# Penetration, Distribution, and Metabolism of $^{14}$C-Endrin in Resistant and Susceptible Tobacco Budworm Larvae

Sammy G. Polles[2] and S. Bradleigh Vinson*[1]

The rate and nature of penetration, localization, and metabolism of endrin in third instar larvae of susceptible (S) and resistant (R) strains of the tobacco budworm, *Heliothis virescens* (Fabricius), were investigated as possible factors in resistance. The rate of endrin penetration both *in vivo* and *in vitro* was greater in S larvae. Differences were also noted in the lateral migration of topically applied endrin, with movement being more rapid in S cuticles. No differences were found in distribution or storage of endrin within S and R larvae. More endrin was extractable with *n*-hexane from S nerve chords than from R chords, suggesting a tighter binding of endrin to nerve components in the R strain. It is suggested that binding occurs with proteins not involved in the toxic action, decreasing the effective insecticide concentration in the nerve of R insects. Most of the radioactivity excreted by both strains was unchanged endrin.

The mechanisms of endrin intoxication and resistance in insects are virtually unknown. Investigations of these mechanisms are complicated by the deficiency of information of the precise mode of action of the organochlorine family. Accordingly, metabolic differences between resistant and susceptible strains have not been indicated as the major cause of cyclodiene resistance (Brooks, 1960; Gerolt, 1965; Earle, 1963; Perry *et al.*, 1964; Winteringham and Harrison, 1959). Nor have studies on the fate of various cyclodienes in insects with respect to excretion, selective storage, or distribution uncovered any significant differences between resistant and susceptible strains (Perry *et al.*, 1964; Gerolt, 1965; Sun *et al.*, 1967; Ray, 1967).

Other physiological mechanisms may be involved. A decrease in nerve sensitivity was suggested in dieldrin resistant houseflies, *Musca domestica* L. (Schaefer and Sun, 1967), and differences in nerve binding were suggested by Matsumura and Hayashi (1966, 1969). Another factor often suggested as contributing to resistance is a decreased rate of absorption of insecticide (Gast, 1961; Chambers, 1970; Vinson and Brazzel, 1966). Lewis (1962) showed that oil films spread over the entire body of the fly *Protopharmia terraenovae* (Robinson–Desviody) within a very short time of the initial exposure. Quraishi and Poonawalla (1969) demonstrated that after topical application of DDT, the insecticide spread over the entire body. It did not diffuse through the cuticle, but penetrated into the body through membranous areas. Other researchers have suggested that insecticides penetrate the organs by an alternate route *via* the tracheal system (Richards and Weygandt, 1945; Gerolt, 1969).

The object of this study was to determine the fate of endrin in third instar, *Heliothis virescens* (Fabricius), larvae with respect to its mode of entry, absorption, metabolism, and excretion in susceptible (S) and resistant (R) larvae.

Mississippi State University, State College, Mississippi 39762.
[1] Present address: Department of Entomology, Texas A&M University, College Station, Texas 77843.
[2] Present address: Coastal Plain Experiment Station, Box 748, Tifton, Georgia 31974.

## MATERIALS AND METHODS

Laboratory-reared *H. virescens* larvae were used in all experiments. The resistant strain was obtained from a stock State College culture maintained at the Mississippi State University Entomology Research Laboratory. The susceptible strain was obtained from the USDA Laboratory at Florence, S.C. Larvae were reared on an artificial medium described by Brazzel *et al.* (1961) and Berger (1963). Third instar larvae having a weight of 30 to 40 mg were used in this investigation.

The $^{14}$C-endrin used in these studies had a specific activity of 2.62 mCi per mmol. A small amount of one impurity was revealed by thin-layer chromatographic analysis. The impurity was removed by tlc and the purified endrin used in all studies. A stock solution of 5 $\mu$g endrin/$\mu$l containing 2 $\mu$g of $^{14}$C-endrin/$\mu$l in acetone (14,000 cpm/$\mu$l) was used for the topical treatment of the resistant strain. Since the stock solution was too toxic to the susceptible strain, the stock solution was diluted to 2.5 $\mu$g of endrin/$\mu$l (1 $\mu$g of $^{14}$C-endrin/$\mu$l) and 1.25 $\mu$g of endrin/$\mu$l (0.5 $\mu$g of $^{14}$C-endrin/$\mu$l) for topical treatments of susceptible larvae and injected treatments, respectively. Samples of the ketone (SD 2614), aldehyde (SD 7442), and alcohol (SD 12182) metabolites of endrin (Phillips *et al.*, 1962) were obtained from Shell Development Co. These metabolites were used in chromatographic studies as standards for comparison with the various endrin products obtained from treated larvae.

For dosage–mortality studies, the endrin was applied as serial dilutions in acetone. Third instar larvae were treated by injection or topical application with a semiautomatic microapplicator calibrated to deliver 1.4 $\mu$l of endrin solution. Three replicates of ten insects per replicate were made for each dosage.

Injections were accomplished by immobilizing third instar larvae with $CO_2$ and inserting a 27-gauge needle into the first abdominal proleg. Third instar larvae were treated topically on the dorsal surface between the first and second abdominal segments. After treatment, the larvae were returned to the artificial medium in shell vials. Mortality was determined 48 hr posttreatment.

Topical application of $^{14}$C-endrin to larvae for penetration

**Table I. Results of Dosage Mortality Assays with Susceptible and Resistant *Heliothis virescens* Larvae**

| Strain | Treatment | $LD_{50}$ μg/insect |
|---|---|---|
| Susceptible | Topical | 1.25 |
| | Injected | 0.234 |
| Resistant | Topical | >320.00 |
| | Injected | 94.0 |

and distribution studies was similar to that described for dosage–mortality studies, except after treatment the larvae were placed in empty shell vials and held without food until their removal for analysis at 0, 1, 2, 4, 12, 24, and 48 hr. The extraction procedure described by Vinson and Brazzel (1966) was employed. This consisted of an acetone surface wash followed by removal of the internal tissues. The vial wash and feces were combined. The cuticles, internal tissues, and feces were ground and extracted with acetone. The acetone extract was evaporated by a stream of $N_2$ and the residue quantitatively transferred to scintillation vials with hexane.

In order to investigate penetration through isolated integument, third instar larvae were decapitated and the tip of the abdomen was severed. The cuticle was then opened longitudinally on the ventral side. After moving the internal organs and adhering tissues, the cuticle was cleaned with a saline solution. The cleaned cuticle was then placed over a 3 × 2 mm vial containing larval hemolymph treated with phenylthiourea to prevent melanization of the hemolymph (Jones and Wilson, 1959). Next $^{14}$C-endrin (2 μg/μl) was topically applied to the cuticle which was in contact with the hemolymph in the vial. After predetermined intervals, the preparation was rinsed with methanol to remove the nonabsorbed insecticide, and the integument ground with a mortar and pestle and extracted with acetone. The hemolymph and vial were extracted with ethanol. These extracts were evaporated to dryness under a gentle stream of dry air and then quantitatively transferred to scintillation vials with hexane until assayed.

Because of the slow rate of cuticular penetration, it was necessary to inject the radio-labeled insecticide into larvae in order to obtain sufficient internal concentrations of insecticide for localization studies. Injection of endrin for localization studies was similar to that described for dosage–mortality studies. Following injection, the insects were held in empty vials until their removal for analysis at 0, 1, 2, 4, and 24 hr.

Localization of endrin was determined for the head, digestive tract, hemolymph, feces, ventral nerve chord, and remaining tissue. The hemolymph was first removed *via* an amputated proleg with the aid of a small capillary tube. The head was then removed. The digestive tract was removed by slitting the larvae along the dorsal midline and lifting the gut out. The ventral nerve chord was excised leaving the fat body and remaining tissues which were processed together. All tissues were rinsed immediately, following removal in distilled water to remove the body fluids. The distilled water rinse was added to the hemolymph sample. Three replicates of 20 insects were analyzed.

All samples were macerated and quantitatively transferred to 50-ml Erlenmeyer flasks, covered with *n*-hexane, and extracted for 24 hr on a mechanical shaker. This was followed by a second 24-hr extraction with a mixture of chloroform–methanol (3:3 v/v). The extracts were filtered through a sintered glass filter, evaporated under dry air, taken up in hexane, and placed in a scintillation vial for analysis.

The movement of topically applied $^{14}$C-endrin in the integument of tobacco budworm larvae was studied. The treated insects were sacrificed at various intervals to facilitate removal of the longitudinal tracheae and integument.

The integument was placed between two sheets of cellophane and exposed to X-ray film. The X-ray film was placed on both sides of the cellophane-wrapped integument and held at −20° C to reduce shrinkage and to prevent movement of the insecticide. The films were exposed for 2 weeks in cassettes.

The tracheal trunks were rinsed with a saline solution and then placed on microscope slides previously cleaned with a "subbing" solution. The stripping film technique described by Rogers (1967) was employed. The film (Kodak Autoradiographic Stripping Plate AR. 10) was held at −20° C and exposed for 3 weeks. Following exposure, the films were developed and the distribution of the insecticide was noted.

**Analytical Methods.** Quantitative analysis of all extracts was made by liquid scintillation counting (Nuclear-Chicago Instrumentation, Mark I Series) with a 15-ml aliquot of counting solution—a mixture of toluene (2.37 l.), PPO (4 g), and POPOP (50 mg). Radioactivity in the extracts was determined by the channel ratio method after correcting for background.

Cleanup of the extracts for penetration and metabolism studies was similar to that described by Vinson and Brazzel (1966). Following the clean-up procedure, the endrin and metabolites were separated by applying 100-μl aliquots of the extracts to precoated tlc sheets (Eastman Chromatogram 6060). The chromatograms were developed in two systems. System A consisted of 9 parts *n*-hexane and 1 part benzene. System B consisted of 16 parts petroleum ether, 3 parts ethyl ether, and 1 part glacial acetic acid. The chromogenic reagent consisted of 0.025% solution of 2,2-dichlorofluorescein in ethanol. After chromatograms had been developed, radioactivity was determined by scanning the chromatogram with a Varian aerograph Radio Scanner (LB 2722). The chromatograms were then stapled to a sheet of X-ray film and held in cassettes for 2 weeks and developed and fixed. The spots were located on the chromatograms cut out, eluted with acetone into scintillation vials, and the activity of the extracts was quantitated as previously described.

## RESULTS AND DISCUSSION

Dosage–mortality data (Table I) show high levels of resistance to endrin in resistant (R) budworms by both routes of application. Previous studies of insecticide resistance have implicated reduced rates of penetration of insecticide through the cuticle as a mechanism of resistance. The injection studies reported here clearly show that reduced penetration alone cannot account for the observed resistance, although this phenomenon was investigated as a possible contributing factor.

The average recovery of radio-labeled endrin and degradation products based on specific activity of the applied dose was 76% with a range of 60–110%. The results of the penetration and distribution of radioactivity are presented in Table II. In order to obtain enough counts in the internal fraction of topically treated resistant larvae, a high dose of endrin was applied (5 μg of endrin/μl). This dose was found to be toxic to susceptible larvae and was diluted to 2.5 μg of endrin/μl. At 48 hr posttreatment, 72% (3.5 μg) of the endrin applied to R larvae was recovered in the external rinse, while this frac-

Table II. Absorption and Distribution of Radioactivity in *Heliothis virescens* Larvae Following a Topical Application of $^{14}$C-Endrin

| | % Applied dose recovered hours after treatment | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Susceptible, 2.5 μg of endrin/μl | | | | | | | Resistant, 5 μg of endrin/μl | | | | | | |
| Fraction | 0 | 1 | 2 | 4 | 12 | 24 | 48 | 0 | 1 | 2 | 4 | 12 | 24 | 48 |
| External rinse | 92.0 | 85.0 | 77.5 | 74.0 | 66.0 | 47.7 | 35.5 | 97.0 | 96.0 | 94.5 | 92.7 | 85.0 | 80.6 | 72.1 |
| Cuticle | 1.0 | 4.0 | 1.5 | 1.3 | 6.3 | 14.0 | 22.0 | 0.6 | 0.5 | 1.0 | 1.8 | 1.8 | 2.4 | 4.2 |
| Internal | 1.0 | 1.4 | 9.2 | 9.3 | 14.2 | 16.6 | 15.0 | 0.2 | 0.4 | 1.0 | 1.2 | 1.9 | 5.6 | 6.8 |
| Feces and container | 6.0 | 9.6 | 11.8 | 15.4 | 13.5 | 21.7 | 27.5 | 1.3 | 3.1 | 3.6 | 4.4 | 11.2 | 11.3 | 16.6 |

% Recovery averaged 83%.

Table III. $^{14}$C-Endrin (2 μg/μl) Penetration Through Isolated Cuticle of Susceptible and Resistant Larvae of *Heliothis virescens*

| | Recovery (%) $^{14}$C-endrin per insect hours after treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Susceptible strain | | | | | Resistant strain | | | | |
| Fraction | 0.25 | 1 | 2 | 24 | 48 | 0.25 | 1 | 2 | 24 | 48 |
| External | 97.0 | 82.1 | 69.0 | 60.0 | 33.0 | 99.0 | 99.0 | 80.0 | 66.0 | 60.0 |
| Cuticle | 1.5 | 12.0 | 23.0 | 23.0 | 50.0 | 1.0 | 0.5 | 19.5 | 33.0 | 39.0 |
| Hemolymph | 1.5 | 6.0 | 8.0 | 17.0 | 17.0 | 0.0 | 0.5 | 0.5 | 1.0 | 1.0 |

Average % recovery was 88%.

tion from S insects contained only 36% (0.96 μg) of the applied dose.

A comparison of the penetration of an identical dose of $^{14}$C-endrin through the isolated cuticle from R and S larvae showed greater penetration of radioactivity in the S cuticles (Table III). At 24 hr posttreatment, 0.34 μg of endrin (17% of the applied dose) was found in the hemolymph beneath S cuticles, and 0.45 μg (23%) was recovered from the cuticle. Comparable values for R cuticles were 0.02 μg (1%) in the hemolymph and 0.66 μg (33%) in the cuticle. The quantity of insecticide penetrating through the S cuticles within 24 hr exceeded the $LD_{50}$ dosage (*via* injection) for S larvae. Applying the reverse relationship, the anticipated $LD_{50}$ interpolated from Table I for injected endrin to R larvae would be 9400 μg/larvae.

The results obtained *in vivo* were confirmed by *in vitro* studies. However, less endrin was found in the cuticle in the *in vivo* studies than in the *in vitro* studies. The decreased cuticular endrin found in living insects may result from increased partitioning of the insecticide from the cuticle into the hemolymph due to circulation. In both instances, however, the S cuticles contained more insecticide at 48 hr posttreatment than did R cuticles, suggesting that the endrin does not readily penetrate the cuticle of R insects.

It has been suggested that the slower penetration into the R insect is due to the presence of inbred insecticide in the tissue of these insects. This explanation, suggested by Brooks (1960), is subject to considerable doubt. For inbred insecticide to have a measurable effect on endrin absorption, the tissues must necessarily be already saturated with endrin. It is highly unlikely that sufficient endrin would be carried transovarially to saturate the tissues of third instar larvae.

The suggestion by Gerolt (1969) that topically applied insecticides move within the cuticle and gain entrance *via* the trachea was investigated. Endrin applied to S budworm larvae readily migrated from the site of application. Movement in the cuticle of R larvae was much slower. Film exposed to the inner surface of the cuticle of treated S insects was darkened, but film similarly exposed to R cuticles was not. This indicates that the insecticide penetrates into the endocuticle more rapidly in S larvae than in R larvae.

Experiments designed to demonstrate the movement of endrin through the trachea were largely inconclusive, since endrin could not be detected in the trachea by the methods employed. Further tests employing more sophisticated methods and refined detection techniques are needed to provide a final answer to this interesting problem.

Though based on the above results that reduced penetration alone cannot account for the observed resistance, this factor must surely contribute. In the first 2 hr posttreatment, endrin penetration into R larvae is six times less than that into S larvae. This sixfold decrease in the amount of absorbed insecticide would be amplified by an internal resistance mechanism to yield the extremely high levels of resistance to topically applied endrin observed in these insects.

The endrin recovered from the tissue extracts following topical application of the endrin increased steadily in both strains (Table II). The holding vials of the S strain contained 27.5% of the applied dose within 48 hr, while only 16.9% was found in the vial of the R strain. The vial represents both the activity rubbed off the treated larvae which would be expected to be similar in both strains and the excreted dose. These results suggest more rapid excretion in the S strain. These data are misleading, however, since penetration is also more rapid in S insects. If percent elimination is based on the dosage absorbed, rather than the applied dosage, it was found that 67% of the absorbed dose was excreted by R strain while 57% was excreted by susceptible larvae in 24 hr. These results were confirmed since a greater percentage of the injected endrin was also excreted by R larvae (Table IV) where rub-off did not occur.

No differences were noted in the localization of endrin in various tissues following the injection of endrin into the hemocoel of S and R larvae (Table IV). The large amount of endrin recovered from the fraction termed "remaining tissues" was expected since this fraction is largely composed of fat body. Storage of endrin in these tissues, however, seems to be of no importance as a resistance mechanism since S larvae stored as much as did R larvae. Similarly, the affinity of endrin for the nerve chord and the head (*i.e.*, the brain) was similar in both strains. The amount of endrin recovered from the hemolymph decreased during the first 6 hr, but increased in the 24-hr sample in both strains. The initial decrease was anticipated since the injection procedure placed all of the insecticide in the hemolymph. The subsequent increase may be due to a concentration of the

Table IV. Distribution of Injected $^{14}$C-Endrin in *Heliothis virescens* Larvae

| Body parts or extracts | % Applied dose recovered hours after treatment 1 | 4 | 6 | 24 |
|---|---|---|---|---|
| | Susceptible | | | |
| Head | 4.2 | 5.6 | 5.1 | 8.3 |
| Digestive tract | 9.7 | 13.9 | 15.4 | 12.5 |
| Hemolymph | 7.2 | 4.9 | 5.7 | 8.3 |
| Feces | 2.2 | 8.3 | 7.1 | 9.3 |
| Nerve | 1.8 | 1.7 | 1.7 | 4.9 |
| Remaining tissues | 74.9 | 65.6 | 65.0 | 56.7 |
| | Resistant | | | |
| Head | 1.5 | 3.2 | 5.0 | 10.0 |
| Digestive tract | 3.0 | 20.5 | 19.5 | 10.0 |
| Hemolymph | 13.0 | 11.0 | 5.0 | 13.0 |
| Feces | 3.3 | 4.7 | 10.0 | 14.3 |
| Nerve | 1.9 | 1.6 | 1.5 | 4.1 |
| Remaining tissues | 77.3 | 59.0 | 59.0 | 49.0 |

Table V. The Quantity of $^{14}$C-Endrin in S and R Larvae of *Heliothis virescens* Central Nervous Systems Extracted with *n*-Hexane and Chloroform–Methanol

| Extraction solvent | % $^{14}$C-endrin recovered at postinjection[a] Susceptible 1[b] | 6 | 24 | Resistant 1[b] | 6 | 24 |
|---|---|---|---|---|---|---|
| *n*-Hexane | 94.0 | 88.0 | 62.0 | 84.0 | 66.0 | 30.0 |
| $CHCl_3CH_3OH$ | 6.0 | 12.0 | 38.0 | 16.0 | 34.0 | 70.0 |

[a] Percentage represents total amount in nerve cord. [b] Hours.

insecticide resulting from the observed loss of blood volume in later hours, leading to more efficient recovery. The importance and cause of the loss of blood volume is unknown.

As shown in Table V, there is no appreciable difference in the degree to which endrin is accumulated in the nervous system. In order to investigate possible qualitative differences between S and R insects, the relative affinity of the insecticide for components of the nerve tissue was analyzed by differential extraction. The removed nerve chords were first homogenized in *n*-hexane, which was radioassayed and then reextracted with a mixture containing equal parts of chloroform and methanol (CM) which was radioassayed. In both strains the percentage of hexane-extractable endrin decreased with time; the CM-extractable fraction increased correspondingly (Table VI). It seems likely that the hexane fraction represents the insecticide which is not tightly bound to nerve components. The CM extraction caused considerable coagulation of the nerve proteins and probably disrupted any existing endrin-containing complexes.

Assuming that the CM-extractable endrin truly represents the bound insecticide, binding is greater in R insects than in the S strain. Though very little is known about insecticide binding to nerve components, there is considerable evidence that organochlorine insecticides form protein complexes. If this phenomenon is of significance in resistance, it can be concluded from the data presented here that endrin complexes with proteins not involved in the toxic action in R nerves. The binding of endrin to proteins may result in decreasing the concentration of insecticide available to exert its toxic effect.

The results of the metabolism study suggest that two metabolites of endrin are produced in both S and R larvae and an additional two metabolites are produced only by R insects.

An attempt was made to identify the metabolites by comparing their chromatographic mobilities to known compounds. The metabolites which were common to both R and S strains (metabolites I and II, Table VI) were tentatively identified as endrin–aldehyde and endrin–ketone. There was no evidence of formation of endrin–alcohol by either strain. The metabolites which were peculiar to the R larvae (metabolites III and IV) remain unidentified. $R_f$ values for standards and metabolites are given in Table VI.

Table VI. $R_f$ Value of Endrin and Various Metabolites in Susceptible and Resistant *Heliothis virescens* Larvae, Using Two Solvent Systems

| Compound | $R_f$ System (A) | System (B) |
|---|---|---|
| Endrin | 0.51 | 0.85 |
| Endrin–alcohol | 0.00 | 0.35 |
| Endrin–aldehyde | 0.00 | 0.54 |
| Endrin–ketone | 0.00 | 0.59 |
| Metabolite I[a] | 0.00 | 0.53 |
| Metabolite II[a] | 0.00 | 0.59 |
| Metabolite III[b] | 0.74[c] | 0.90 |
| Metabolite IV[b] | | 0.95 |

[a] Recovered from both S and R larvae. [b] Recovered from R larvae only. [c] This spot probably includes both metabolites III and IV.

These data show a qualitative difference between the metabolic degradation in S and R insects. Due to the small quantities of metabolites produced, it was not possible to quantitatively measure differences in the rates of metabolism and the importance of detoxication, as a resistance mechanism remains unknown. The very low degree of metabolism in both strains, however, suggests that this mechanism is probably of little significance.

ACKNOWLEDGMENT

Appreciation is expressed to H. M. Taft, USDA Laboratory, Florence, S.C., for supplying the susceptible strain of tobacco budworms. Thanks is also expressed to Shell Development Co., Modesto, Calif., for supplying the labeled endrin and endrin metabolites.

LITERATURE CITED

Berger, R. S., *U. S. Dept. Agr. Ser.* **33,** 84 (1963).
Brazzel, J. R., Chambers, H., Hammond, P. J., *J. Econ. Entomol.* **54,** 949 (1961).
Brooks, G. T., *Nature (London)* **186,** 96 (1960).
Chambers, H., Mississippi State University, State College, Miss., personal communication, 1970.
Earle, N. W., J. AGR. FOOD CHEM. **11,** 281 (1963).
Gast, R. T., *J. Econ. Entomol.* **54,** 1203 (1961).
Gerolt, P., *J. Econ. Entomol.* **58,** 849 (1965).
Gerolt, P., *J. Insect. Physiol.* **15,** 563 (1969).
Jones, B. M., Wilson, R. S., *Biol. Bull.* **117,** 482 (1959).
Lewis, C. T., *Nature (London)* **193,** 904 (1962).
Matsumura, F., Hayashi, M., *Science* **153,** 757 (1966).
Matsumura, F., Hayashi, M., J. AGR. FOOD CHEM. **17,** 231 (1969).
Perry, A. S., Pearce, G. W., Buckner, A. J., *J. Econ. Entomol.* **57,** 867 (1964).
Phillips, D. D., Pollard, G. E., Soloway, S. B., J. AGR. FOOD CHEM. **10,** 217 (1962).
Quraishi, M. S., Poonawalla, Z. T., *J. Econ. Entomol.* **62,** 988 (1969).
Ray, J. W., *Biochem. Pharmacol.* **16,** 99 (1967).
Richards, A. G., Weygandt, J. L., *J. N. Y. Entomol. Soc.* **53,** 153 (1945).
Rogers, A. W., Elsevier Publishing Company, New York, N. Y., 1967, p 335.
Schaefer, C. H., Sun, Y. P., *J. Econ. Entomol.* **60,** 1580 (1967).
Sun, Y. P., Schaefer, C. H., Johnson, E. R., *J. Econ. Entomol.* **60,** 1033 (1967).
Vinson, S. B., Brazzel, J. R., *J. Econ. Entomol.* **59,** 600 (1966).
Winteringham, F. P. W., Harrison, A., *Nature (London)* **184,** 608 (1959).

*Received for review November 9, 1970. Accepted July 30, 1971. Publication No. 2030, Mississippi Agricultural Experiment Station, in cooperation with the ARS, USDA, Boll Weevil Research Laboratory.*