Dimbulah district of north Queensland.

2. A mature flowering plant generally has a leaf area of $1 \cdot 6 \, \mathrm{m}^2$ to $2 \cdot 4 \, \mathrm{m}^2$ before the start of harvesting. If larvae on the leaves as well as on the inflorescence have to be detected, counting is very time consuming. This problem can be avoided by using the residual part of the crop after harvesting the leaves. These crop residues consist of the stalk, lateral suckers, a small number of leaves and the flowerheads (figure 3). As the flowerheads are the dominant component, and as they remain unsprayed, they are an ideal site for increase in budworm populations.

Figure 3.—Close-up of part of a tobacco inflorescence showing the bell shaped flowers, and developing seed capsules.

B. Choice of Trial Site

All farms have crop residues at the end of a tobacco season (usually November to January depending on climatic conditions). As growers are required by the Tobacco Industry Protection Act of 1965 to destroy these residues (Currie 1974), the time interval in which the trial site can be selected is usually limited. The following criteria have assisted in the choice of trial areas:

1. Residues should be uniformly distributed in the paddock. Sites with uneven plant stands caused by disease, waterlogging and other factors, are unsuitable as they may result in overdispersed budworm distributions (Southwood 1971).

2. Residues over 2 m high, even when uniform are tiring to count. It is difficult to look directly into the uppermost flowers, and this can lead to counting errors. If the flowers are bent to eye level, the stalks may break, especially when they are brittle in the cool conditions of early morning.

3. Older inflorescences with brown, dry seed capsules are not suited for insect development. An ideal site is one in which the inflorescences contain a moderate number of flowers, combined with an excess of immature seed capsules. These capsules have a light-green pigmentation and feel spongy when compressed between the fingertips.

By selecting the trial location on the basis of the physiological state of the inflorescence, there is a good chance that a high population of larvae will be automatically located. Insecticides can then be evaluated under the rigorous conditions of high population pressure.

4. It is important that the block have an adequate soil moisture content. Water stress during the trial period can lead to a shedding of flowers and developing seed capsules containing budworm larvae.

5. At present, there is no practical method for distinguishing between all stages of *H. armigera* and *H. punctigera* larvae in the field, although methods of separating larvae, pupae and adults have been developed (Common 1953, Kirkpatrick 1961, Hardwick 1965). As both species occur simultaneously on tobacco in north Queensland (Broadley 1974a) it is difficult to determine the proportion of each in the experimental area before the start of a trial. Therefore blocks with either *H. armigera* or *H. punctigera* predominating cannot be preferentially selected. However, the experimental situation simulates what could reasonably be expected in the field during the tobacco season i.e. the presence of both species.

The relative incidence of the two species of *Heliothis* can be approximately determined by identification of pupae. For this purpose, at least 100 larvae are required for rearing, so that allowance for the cumulative effects of disease, parasitism and other mortality factors can be made. Care must be taken to ensure that larvae are a representative sample of the population. Collection of large larvae only, for example, must be avoided.

6. The stage of development of the larvae is another trial-site consideration. It is important to ensure that numbers of larvae are maintained in the untreated plots, and the age structure of the larvae is such that an adequate insecticide test can take place. For example, some chemicals are known to control smaller larvae only, and not the penultimate and ultimate instars.

The duration of the larval stage of *Heliothis* spp. varies from 15 to 17 days in mid summer in south Queensland (Kirkpatrick 1962) but is shorter in the hotter conditions in north Queensland. To ensure that larger larvae are included in the test and because a trial usually takes 7 days, a population with a mean age of about 7 days (third and fourth instars) should be chosen. A site in which the majority of larvae are less than 6 days old is normally unacceptable as the young larvae are difficult to find and count accurately on the flowerheads.

A collection of larvae from the flowerheads can be used to determine accurately the age structure of the population at the start of the trial. This must be performed so that all larvae have an equal chance of being included in the sample. In practice, this is accomplished by examining every bud, flower and seedcapsule in a number of inflorescences. At least 50 larvae are required for age distribution studies of the larvae.

7. There are times when mortality agents exert profound effects on the survival of larvae. Nuclear polyhedrosis viruses (Teakle 1973) can decimate large populations of *Heliothis* spp. larvae. A wasp *Microplitis* sp. (Braconidae), which forms a brown pupal case beside the paralysed fourth instar also has a significant effect at times. Locations where mortality agents have caused or could cause catastrophic effects should be avoided where alternatives are available.

C. Experimental Design

The most common experimental design used in budworm screening trials in north Queensland is the randomized complete block design, but this is only one of many which can be used. Details of possible designs, their applications and analysis can be found in a number of text books e.g. Cochran and Cox 1957.

The following preparations of the trial site have been found to be useful, and are applicable to most designs:

1. Suckers and unharvested leaves which hinder counting operations, and show considerable variation from plant to plant, should be stripped from the datum plants (figure 4). This saves time during the assessment of larval numbers, as very few budworms are found on these parts of the plant. It is important to leave at least one healthy sucker per plant, presumably photosynthesizing, to ensure that early death of the residues does not occur.

Removal of leaves and excess suckers is best completed 2 days before the first (pre treatment) count. Plant material should be discarded some distance away from the datum plots, to prevent reinfestation of datum plants.

2. The number of datum plants per plot can be calculated after preliminary counting of several plants in the block has given an estimate of the mean number of larvae per plant. In practice, it has been found that between 8 and 15 plants are required to give an acceptable number of larvae, which is normally more than 80 per plot.

The total number of datum plants to be counted can be used to compute the approximate value of the labour input necessary to complete the trial. A thorough inspection of a tobacco plant and count of larvae present takes 5 to 8 min. Thus a 5×4 randomized block trial with 10 plants per plot will take approximately 1 000 min. (calculated at 5 min. per plant) to count. However, where a large number of flowers is present the labour input may be doubled i.e. it might take 10 min. per plant to count the number of larvae present.
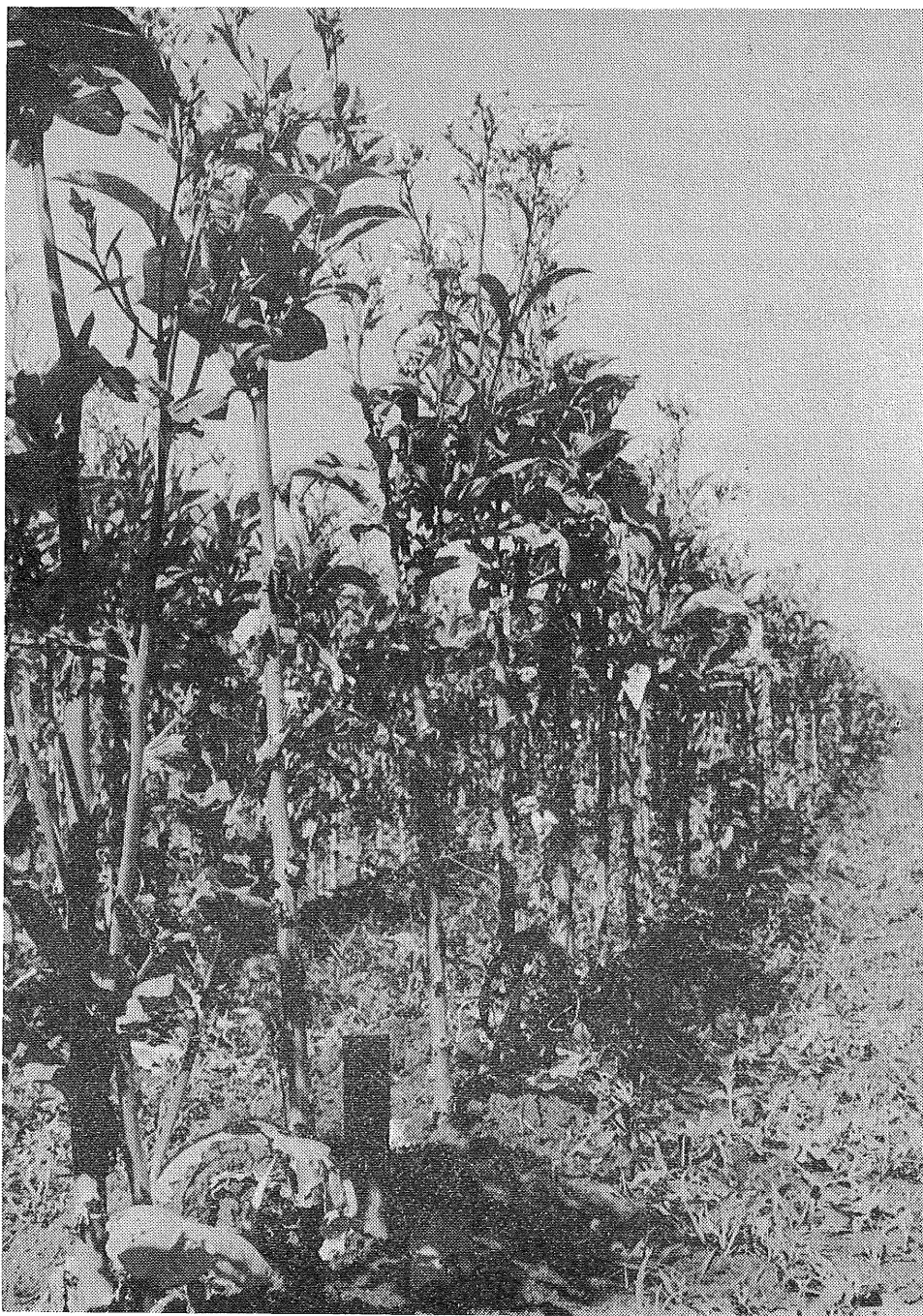
Figure 4.—Plants in the Marked plot (peg is in the foreground) were stripped of excess suckers before counting of larvae commenced.

3. Datum rows should be separated by two or more guard rows to eliminate drift insecticide at treatment application. Similarly, datum plots in the same row should be three to five guard plants apart.

D. Methodology

The methods described here indicate the efficiency of a single spray application to the flowerheads. Typically, there is a single pretreatment count, the insecticide application and three post-treatment counts.

The following factors can influence the outcome of the single spray trial, and therefore require consideration:

1. Effective counting requires a detailed knowledge of the biology of the budworm larvae. The feeding habits of these are such that both small and large larvae can be found completely concealed inside the bud, flower or developing seed capsule. In addition the green forms of the tobacco budworms blend in with the light-green background of the plant tissues, and are well camouflaged.

Young larvae (first, second and third instars) in particular prefer to feed in the sheltered environment provided inside the elongate, bell-shaped flowers. Small larvae are easily detected in the fully opened flowers as their dark-coloured frass and dark head-capsules contrast with the whitish background of the corolla. Occasionally, first instar larvae bore directly into unopened buds leaving only a small, circular puncture in the corolla to indicate their presence. Older, more mature budworm larvae are usually located on the external surfaces, though some bore within the developing seed capsules. In these instances the only indication of their presence is fresh frass around the entrance to the feeding tunnel.

Every flower and seed capsule must therefore be inspected for budworms. With practice an operator can become very skilful in detecting concealed larvae.

2. Certain methods of counting are more prone to error than others. For example in the random approach it may be difficult to remember which flowers have been examined and which have not. The following methodical approach has been found to be effective. The lowest flower stalk is selected, and the larvae are counted by starting with the lowest stalklet and moving towards those at the tip. In this manner all stalks comprising the flowerhead can be dealt with progressively.

3. The timing of the pretreatment count usually cannot be varied because of the brief life-span of the larvae in the field in north Queensland. However, the timing and number of post-treatment counts can be adapted to suit the trial objectives and the insecticides used. For most purposes counts on the first, third and fifth (or seventh) days following treatment application are sufficient. Where highly residual chemicals are being used (on tobacco the chances of this happening are low) or an assessment is required for some other reason, a further count can be made after 14 days.

4. No record is kept of the number of eggs per plant in normal trial work. Where eggs occur on the plants it must be realized that an increase in numbers of larvae may occur during the trial.

5. A record of the effect of insecticides on certain biological mortality agents may also be kept. The number of pupal cocoons of the wasp *Microplitis* sp. (Braconidae) on the datum plants can indicate whether an insecticide has selective insecticidal properties.

E. Insecticide Application

In north Queensland, insecticide treatments are applied by knapsack sprayers. The following points are relevant to spraying technique:

1. Spraying should take place during cool, calm weather only. This will ensure that the insecticides will be deposited in the required manner and little contamination of other plots will occur, provided an adequate number of guard rows and guard plots are being used. Early morning is a suitable time to spray.

2. Thorough coverage with insecticides is essential. Special attention must be directed to placing insecticides inside the flowers, where most of the young larvae, as well as some mature ones, are feeding.

3. A constant spray concentration e.g. 0·01%, 0·05%, active concentrate spray, is normally used during insecticide application.

F. Meteorology

A record of meteorological conditions during the trial is kept for the following reasons.

1. The activity of insecticides can be directly influenced by temperature. For example, methomyl activity against certain insect species is not affected to the same extent as is DDT (Chalfant 1973).

2. Rain can cause heavy mortality of young larvae which drown when the bell shaped flowers fill with water.

G. General

If insecticides are tested under a range of conditions on high budworm populations, and prove to be efficacious, it is likely that they can be used confidently by growers when following the strategic spray applications of the insect-activity forecasting service (Broadley 1974b). In addition the results could be applicable to other tobacco growing districts in Queensland.

III. DISCUSSION

Ideally, field trials should be the natural extension of laboratory toxicity studies under controlled conditions, which would give some indication of the potential of an insecticide. In many instances this is not possible and consequently, field experiments may have to partially replace preliminary laboratory investigations. The type of trial which evaluates a single spray treatment has advantages over one employing a series of sprays applied on a schedule basis e.g. every 6 days. These advantages are:

1. The single spray trial can be interpreted with less ambiguity than the schedule spray trial (Smith 1961, Cunningham 1971). Variables such as percentage incidence of *Heliothis* spp., age structure of the population of the *Heliothis* spp. larvae, and influence of biological mortality factors can be defined in a precise manner. A meteorological record can be used to separate the influences of physical effects from those of insecticide effects.

78866—B

R. H. BROADLEY

2. The single spray trial is not biased towards the more persistent chemicals. Non-residual chemicals (Waite and Passlow 1971) can be thoroughly tested by this method. It must be noted, however, that because of the complex physical structure of the flowerheads, the chances of obtaining 100% mortality even with the most efficacious chemical are quite small. Also their structure can vary from site to site (for example, one block may have a higher proportion of flowers in the inflorescences than another). For this reason when a new insecticide is being evaluated it is better to compare its performance with that of the current standard recommended chemicals in conjunction with percentage mortality, than to talk solely in terms of percentage mortality. It is common to achieve 90% (or higher) control of larvae. An efficacious candidate material which controls high budworm populations in the flowerheads, will also control budworms in the crop before flowering, provided thorough coverage is achieved (Broadley 1974a).

3. If the experimental site has been carefully selected to ensure a *Heliothis* spp. larval population with the correct age structure, it is probable that efficacious insecticides will be active against mature and immature larvae. No attempt to determine the age of individual larvae is attempted during counting because of the difficulties in determining the correct larval stadiums, and in the subsequent analysis of data. Also it would involve handling of larvae and this is not desirable.

4. The single spray application approach can readily be incorporated into the concept of integrated control (Smith and van den Bosch 1967). It does not rely on the cumulative action of a series of repetitive sprays which are not only ecologically disruptive, but usually uneconomical (Stern 1973). Such a spraying schedule might also hasten the advent of insecticide resistance. The recommendation of single applications of non-residual insecticides, which are desired end products of the screening trials outlined in this article should result in minimum disturbance of the biological systems operating in the tobacco crop.

IV. CONCLUSIONS

The single spray trial is a reasonably precise method of evaluating an insecticide. Efficacy data can be interpreted in relation to the species of *Heliothis* present at the trial site, the age of larvae and prevailing meteorological conditions. In addition, effects on biological control agents can also be obtained. More importantly, the single spray trial offers a means of testing insecticides against high populations of budworms on tobacco plants.

REFERENCES

BROADLEY, R. H. (1974a).—Tobacco budworms and their control. *Australian Tobacco Journal, Mareeba* 15:7-10.

BROADLEY, R. H. (1974b).—Insect activity prediction service. *Australian Tobacco Journal, Mareeba* 15:28-30.

BROADLEY, R. H. (1974c).—The importance of destroying unwanted plants. *Australian Tobacco Journal, Mareeba* 15:21-23.

BURNETT, W. M., and INGLIS, I. M. (1971).—Considerations in the development of foliar insecticides for use in Queensland tobacco. Australian Tobacco Research Conference, Albury, 1971.

CHALFANT, R. B. (1973).—Cabbage looper: Effect of temperature on toxicity of insecticides in the laboratory. *Journal of Economic Entomology* 66:339-341.

COCHRAN, W. G., and COX, G. M. (1957).—'Experimental Designs.' 2nd ed. Wiley and Sons, Inc., New York.

COMMON, I. F. B. (1953).—The Australian species of *Heliothis* (Lepidoptera:Noctuidae) and their pest status. *Australian Journal of Zoology* 1:319-344.

CUNNINGHAM, I. C. (1971).—Tobacco insecticide screening trials, 1961-1966. *Queensland Journal of Agricultural and Animal Sciences* 28:13-18.

CUNNINGHAM, I. C. (1975).—Tobacco pests—budworms. *Australian Tobacco Growers' Bulletin* 22:9-12.

CURRIE, N. T. (1974).—The law and tobacco crop residue production. *Australian Tobacco Journal, Mareeba* 15:34-36.

GIRARDEAU, J. H. (1968).—The influence of variety in flue cured tobacco on damage by the tobacco budworm *Heliothis virescens* (F.) (Lepidoptera:Noctuidae). *Journal of the Georgia Entomological Society* 3:51-55.

HARDWICK, D. F. (1965).—The corn earworm complex. *Memoirs of the Entomological Society of Canada* 40:1-247.

KIRKPATRICK, T. H. (1961).—Comparative morphological studies of Heliothis species (Lepidoptera:Noctuidae) in Queensland. *Queensland Journal of Agricultural Science* 18:179-194.

KIRKPATRICK, T. H. (1962).—Notes on the life histories of species of *Heliothis* (Lepidoptera: Noctuidae) from Queensland. *Queensland Journal of Agricultural Science* 19:567-570.

McNEE, D. A. K. (1967).—Tobacco crop residues must be destroyed. *Queensland Agricultural Journal* 93:84-85.

PARSONS, F. S. (1940).—Investigations on the cotton bollworm, *Heliothis armigera* Hubn. Part III. Relations between oviposition and the flowering curves of food plants. *Bulletin of Entomological Research* 31:147-177.

REAGAN, T. E., RABB, R. L., and COLLINS, W. K. (1974).—Tobacco budworm: Influence of early topping and sucker control practices on infestations in flue cured tobacco. *Journal of Economic Entomology* 67:551-552.

SMITH, R. F. M., and VAN DEN BOSCH, R. (1967).—Integrated control. In Kilgore, W. W., and Doutt R. L. (eds.), 'Pest Control. Biological, Physical and Selected Chemical Methods.' Academic Press, New York.

SMITH, W. A. (1961).—Tobacco leaf pest control investigations, 1949-1955. *Queensland Journal of Agricultural Science* 18:33-56.

SMITH, W. A., CHAMP, B. R., and SAUNDERS, G. W. (1961).—Methods of testing insecticides for use in tobacco fields in Queensland. Australian Tobacco Research Conference, Canberra.

SOUTHWOOD, T. R. E. (1971).—'Ecological Methods.' Chapman and Hall, Lond.

STERN, V. M. (1973).—Economic thresholds. *Annual Review of Entomology* 18:259-280.

TEAKLE, R. E. (1973).—A nuclear polyhedrosis virus from *Heliothis punctigera* Wallengren (Lepidoptera:Noctuidae). *Queensland Journal of Agricultural and Animal Sciences* 30:161-177.

TWINE, P. H., and KAY, I. R. (1973).—*Heliothis* species and DDT resistance. *Queensland Agricultural Journal* 99:520-522.

WAITE, G. K., and PASSLOW, T. (1971).—Non-residue producing insecticides. *Queensland Agricultural Journal* 97:409-412.

WILSON, A. G. L. (1974).—Resistance of *Heliothis armigera* to insecticides in the Ord irrigation area, north western Australia. *Journal of Economic Entomology* 67:256-258.

    The author is an officer of Entomology Branch, Queensland Department of Primary Industries, and is stationed at Toowoomba, Q., 4350.