STUDIES ON THE DEVELOPMENT OF INSECT RESISTANT SORGHUM VARIETIES AND HYBRIDS

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STUDIES ON THE DEVELOPMENT OF INSECT RESISTANT

SORGHUM VARIETIES AND HYBRIDS

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PREFACE

I have been interested in insect resistance in crop plants since I worked on my Master of Science thesis, "Evaluation of barley varieties for resistance to the corn leaf aphid, Rhopalosiphum maidis (Fitch)," during 1961-62. Early in 1964, Dr. Harvey L. Chada, Professor of Entomology at Oklahoma State University and Investigations Leader, Entomology Research Division, United States Department of Agriculture, had called to my attention the need for studies on the development of insect resistance in sorghums at Oklahoma State University.

I am deeply indebted to my major adviser, Dr. Harvey L. Chada, for making this study possible, for his competent instruction and guidance, generous encouragement, helpful suggestions and assistance in the preparation of this manuscript.

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INTRODUCTION

Because of recent publicity on the dangers of toxic residues resulting from treatment of crop plants with insecticides, emphasis is now being placed on development of insect controls by means other than the use of chemicals. These involve cultural operations, biological control through use of parasites and predators, use of sex attractants and chemosterilants, light traps, and the breeding of crop plants having resistance to insects. The latter probably is one of the most important, because use of resistant varieties usually precludes use of other control methods, is relatively inexpensive to utilize, and usually is permanent (Painter 1951). Dr. E. F. Knipling (1966) stated that:

> The development of crop varieties that resist insect attack is one of the oldest, most desirable, and most economical ways to meet insect problems. Unfortunately, relatively little research work has been directed toward this approach to insect control.

Grain sorghums, *Sorghum bicolor* (Linn.) Moench, are frequently heavily infested with a number of insects, and damage resulting can be severe. Because of the low value per acre of sorghums, danger of toxic residues on grain and forage following use of insecticides, and impracticability of use of other control methods, the development of sorghums with resistance to insects would appear to be the most practical approach to control.

The corn earworm, *Heliothis zea* (Boddie), fall armyworm, *Spodoptera* frugiperda (J. E. Smith), and the southwestern corn borer, Zeadiatraea grandiosella (Dyar) are species that cause considerable damage to grain sorghums. In order to test sorghums for resistance to these insects, it is necessary to have available a good supply of eggs or larvae of the insects for manually infesting the test plants. Natural infestation cannot be relied on for this purpose. Consequently, it was necessary to develop techniques for rearing these insects in mass numbers in order to have them available when needed. It was also necessary to determine the optimum stage of development of the test plants for infestation by the several insects concerned before satisfactory resistance rating could be made. This investigation was undertaken to achieve the above objectives and to add to the knowledge in this regard developed previously by Bailey (1964).

REVIEW OF LITERATURE

With the need to maintain lepidopterous insects in culture for research in both the greenhouse and field for biological control and toxicological studies, a trend has been established in developing an artificial food substratum which allows culturing of insects without dependency on their natural host plants. An artificial diet is advantageous over the plant material diet in that it provides a constant food supply at all times of the year. Also, facilities and labor necessary to manufacture the artificial diet are not usually of the magnitude required for maintenance of plants in greenhouse cultures.

Rearing Lepidopterous Larvae on Artificial Diets

A very good review of the literature on the subject of rearing insects on artificial diets was made by Bailey (1964). Literature reviewed here has more particular reference to the present studies.

George et al. (1960) developed an artificial diet for rearing the European corn borer, *Ostrinia nubilalis* (Hubner), following early work reported by Bottger (1942) and Beck et al. (1949). George et al. (1960) found that an artificial diet which was satisfactory for the European corn borer was also adaptable for rearing other lepidopterous larvae, including the corn earworm and fall armyworm. Newly hatched corn earworm larvae did not survive on this diet, but when fed corn silks until after the first molt, growth was nearly normal. They also stated that late instars of fall armyworm larvae completed the life cycle on the diet mentioned.

Vanderzant et al. (1962) was the first to rear the corn earworm through its complete larval cycle without depending on natural food. Their diet was modified from that developed by Adkisson et al. (1960) for the pink bollworm, Pectinophora gossypiella (Saunders). Berger (1963) reared 18 consecutive generations of cotton bollworm on a laboratory-prepared artificial diet developed from Vanderzant et al. (1962). Bailey (1964) reared corn earworm 14 consecutive generations in the laboratory on an artificial diet developed from Vanderzant et al. (1962).

The first report of artificial rearing of fall armyworm was reported by Bailey (1964) when he reared this insect continuously in the laboratory during 1963-64.

The southwestern corn borer was reared 12 consecutive generations in the laboratory on wheat germ diet (Bailey 1964). Keaster and Harrendorf (1965) described a technique for rearing 18 successive generations of the southwestern corn borer on an artificial diet developed from Vanderzant et al. (1962) and Berger (1963).

Mass Rearing

One of the important objectives of this study was to develop a mass rearing technique. Vanderzant and Ivy (1956) mass reared the pink bollworm by using sprouted peas and beans as food. Pan and Long (1961) mass reared the sugarcane borer, Diatraea saccharalis (Fabricius), on an artificial diet called the "standard diet". Ignoffo (1963) described a successful technique for rearing cabbage loopers on a semi-synthetic diet. Richmond and Ignoffo (1964) used three types of containers; 8-oz glass jars, plastic-coated paper cups, and 9- and 6-oz wax-coated paper cups for mass rearing of the pink bollworm on a semi-synthetic diet. Bailey (1964) studied mass rearing of the corn earworm and fall armyworm on a wheat germ diet. Lingren (1964) also described the mass rearing technique of the cotton bollworm and tobacco budworm on artificial diet developed by Adkisson et al. (1960) and modified by Berger (1963).

Shorey and Hale (1965) made a significant study on mass rearing of the larvae of nine noctuid species on a simple artificial diet which was based on a minimum expenditure of time and money. Mendoza and Peters (1963) described the mass rearing of the southern corn rootworms, *Diabrotica undecimpunctata howardi* Barber.

Resistance Studies

Sorghum plants were attacked by a number of insects including the corn earworm, fall armyworm,and southwestern corn borer Walton and Bieberdorf (1948), Luginbill (1950), Painter (1951), Howell (1953), Arbuthnot (1953), Rolston (1955), Quinby et al. (1958) and Buckley and Burkhardt (1962). These insects became serious pests and damaged sorghums in Oklahoma and other states for some years. Resistance of sorghum plants to insect attack has been described by Snelling et al. (1937), Snelling and Dahms (1940), Dahms and Martin (1940), Dahms (1943), and Painter (1951).

There is little information in the literature regarding the resistance of sorghum to lepidopterous insects. Dicke et al. (1963) evaluated the resistance of sorghum varieties and hybrids to the European corn borer during a period of three years. They and Guthrie et al. (1960) used the same criteria for determining the degree of infestation developed for evaluating plant injury by the European corn borer in corn. This involved rating the plants for relative amount of leaf feeding, midrib, sheath, and collar lesion counts, and stalk burrows.

Due to lack of information in the literature on evaluation of resistance of sorghum varieties to lepidopterous insects, techniques developed here for this study were based on those used for insects attacking other small grains such as wheat, oats, and barley.

Several workers have screened a large number of small grains to find germ plasm with resistance to insects. Dahms et al. (1955) tested several hundred varieties and hybrids of small grains in searching for resistance to the greenbug. Painter and Peters (1956) reported that 2000 wheat strains tested were more susceptible to greenbug, *Schizaphis graminum* (Rondani) than Pawnee, but about 4 percent carried some resistance. A single factor difference for resistance was indicated. Wood (1961) screened 4600 wheat lines and found 19 varieties which showed a high degree of resistance to the greenbug.

Chada et al. (1961) screened a large number of barley varieties for greenbug resistance. Among 1,230 winter and intermediate winter barleys, 76 were found with significant resistance. Among 4,445 spring-type barleys of the 6,174 varieties in the USDA World collection, they reported 36 with resistance equal to or superior to that of Omugi. They also reported 74 oat varieties from the USDA World collection with resistance of significance. Chada et al. (1961) screened the World collection of 10,010, 5,105, and 18,860 varieties of barley, oats and wheat, respectively, for resistance to the greenbug. They found several lines with a high degree of resistance. Hormchong and Wood (1963) evaluated 121 barley varieties for resistance to the corn leaf aphid, *Rhophalosiphum maidis* (Fitch), and found 7 varieties with a high degree of resistance.

Neiswander (1948) described a quick way of evaluating differences in susceptibility or resistance of corn strains to European corn borer by using the rating system 1-5. Guthrie et al. (1960) used a nine-class rating scale (0-9) to evaluate leaf feeding by the European corn borer. Wiseman et al. (1966) detected corn seedling differences in the greenhouse by visual classification of damage by the fall armyworm using a rating system 0-10.

Many references on the complex of insect resistance in crop plants are presented by these outstanding authors: Painter (1941, 1951, 1954, 1958, 1960, 1966), Snelling (1941a, 1941b), Dicke (1954), and Beck (1965).

MATERIALS AND METHODS

The following lepidopterous insect species were used in this study: (1) corn earworm, H. zea (Boddie), (2) fall armyworm, S. frugiperda (J. E. Smith), and (3) southwestern corn borer, Z. grandiosella (Dyar).

Corn earworm

Source of insects - One hundred and twenty corn earworm pupae were obtained from Dr. Erma S. Vanderzant, Texas A & M University, College Station, Texas, on January 20, 1965. This culture was reared continuously in the laboratory at Stillwater, Oklahoma for 14 generations. It was then discarded because the female moths lost their vigor. Eggs and larvae from this culture were used for manually infesting sorghum plants in the field in connection with resistance studies. From September 1966 to the present time the culture was developed from moths caught in a light trap at Stillwater, Oklahoma.

Rearing technique - Artificial rearing techniques for H. zea have been described by several workers: George et al. (1960), Vanderzant et al. (1962), Berger (1963), and Bailey (1964).

The rearing work was conducted in the Entomology controlled environment insectary on the Oklahoma State University campus, as described by Bailey (1964). The walls, wooden shelves, and tables in the insectary were painted white, and the laboratory was kept clean at all times.

One gallon food cartons with the lid removed were used as mating cages. The sides of the cartons were lined with wax paper. The bottoms of the cartons were covered with sterilized paper toweling. The open end was covered with nylon cloth. After sexing, ten pairs of newly emerged moths were placed in each carton. A small piece of sterilized cotton approximately 1 inch square was placed on the nylon screen cloth covering the open end of the carton, and it was wet with 10 percent honey solution for feeding purposes. To prevent mold contamination and rapid evaporation of the honey solution, the bottom side of a petri dish was placed over the cotton. Diffused light was provided during the night by placing a 60-watt bulb desk lamp under a table, as reported by Bailey (1964).

Oviposition - Female moths deposit their eggs singly. For making manual infestations in the field, 1/3 x 2" paper strips were laid on top of the nylon screen cloth, and eggs were deposited on them (Fig. 1, 2). Eggs on each strip were counted. One or more strips bearing the desired number of eggs were placed into the sorghum plant whorl in making the infestation.

Fall armyworm

Source of insect - The original culture of the fall armyworm was developed from approximately 2500 eggs received from the Entomology Research Division, Southern Grain Insects Research Laboratory, Coastal Plain Experiment Station, Tifton, Georgia on January 15, 1965. These eggs were deposited on January 12, and nearly all hatched on January 17 after being received at Stillwater. The insects were reared very successfully for 12 generations, but because of failure of the thermostat in the 80-degree growth chamber, a temperature of 120° F killed all of the pupae in the 12th generation. A new culture was then started from another shipment of 2000 eggs received from the same

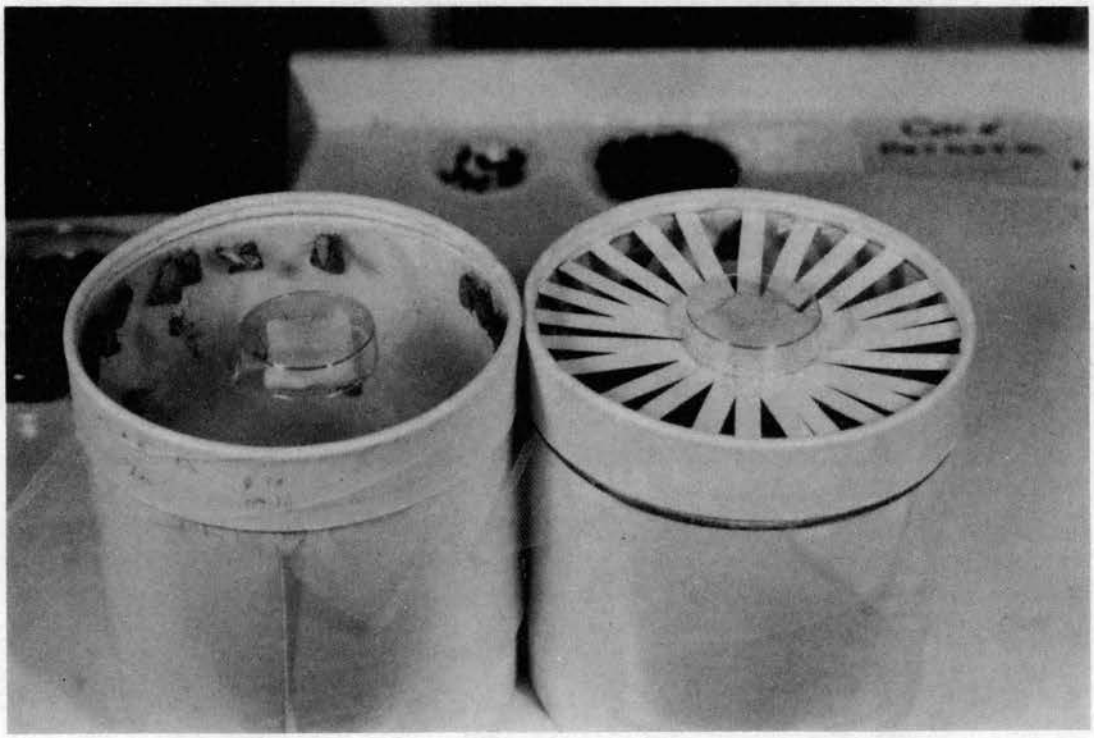

Fig. 1. Corn earworm moth oviposition chambers. L - moths in chamber. R - paper strips on nylon cloth top for oviposition site.

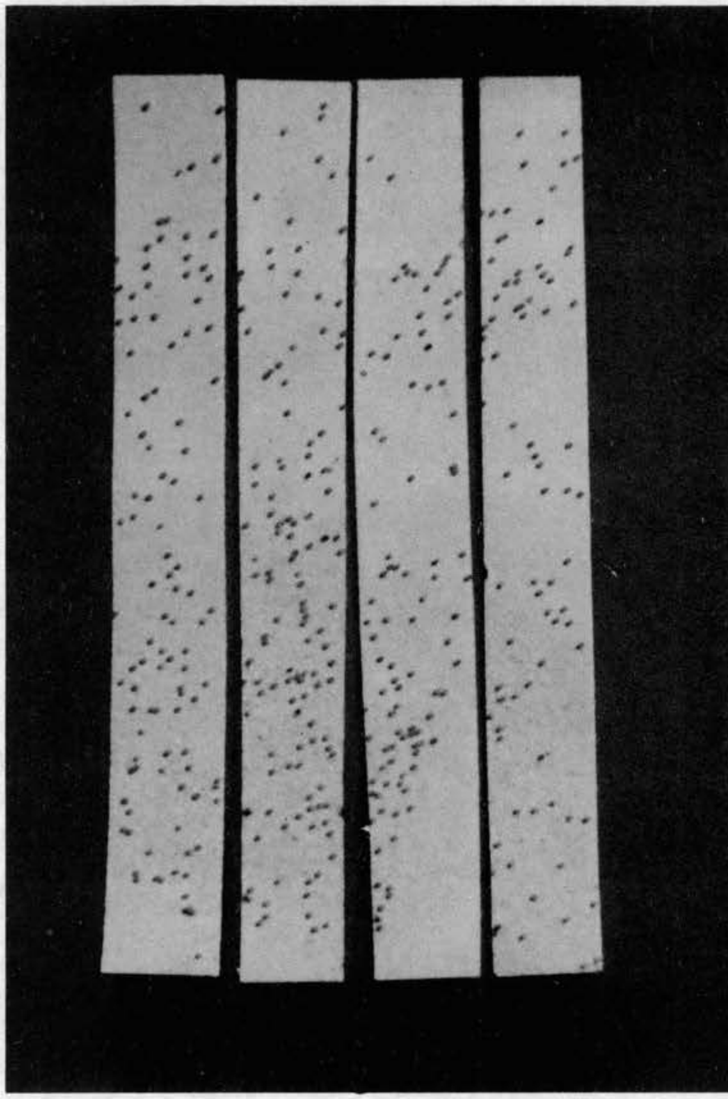

Fig. 2. Paper strips showing eggs of the corn earworm.

source on February 7, 1966.

Rearing Technique - The technique for rearing the fall armyworm was similar to that used for the corn earworm. Mating cages were used as described by Bailey (1964). Pupae were placed in a one-gallon food carton used as the adult emergence cage. After emergence, 20 pairs of moths were put into each mating cage. Moths were fed with 10 percent honey solution in a cotton-stoppered 4-dram vial turned upside down on the top of the cage.

Oviposition - The female moths seemed to prefer to lay their eggs on any object available, but most of them were laid on the wax paper or paper toweling on the outside of the cage (Fig. 3). Sections of the wax paper or paper toweling containing egg masses were used to infest the sorghum plants in the field.

Southwestern corn borer

Source of insect - All of the southwestern corn borer cultures used were developed from larvae and pupae collected from infested corn fields during the winter and spring of 1965. Male and female moths emerged from the pupae kept in the insectary. All of the diapausing immaculate larvae were used in studies on breaking larval diapause, which is discussed later.

Rearing technique - The rearing technique used for the southwestern corn borer was a modification that was developed by Hensley (1956), Bailey (1964), and Keaster and Harrendorf (1965). One-gallon food cartons were used as moth emergence cages. About 80 pupae (male and female) were placed in each cage. After moth emergence, 15 to 20 pairs were placed in oviposition cages. The oviposition cages were one-gallon food cartons lined with wax paper upon which the moths oviposited.

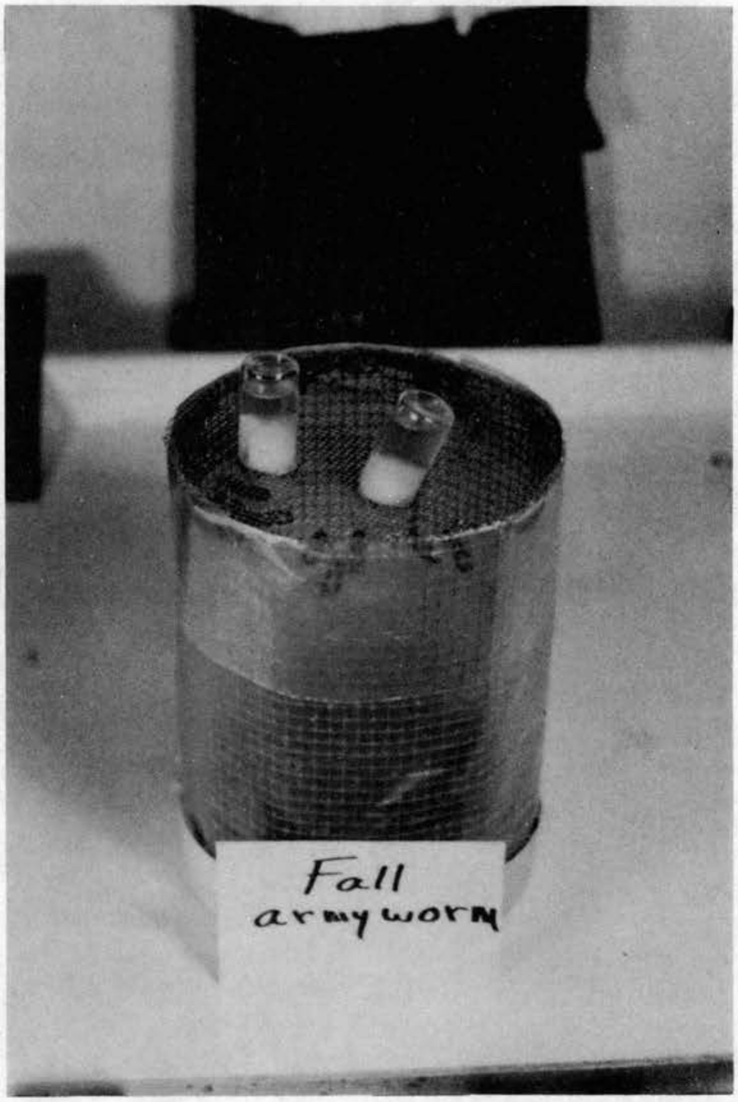

Fig. 3. Fall armyworm oviposition chamber showing honey solution and waxed paper for oviposition.

The open end of the food container was covered with nylon screen cloth fastened with a rubber band. Sterilized filter paper was placed in the bottom of the carton, and it was wet with water morning and evening to provide a high degree of relative humidity. Since the moths seemed to prefer darkness for mating, the lid of the carton was put in place. A piece of sterilized cotton approximately 1 inch square was placed on the top of the screen cloth and wet with 5 percent honey solution as food for the moths. Distilled water was added to the cotton once or twice daily. The moths were transferred to a new cage after 2-3 days. Eggs on the wax paper were stored or placed in the hatching chamber.

Egg Storage

In order to assure an abundance of eggs or larvae of the three lepidopterous species when wanted for infesting sorghum plants being tested for resistance to these insects, they were stored at low temperatures. Approximately 2500 eggs of each of the three species which were oviposited on wax paper or paper toweling were placed in one-gallon food containers. The bottom of the container was lined with paper towels which were moistened with distilled water twice a day to maintain a high relative humidity. The top of the container was covered with paper toweling, a clear plastic sheeting, and the carton lid. This storage was done in a plant growth chamber in which varying environmental conditions could be maintained. The eggs were stored at different temperatures which depended on the demands for larvae. At 80° F ± 2° F the eggs hatched in 4 or 5 days. Increase of the temperature to 85 or 90° F stimulated the development of the eggs. Decreasing the temperature between 40-70 generally delayed the hatching period.

Stewart and Walton (1966) studied the extension of the incubation period by egg refrigeration and found that 48-hour-old Z. grandiosella eggs could be stored at a temperature of 50-55° F for 3 days without materially reducing expected hatching percentage.

Rearing Chambers for Lepidopterous Larvae

In rearing lepidopterous larvae on artificial diets many kinds of containers have been used. Glass shell vials were used in rearing the cotton bollworm (corn earworm), Vanderzant (1962), Bailey (1964); European corn borer, Beck (1949), Becton et al. (1962), Guthrie et al. (1965); pink bollworm, Beckman et al. (1953), Adkisson et al. (1960), Ouye and Vanderzant (1964), Richmond and Ignoffo (1964); and the silkworm, Ito (1962). Bailey (1964) also used ice cream cups, ice trays, and sandwich boxes as rearing chambers for the corn earworm, fall armyworm, and southwestern corn borer. Keaster and Harrendorf (1965) used ½-oz. coffee creamers for rearing the southwestern corn borer. Several other types of containers were used in the present study, including petri dishes, glass cake pans, and clear plastic medicine cups. However, one-ounce clear plastic medicine cups having tight fitting paper lids proved to be best, and they were used for rearing the larvae of the three species.

Artificial Diets

Artificial media for rearing lepidopterous larvae have been known for a few decades. Several synthetic diets were developed for rearing the European corn borer, O. nubilalis (Hübner), by Bottger (1942) and Beck et al. (1949); pink bollworm, Pectinophora gossypiella (Saunders), Vanderzant (1956), Adkisson et al. (1960), Clark et al. (1961); cabbage looper, Trichoplusia ni (Hübn.), Chippendale (1965); gypsy moth, Porthetria dispar (Linn.), Leonard and Doane (1966).

Artificial diets for the corn earworm were developed by several workers: George et al. (1960), Vanderzant (1962), Berger (1963), and Bailey (1964). Most of these diets were based on the European corn borer diet developed by Bottger (1942) and Beck (1949), and the pink bollworm diet developed by Adkisson et al. (1960).

All of the larvae used in these studies were reared on an artificial diet in controlled environment chambers or insectaries which were described by Bailey (1964). Modifications in the diet from that described by him included the use of less sucrose, use of a standard vitamin fortification mixture, and the addition of a small amount of propionic acid and tetracycline as antimicrobial agents. The composition of the diet was as follows:

Table 1. Composition of corn earworm, fall armyworm, and southwestern corn borer diet.[1]

Constituents	Amount
Wheat Germ	108 g
Sucrose	96 g
Casein (Vitamin Free)	126 g
Salt Mixture (Wesson's)	36 g
Alphacel	18 g
Vitamin Fortification Mixture	36 g
Ascorbic Acid	15 g
Agar	80 g
KOH Solution 22.5%	18 ml
Formaldehyde 10%	15 ml
Methyl Parahydroxyberzoate 15% in 95% Ethyl Alcohol	36 ml
Propionic Acid	5 ml
Tetracycline (250 mg per capsule)	3 capsules
Water	3000 ml

[1]This diet was developed from Vanderzant et al. (1962), with modification.

Mass Production of Lepidopterous Larvae and Pupae

The main purpose of this study was to produce a large number of larvae and pupae with a high survival percentage, low cost of production, and saving of time and labor.

The rearing chambers used were: (1) glass cake pans (8½ x 8½ x 2½ in.), (2) plastic petri dishes (100 x 15 mm), (3) clear medicine cups (1 oz). Glass cake pans with ½ inch of artificial (wheat germ) diet were infested with 100, 200, and 300 first-instar fall armyworm larvae, respectively. There were 3 replicates of each rate. The top of the glass cake pan was covered with sterilized paper toweling and a piece of glass.

Plastic petri dishes approximately ½ full of diet were infested at the rate of 3, 5, 10, 15, and 20 first-instar larvae. To prevent the tiny larvae from escaping, filter paper (No. 7760) was put on the top of the petri dish and covered with the upper half of the dish. In this experiment there were 6 replicates.

The tests with clear medicine cups were divided into 3 experiments: (1) large numbers of the medicine cups with the diet were infested with 3 first-instar fall armyworm larvae, (2) the same procedure as described above was followed using one first-instar corn earworm larva, and (3) 2 first-instar southwestern corn borer larvae. Numbers of developing pupae were recorded.

Later, a commercially prepared diet, known as the Vanderzant-Adkisson wheat germ diet,[2] was used. Results obtained were somewhat similar to those obtained with the diet described above, but it had little advantage over the diet prepared from basic chemicals.

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[2]Product of Nutritional Biochemicals Corp., Cleveland, Ohio.

Tests with Brewer's Yeast

Because of the high cost of the vitamin complex fortification mixture used in the artificial diets (currently $9.10 per pound), tests using brewer's yeast (63¢ per pound) as a substitute were conducted. Brewer's yeast contains thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid, biotin, choline, and inositol. These ingredients are found in the vitamin diet fortification mixture, but in different amounts. Also, the vitamin A, D, and B_{12}, and alpha tocopherol, menodione, and aminobenzoic and ascorbic acids in the vitamin complex fortification mixture were missing in the brewer's yeast.

The synthetic diet described above was used, but the brewer's yeast was substituted for the vitamin complex fortification mixture. Larvae of the corn earworm and fall armyworm hatching from eggs deposited by one female moth were used in the test. They were reared on the diet according to the procedure described previously, and data on their development were recorded.

Tests with Propionic Acid as an Antimicrobial Agent

Considerable difficulty in rearing larvae on artificial diets has been encountered because of the development of molds and bacteria. Even with the best of sanitation, diet contamination frequently occurs. This interferes with development of the larvae and results in poor larval and pupal survival. Dr. N. N. Durham, Professor of Microbiology and Dean of the Graduate School of Oklahoma State University, suggested the use of propionic acid in the diet as an antimicrobial agent, since it is used with safety in bakeries and in food processing.

Feeding tests involving third-instar corn earworm larvae were set up in which propionic acid (95%) was added at rates of 5, 6, 7, 8, and

9 ml per 3,600 grams of diet. The standard synthetic diet was used as the check. Each test involved 30 medicine cups with diet, each containing one larva. Data were recorded on larval and pupal development, and pupal weights were recorded.

Extension of the Larval Period of the Corn Earworm and Fall Armyworm

Under natural field conditions larval periods of lepidopterous larvae are somewhat different from those reared in the laboratory. Corn earworm larvae attained full growth in 14-28 days under field conditions (Metcalf and Flint 1964), while it took 18-20 days on the wheat germ diet in the laboratory at 80 ± 2° F (Bailey 1964). About the same was also true for the fall armyworm. The longer duration of larval period under field conditions probably was due to differences in day and night temperatures in nature, as compared to the fairly constant temperatures in the laboratory. Low temperature slows the body metabolic and enzymatic action and also is involved in the hormone production of the insects, which prevents them from molting.

The tests were conducted in a growth chamber in which temperatures ranged from 35° F at midnight to 65° F at noon. The relative humidity was around 60%. Light intensity was 3000 candlepower during the daytime, and the lights were off at night. First-instar larvae of the fall armyworm and corn earworm were used in this study. Newly hatched larvae at rates of 5, 10, 15, 20, and 25 per medicine cup containing diet were stored in the growth chamber. There were 10 replicates for each rate. Numbers of larvae surviving at the end of 7-day periods were recorded.

Breaking and Inducing Diapause of Southwestern Corn Borer

Frequently, southwestern corn borer larvae collected in the field are in a state of diapause. Before they can be used for studies in the laboratory it is necessary that they be in an active, developing stage. Several types of containers were used in an experiment designed to break diapause. These were one-ounce medicine cups, plastic petri dishes, and 1 x 3 3/4 inch glass shell vials. The containers were filled 1/3 to 1/2 of their depth with sterile cotton which was then thoroughly wet with sterile water. Larvae used were sterilized by placing them in a 1% sodium hypochlorite for about one minute. One larva was placed in each container, and there were 36 containers of each type. The glass shell vials were plugged with cotton, and covers were placed on the respective petri dishes and medicine cups. All tests were conducted in the laboratory having a temperature varying from 80 to 85° F. The larvae were examined weekly, and data on molting and pupation were recorded.

The diapause induction experiment was based on preliminary work during the summer of 1965 in which petri dishes were used for rearing second generation southwestern corn borer larvae at room temperatures ranging between 85-90° F. Under these conditions, about 75 percent of the larvae entered diapause. This was rather unusual as far as the season and the temperature were concerned. Presumably, the factors which induced diapause were (1) low humidity, or (2) insufficient diet. An experiment was conducted to determine whether inducing diapause of southwestern corn borer larvae could be done under laboratory conditions. Petri dishes and medicine cups were used as rearing chambers. The diet was similar to that used for mass rearing, except that it contained 90, instead of 80 g of agar. This addition of more agar provided for a drier and thicker diet.

Preliminary Screening of Sorghum Varieties and Hybrids for Resistance to Corn Earworm, Fall Armyworm, and Southwestern Corn Borer

Preliminary studies attempting to search for lines of sorghum with resistance to the corn earworm, fall armyworm, and southwestern corn borer were conducted in the field, greenhouse, and laboratory during the 1966-1967 period. The aim of these studies was to find an effective method of evaluating the degree of resistance exhibited by a particular variety. Varieties which exhibited a high degree of resistance were selected for future intensive study to obtain resistant germ plasm for use by plant breeders.

Sorghum Varieties and Hybrids - Professor Frank Davies and Dr. D. E. Weibel of the Oklahoma State University Agronomy Department provided 144 varieties of sorghums for this study. These varieties represented many types of sorghum, including milos, kafirs, feteritas, kaoliangs, hegaris, sorgos, and other sorghums. Many of these varieties have been developed by breeding and are of commercial importance.

During the summer of 1966 a test was conducted on the Agronomy Farm south of the Oklahoma State University water treatment plant. The sorghums were planted in single 10-foot rows on June 18. Each variety consisted of a 3-row plot. Five plants in each of the three rows were infested with eggs of the corn earworm, fall armyworm, and the southwestern corn borer, respectively. The plants emerged from the soil on June 25, and after the seedlings were about 10 days old, plots were thinned to 10-12 plants per row.

Infestation of the Whorls and Sorghum Heads - When the plants were about 4 weeks old, they were manually infested at approximately 5:00 p.m., thus avoiding the great change of temperature and light intensity differences between laboratory and field conditions. Infestation was made by inserting a paper strip containing 25 fertile eggs of the corn earworm and 50 of the fall armyworm (which had been counted previously) inside the whorl of the sorghum plant. Waxed paper strips with approximately 25 eggs of the southwestern corn borer were used to infest test plants. Eggs in all cases were in the "black-head" stage, just prior to hatching. Five plants were infested, and five plants were left as uninfested checks.

As the sorghum headed, the check plants were bagged and the heads of the whorl infested plants were reinfested with 25 "black-head" stage eggs. After infestation the sorghum heads were covered with paper sacks.

Injury Ratings - Leaf injury ratings were made 7 and 14 days after the whorls had been infested. A rating system 0-5, as described by Chada et al. (1961), which is a measure of the estimated percentage of leaf area damaged, was used as follows:

Rating	Percent Damage
0	0-10
1	11-20
2	21-40
3	41-60
4	61-80
5	Beyond recovery

This system has been used in screening wheat, barley, and other small grains for insect damage, and it appeared to be acceptable for screening for insect resistance of sorghums.

Greenhouse Screening Test - The same entries of sorghum varieties and hybrids which were tested in the field were also used in greenhouse testing. The sorghum varieties to be evaluated were planted in sand in benches (8'4" x 4'2" x 5½"). Each bench contained 24 varieties in rows 24" long and 9" apart. Each variety was thinned to 12 plants a few days after emergence. When the seedlings were about one week old, each was infested with 2 newly hatched first-instar larvae. To prevent migration of larvae from one variety to another, they were separated by means of clear plastic plate barriers 8½" tall (Fig. 4). Plant injury ratings, using a scale of 0-10, based on work by Wiseman et al. (1966), were made 3 and 5 days after infestation. Data on numbers of surviving larvae and larval weight were recorded.

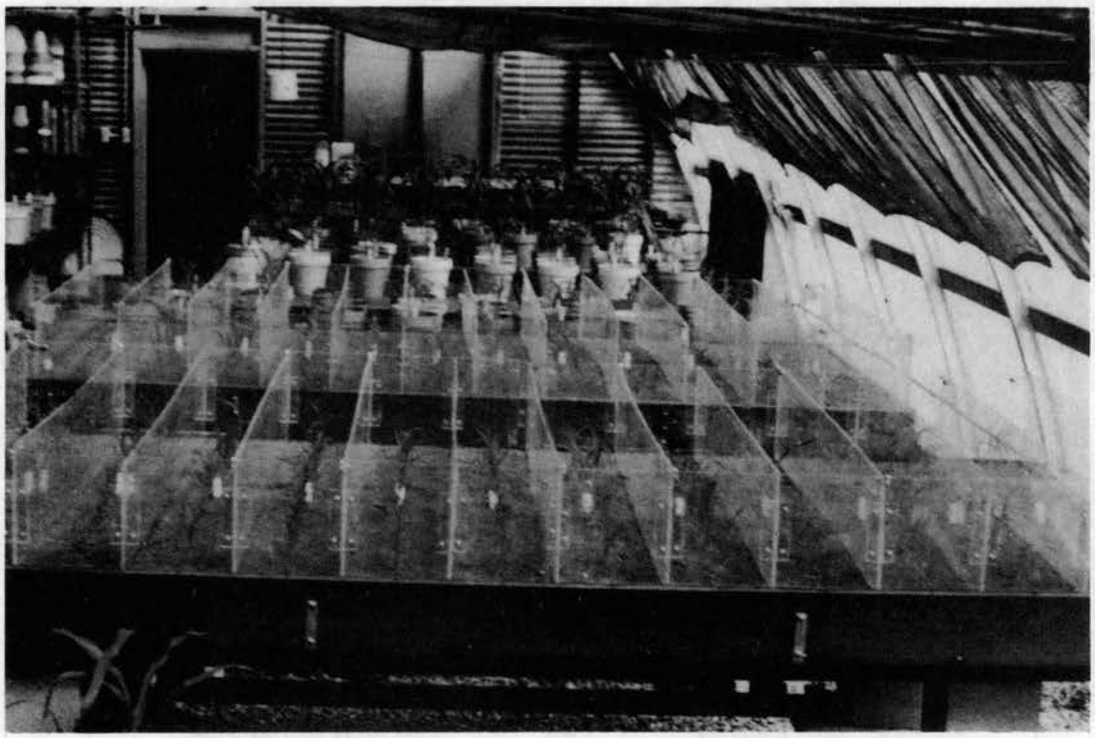

Fig. 4. Greenhouse technique for testing seedling sorghum plants for resistance to lepidopterous larvae.

RESULTS AND DISCUSSION

Mass Production of Eggs and Larvae of Three Lepidopterous Species

In screening sorghum lines and varieties for insect resistance, a large number of eggs or first-instar larvae of the insects used in making manual infestations are required. Before starting an experiment in the greenhouse or in the field, one needs to calculate the numbers of eggs or larvae required at definite periods. In order to have enough eggs or larvae when needed, planning in rearing the insects should be taken into consideration.

Corn earworm - Mass production of eggs and larvae of this insect is more difficult than for the fall armyworm and southwestern corn borer. Several factors, such as mating habits, temperature, relative humidity, light intensities, and adult food are involved. However, by following the techniques suggested by several workers mentioned previously, satisfactory results have been obtained.

Data on corn earworm moth oviposition are presented in Table 2. Total number of eggs deposited on paper strips, nylon cloth, and on the side of 5 cages during a 5-day observation period were 3415, 2663, 3681, 3204, and 3647, respectively, giving a grand total of 16,610 eggs deposited by the 50 females involved. This was an average of 332 eggs per female.

Normally corn earworm moths lay eggs singly and prefer to deposit them on the upper part of the chamber. Paper strips 1/3 x 2 inches were satisfactory for ovipositions. Sixteen of the strips were placed

Table 2. Number of eggs from 10 pairs of laboratory reared corn earworm moths during the third, fourth, and fifth days of oviposition.

Paper Strip No.	Oviposition Chamber No. 1: 3rd,4th day	1: 5th day	2: 3rd,4th day	2: 5th day	3: 3rd,4th day	3: 5th day	4: 3rd,4th day	4: 5th day	5: 3rd,4th day	5: 5th day
1	64	34	98	65	110	24	70	24	70	55
2	115	50	43	31	125	41	84	41	84	18
3	59	56	71	29	130	57	100	57	100	25
4	48	52	41	23	84	23	52	23	52	48
5	79	34	84	24	42	48	24	48	24	58
6	68	42	25	38	65	40	26	40	26	23
7	75	22	75	32	65	24	54	24	54	30
8	65	45	125	34	50	110	90	110	90	24
9	116	14	81	22	80	18	51	18	51	78
10	80	53	55	34	105	37	36	37	36	62
11	90	62	118	28	65	39	104	39	104	27
12	165	24	82	28	108	30	77	30	77	36
13	85	36	29	21	24	37	88	37	88	23
14	55	57	38	16	63	78	62	78	62	26
15	85	29	32	26	88	43	155	43	155	34
16	94	63	65	25	79	68	117	68	117	52
Paper Strip Total	1342	673	1062	476	1282	717	1190	614	2016	741
No. eggs on side and screen top	850	550	825	300	1092	590	950	450	590	300
Total	2192	1223	1887	776	2374	1307	2140	1064	2606	1041
Grand Total		3415		2663		3681		3204		3647

on the screen top of each oviposition chamber (Fig. 1). The number of eggs per chamber laid on the paper strips on the third and fourth day were approximately 1342, 1062, 1282, 1190, and 2016, and on the fifth day, 673, 476, 717, 614, and 741, respectively, for the 5 chambers (Table 2). This gives an average of 63.20 eggs per strip. These eggs on the paper strips were used for manually infesting test plants both in the field and greenhouse. The eggs laid on the third, fourth, and fifth days were almost one hundred percent fertile.

Fall armyworm - Large numbers of fall armyworm eggs and first-instar larvae could be produced in the laboratory with little difficulty. Data showing the results of egg oviposition 5 days after emergence of moths are given in Table 3. A total of 803 egg masses produced by 82 females gave an average of 9.79 masses per female (Table 3). The maximum length of an egg mass was 7.0 mm, and the minimum was 3.0 mm. The width varied from 2.5 and 5.5 mm. The average egg mass size varied from 10.5 and 30.0 mm^2. The number of egg layers per mass ranged from 1 to 5. It was not possible to count the number of eggs per mass in multi-layered masses. Instead, production was based on the number of first-instar larvae hatching per mass. The average number of first-instar larvae per egg mass was 133.80, as shown in Table 4. Therefore, 107,441 first-instar larvae were produced by 82 female moths in the 5 oviposition chambers.

Southwestern corn borer - Oviposition data for southwestern corn borer moths are given in Table 5, 6, and 7. A total of 1,868 egg masses were produced by 72 female moths (Table 5). These data were analyzed statistically, and there were no significant differences in the average number of egg masses per female among chambers containing 5, 6, 7, 8, or

Table 3. Egg production of fall armyworm moths reared in the laboratory 5 days after emergence.

Oviposition chamber no.	Number of moths		Total no. egg masses per chamber	Average no. egg masses per female
	Males	Females		
1	25	25	232	9.28
2	16	16	163	10.18
3	19	19	188	9.89
4	12	12	142	11.83
5	10	10	78	7.80
Total	82	82	803	9.79

Table 4. Average size of fall armyworm egg masses and average number of first-instar larvae hatching per mass.

Egg mass No.	Length (mm)	Width (mm)	Approx. size (mm^2)	No. egg layers	No. instar larvae hatched
1	3.5	3.0	10.50	2	95
2	4.0	5.0	20.00	3	110
3	4.5	5.0	22.50	3	90
4	3.5	3.0	10.50	2	164
5	5.5	4.0	22.00	3	170
6	6.0	3.5	21.00	2	146
7	5.5	5.0	27.50	3	120
8	7.0	5.0	35.00	3	179
9	6.0	5.5	33.00	3	210
10	3.5	3.0	10.50	3	120
11	3.0	3.0	9.00	2	141
12	3.0	2.5	7.50	1	57
13	4.0	3.0	12.00	2	105
14	4.5	4.0	18.00	2	170
15	5.0	3.0	15.00	4	130
16	4.5	4.0	18.00	3	125
17	4.0	4.0	16.00	3	60
18	5.0	4.5	22.50	3	190
19	4.0	3.0	12.00	5	150
20	3.0	2.5	7.50	3	40
21	3.5	3.0	10.50	2	70
22	3.0	2.5	7.50	2	179
23	6.0	4.0	24.00	3	193
24	5.0	4.5	22.50	4	141
25	5.0	4.5	22.50	3	177
Total					3,332
Average					133.80

Table 5. Egg production of laboratory reared southwestern corn borer moths in concentrations of five to ten pairs per mating chamber.

Mating chamber No.	No. moth pairs	No. egg masses counted after the 4th night	Average no. egg masses per female
1	5	140	28.00
2	5	173	34.60
3	6	132	22.00
4	6	166	27.66
5	7	159	22.71
6	7	182	26.00
7	8	194	24.25
8	8	269	33.62
9	10	247	24.70
10	10	206	20.60
Total	72	1868	- - -
Average			25.94

Table 6. Egg and larval production of 10 pairs of southwestern corn borer moths in individual oviposition chambers.

Pair no.	No. of egg masses deposited 4 days after emergence	No. of fertile egg masses	Total no. of fertile eggs (in black-head stage)	No. of first instar larvae emerging	Percent hatching
1	32	27	179	143	85.4
2	67	42	254	237	93.3
3	48	44	279	255	91.3
4	38	29	192	175	91.1
5	23	22	140	126	90.0
6	55	47	285	260	91.2
7	28	20	121	107	88.4
8	37	32	195	155	79.4
9	36	33	203	185	91.1
10	34	28	199	178	89.4
Total	398	324	2,047	1,821	88.9
Average	39.8	32.4	204.70	182.1	

Table 7. Average number of southwestern corn borer "black-head" stage eggs per mass produced in the laboratory.

Egg mass no.	Chamber number 1	2	3	4	5	6	7	8	9	10	Total no. eggs
1	4	6	4	5	6	10	8	6	7	9	
2	4	8	7	8	5	3	3	5	5	3	
3	4	3	7	5	3	6	7	2	4	11	
4	7	14	5	2	7	5	5	7	8	3	
5	7	5	7	6	13	5	6	12	15	12	
6	6	7	4	3	11	11	6	9	3	6	
7	3	9	2	10	7	8	10	7	5	7	
8	3	13	4	4	6	5	10	7	8	9	
9	3	7	5	7	6	7	13	3	3	8	
10	4	6	7	15	4	3	2	5	7	7	
11	5	3	3	4	10	8	2	6	8	9	
12	6	10	4	4	3	6	3	6	6	7	
13	10	5	3	13	5	7	12	11	18	7	
14	6	8	2	3	9	4	2	9	13	2	
15	5	3	8	11	3	10	4	2	7	4	
16	4	3	4	8	3	7	5	9	4	6	
17	4	2	6	3	2	5	3	24	10	8	
18	5	2	7	6	11	9	8	6	4	6	
19	6	12	3	6	21	2	5	6	3	4	
20	3	6	2	11	7	5	10	4	5	4	
21	5	6	4	4	4	6	5	18	5	10	
22	9	8	3	13	5	4	5	4	5	4	
23	6	2	8	8	5	3	6	3	13	10	
24	6	2	3	7	8	7	10	6	2	10	
25	12	5	5	5	16	20	12	3	4	3	
Total	137	155	117	171	179	168	162	180	182	179	1,630
Average	5.48	6.20	4.68	6.84	7.16	6.72	6.48	7.20	7.28	7.16	6.52

10 pairs. However, the average number of egg masses per female moth was greater in oviposition chambers having fewer pairs of moths. The average number of egg masses produced by one moth pair per cage was 39.8 which exceeded production in chambers having multi-pairs (Table 5). Twenty-five egg masses taken at random in the black-head stage from each of the chambers shown in Table 5 were counted. The data in this regard are presented in Table 7. The over-all average number of fertile eggs per mass was 6.52. Therefore, the average number of fertile eggs produced by 72 female moths would be 12,179, or 169 eggs per female. Ten females in separated cages produced 2,047 fertile eggs or 204.70 fertile eggs per female (Table 6).

<u>Mass Production of Pupae of Three Lepidopterous Species</u>

In order to maintain a supply of adults, eggs, and larvae for use in the laboratory, greenhouse, and field, calculations on the production of these forms and emergence data on adults are of importance.

Data on calculated and observed pupation and emergence of the corn earworm, fall armyworm, and southwestern corn borer are presented in Table 8. It is shown that the actual pupation and emergence dates were fairly close to calculated dates.[1] This information is of importance in connection with correlating availability of eggs or larvae for use in manual infestations of test plants with stage of development.

<u>Rearing Chamber Studies</u>

<u>Corn earworm</u> - Many types of rearing containers, including glass shell vials, ice cream cups, ice trays, and medicine cups, mentioned by

[1] Calculated dates were based on previous data presented by Bailey (1964), and from the observation of the author.

Table 8. Prediction of pupation and emergence dates of corn earworm, fall armyworm, and southwestern corn borer.

Insect species	Hatching date	No. first-instar larvae	Calculated pupation date	Observed pupation date	No. pupae	Calculated emergence date	Observed emergence date
H. zea 1/	6/5	275	6/25	6/23	210	7/2	7/3
	6/11	500	7/1	7/3	425	7/14	7/14
	6/12	400	7/2	7/3	332	7/15	7/15
Total		1175			967		
S. frugiperda 2/	6/8	1000	6/27	6/26	730	7/6	7/5
	6/10	800	6/29	6/28	682	7/8	7/6
Total		1800			1412		
Z. grandiosella 2/	6/20	160	7/8	7/10	91	7/18	7/17
	6/21	192	7/9	7/11	112	7/19	7/18
	6/22	380	7/10	7/11	208	7/20	7/19
	6/23	612	7/11	7/13	345	7/21	7/21
	6/24	250	7/12	7/13	139	7/22	7/23
	6/25	628	7/13	7/14	353	7/23	7/24
Total		2222			1248		

1/ One larva per cup.

2/ Two larvae per cup.

several workers for rearing corn earworm larvae, have been used in this study. Each type of container was used successfully for rearing from the first instar to pupation, but medicine cups have some advantages. Due to the cannibalistic habits of this insect, one larva was transferred to an individual cup containing diet, and it was reared to pupation.

Data on rearing the corn earworm are presented in Table 9. The average percent pupation was 75.3. Pupation of three-fourths of the larvae is an indication that the technique and diet are satisfactory.

Table 9. Pupation of the corn earworm reared from first-instar larvae on artificial diet in individual one-ounce medicine cups.

Test no.	Total no. larvae	Pupation	
		No. pupae	Percent
1	200	163	81.5
2	200	141	70.5
3	200	148	74.0
Average			75.3

Fall armyworm - Data on mass production of the fall armyworm are presented in Tables 10, 11, and 12. Fall armyworm could be reared in several types of containers. In using medicine cups containing 3 first-instar larvae on artificial diet, 51.6% pupation was attained. This was 155 pupae per 100 medicine cups. When 1 larva per cup was tried, the percent pupation was above 90 percent. (This is based on observation by Bailey (1946) and the author.) Therefore, in rearing this insect by using medicine cups as a rearing container, 2 larvae per cup

(Table 8) and 3 larvae per cup (Table 10) produced more pupae per 100 cups than when 1 larva per cup was used.

Glass cake pans containing artificial diet were used for rearing the fall armyworm (Fig. 5). Tests were made using 100, 200, and 300 first-instar larvae per pan. Although more pupae were obtained when larger numbers of first-instar larvae per pan were used, the percent pupating was greatly reduced (Table 11). This was due to crowding and some cannibalism in the container.

In a similar test involving the use of 3, 5, 10, 15, and 20 first-instar larvae on diet in plastic petri dishes, more total pupae were produced by using the larger numbers of first-instar larvae per container, but the percent pupation was greatly reduced, and pupal weights decreased greatly (Table 12).

On the basis of the above tests, best results were obtained using 3 first-instar larvae on artificial diet per 1-ounce medicine cup.

<u>Southwestern corn borer</u> - Bailey (1964) found that diet-containing glass shell vials were satisfactory for rearing the southwestern corn borer. The present studies were conducted using 2 first-instar larvae per medicine cup containing artificial diet. Data in this regard are presented in Table 13. The average percent pupation was 59.0, with an average of 118 pupae from 200 first-instar larvae per 100 medicine cups.

<u>Effect of Substituting Brewer's Yeast for the Vitamin Diet Fortification Mixture in the Wheat Germ Diet</u>.

Because brewer's yeast contains most of the vitamins found in the commercially prepared vitamin diet fortification mixture and is less expensive, it was substituted for the vitamin mixture in the 3600 g

Table 10. Pupal production of fall armyworm reared in the laboratory.

Test no.	No. first-instar larvae[1/]	Number of cups containing			Total no. pupae	Percent pupation
		1 pupa	2 pupae	3 pupae		
1	300	29	42	19	170	56.7
2	300	34	35	15	149	49.7
3	300	36	40	10	146	48.7
Total	900	99	117	44	465	
Average					155	51.7

[1/] Three first-instar larvae per cup.

Fig. 5. Fall armyworm larvae (5th and 6th instar) reared in glass cake pan on artificial diet.

Table 11. Pupal production of fall armyworm reared on artificial diet in cake pans.

Test no.	Cake pan no. & no. of 1st-instar larvae			Cake pan no. & no. pupae			Total no. pupae	Percent pupation
	1	2	3	1	2	3		
1	100	100	100	22	19	31	72	24.0
2	200	200	200	36	26	33	95	15.8
3	300	300	300	37	41	44	122	13.6

Table 12. Production of fall armyworm pupae from various numbers of first-instar larvae per petri dish.[1]

Test No.	3 larvae		5 larvae		10 larvae		15 larvae		20 larvae	
	No. pupae	Avg. wt.[2]	No. pupae	Avg. wt.	No. pupae	Avg. wt.	No. pupae	Avg. wt.	No. pupae	Avg. wt.
1	23	0.3011	37	0.2812	45	0.2463	51	0.2256	60	0.2178
2	28	0.2873	38	0.2831	54	0.2359	58	0.2232	66	0.2096
3	20	0.2975	33	0.2899	42	0.2538	46	0.2331	57	0.2319

1/ Ten replicates per test.

2/ Grams.

Table 13. Pupal production of southwestern corn borer reared in the laboratory.

Test no.	No. cups	No. first instar larvae	No. of cups with			Total no. pupae	Percent pupation
			no pupae	1 pupa	2 pupae		
1	100	200	5	76	19	114	57.0
2	100	200	1	74	25	124	62.0
3	100	200	4	76	20	116	58.0
Total			10	226	64	354	
Average			3.3	75.3	21.3	118	59.0

wheat germ diet to determine if this diet would be satisfactory. Data on the effects of using varying amounts of brewer's yeast in the diet are presented in Table 14. It is shown that there was an increase in pupation percentages with each increase in the amount of brewer's yeast used. The highest rate (72 g) resulted in a pupation of 52.5 percent, whereas, average pupation when the standard diet (containing the vitamin diet fortification mixture) was used averaged 74.0 percent. Also, increasing the amount of brewer's yeast resulted in pupae with greater weight. However, the average weight of pupae reared on the standard diet was greater than when larvae were reared on the brewer's yeast. It is possible that use of increased amounts of brewer's yeast would have resulted in increased pupation, equal to that obtained with the diet involving the vitamin diet fortification mixture, which is about 14 times more expensive.

A study involving use of various numbers of third-instar corn earworm larvae per medicine cup when reared on a diet containing 72 grams of brewer's yeast was conducted. The results are presented in Table 15. It is shown that with an increase from 2 to 8 larvae per medicine cup, the pupation percentage dropped from 31.0 to 10.5. This is an indication that overcrowding in the cups becomes a factor in pupation percentages. It resulted in cannibalism and lack of food.

Effects of Addition of Propionic Acid to the Diet as an Antimicrobial Agent

Test with corn earworm. Tests involving addition of propionic acid to diets in the amounts of 5, 6, 7, 8, and 9 ml per 3600 g of standard diet were conducted. One first-instar larva was placed in each medicine cup containing the test diet. Results were based on the

Table 14. Effect of various amounts of brewer's yeast in the wheat germ diet on pupation of corn earworm larvae.

Grams brewer's yeast added to diet	Pupation [1/]		Average pupal weight (g)
	No. pupae	Percent	
36	16	20.0	0.3834
54	30	37.5	0.4046
72	42	52.5	0.4293
Standard diet (check)	148 [2/]	74.0	0.4610

[1/] Each test started with 80 third-instar larvae.

[2/] Test started with 200 first-instar larvae.

Table 15. Effect of a diet containing 72 grams of brewer's yeast on production of pupae from various numbers of corn earworm larvae per cup.

Number of 3rd-instar larvae per 1-oz. medicine cup	Number cups	Number larvae	Number pupae	Percent pupation
2	50	100	31	31.0
4	50	200	33	16.5
6	50	300	37	12.3
8	50	400	42	10.5

pupation percentages obtained. Data in this regard are presented in Table 16. The addition of 5 ml propionic acid resulted in a pupation percentage of 78.0 compared to 46.0 where no acid was used. However, with increased rates, the pupation percentages dropped rapidly to where there was no pupation at the 9 ml rate. Although the pupation percentage was increased at the 5 ml rate, the average weight of the pupae was lower than in the check, and there was a further weight decrease with each increase in the amount of propionic acid used. This would indicate that the propionic acid in large amounts was toxic. However, it was found to be an effective agent in preventing mold, yeast, and bacterial development in the diet, and it was used in all diets prepared subsequently.

Test with fall armyworm. A similar test involving addition of propionic acid to the diets was conducted for rearing the fall armyworm. These data are presented in Table 17. The addition of 5 ml of propionic acid was an effective microbial agent for the fall armyworm diet. Increased amount in the diet resulted in decreased pupation percentages, but the effect was not so drastic as on the corn earworm (Table 16). Similarly, the effect on pupal weight was less important. Propionic acid added to the diet for the fall armyworm at the rate of 5 ml for 3600 g has been used in all subsequent diet preparations.

Extension of the Larval Period of the Corn Earworm and Fall Armyworm

When first-instar larvae of the corn earworm and fall armyworm were placed on artificial diet in medicine cups and stored in a growth chamber at temperatures ranging from 35° F at midnight to 65° F at noon, small percentages survived up to 3 and 4 weeks (Table 18). Beyond this period, mortality was rather rapid. The percent survival decreased as

Table 16. Effect of various amounts of propionic acid in the standard wheat germ diet on corn earworm pupation.

Amount of propionic acid in wheat germ diet (ml)	Pupation [1/]		Average weight of pupae (g)
	No. pupae	Percent	
none (check)	23	46.0	0.4610
5	39	78.0	0.4271
6	28	56.0	0.3914
7	19	38.0	0.3885
8	10	20.0	0.3492
9	0	0.0	------

[1/] 50 first-instar larvae started in each test.

Table 17. Effect on pupation of fall armyworm of various amounts of propionic acid in the standard wheat germ diet.

Amount of propionic acid in wheat germ diet	Pupation [1/]		Average weight of pupae (g)
	Number of pupae obtained	Percent	
none (check)	42	52.5	0.2820
5	62	77.5	0.2719
6	36	45.0	0.2537
7	32	40.0	0.2906
8	38	47.0	0.2995
9	26	32.5	0.3015

[1/] Each test started with 80 first-instar larvae.

the number of larvae per cup increased.

Similar data for the fall armyworm are presented in Table 19. However, surviving numbers at the end of the 21-day period were much greater than for the corn earworm. Also, percent survival was greater when fewer newly hatched larvae per cup were started.

From the data presented in Tables 18 and 19, it is shown that the larval period of the corn earworm and fall armyworm can be extended by storage at fluctuating low temperatures. The larvae did some feeding during the day when the temperature reached 65° F at noon, and this undoubtedly helped to keep them alive. Most of the larvae were in the second and third instar at the end of 42 days.

When the larvae surviving after 42 days were transferred to artificial diet in a growth chamber having a constant temperature of 50° F, most of them reached the third and fourth instars within a period of 42 days. Surviving larvae were then transferred to individual diet containing medicine cups at room temperature. Most of the third-instar corn earworm and fall armyworm larvae fed and continued development and pupated in between 14 to 17 days. Total elapsed period from first-instar larva to pupation was 98 days, whereas at room temperatures, it is about 18-21 days.

Inducing Pupation of Diapausing Southwestern Corn Borer Larvae

The technique used to induce pupation of diapausing southwestern corn borer larvae was a modification of that used by Hensley (1960). Data on the use of medicine cups, petri dishes, and glass shell vials as pupation chambers, using overwintering diapausing larvae are presented in Table 20. The use of glass shell vials for this purpose is shown in Fig. 6. First pupation was observed in the petri dishes 29

Table 18. Extension of corn earworm larval period during storage on artificial diet at low temperatures.

No. larvae per cup	No. 1st-instar larvae	No. of larvae alive after - days					
		7	14	21	28	35	42
5	50	22	22	15	11	8	7
10	100	51	32	23	10	9	7
15	150	70	41	20	9	9	6
20	200	73	57	25	11	8	7
25	250	76	32	14	10	10	10

Table 19. Extension of fall armyworm larval period during storage on artificial diet at low temperatures.

No. larvae per cup	No. 1st-instar larvae	No. of larvae alive after - days					
		7	14	21	28	35	42
10	100	88	71	46	19	18	9
20	200	163	113	75	49	24	13
30	300	223	164	88	61	35	23
40	400	290	180	121	84	46	30
50	500	356	221	109	100	59	34

Table 20. Observation on induction of pupation of diapausing southwestern corn borer larvae collected from corn stalks in the spring in 3 different types of chambers.[1/]

Observed pupation date [2/]	Medicine cups		Petri dishes		Glass shell vials	
	No. of pupae	Percent pupation	No. of pupae	Percent pupation	No. of pupae	Percent pupation
April 13	0	0.0	3	8.3	0	0.0
April 20	2	5.5	21	58.3	13	36.1
April 27	20	55.5	8	22.2	12	33.3
May 4	7	19.4	2	5.5	4	11.1
May 11	1	2.7	1	2.7	2	5.5

[1/] 36 chambers of each type, each with one larva.

[2/] Experiment started March 15.

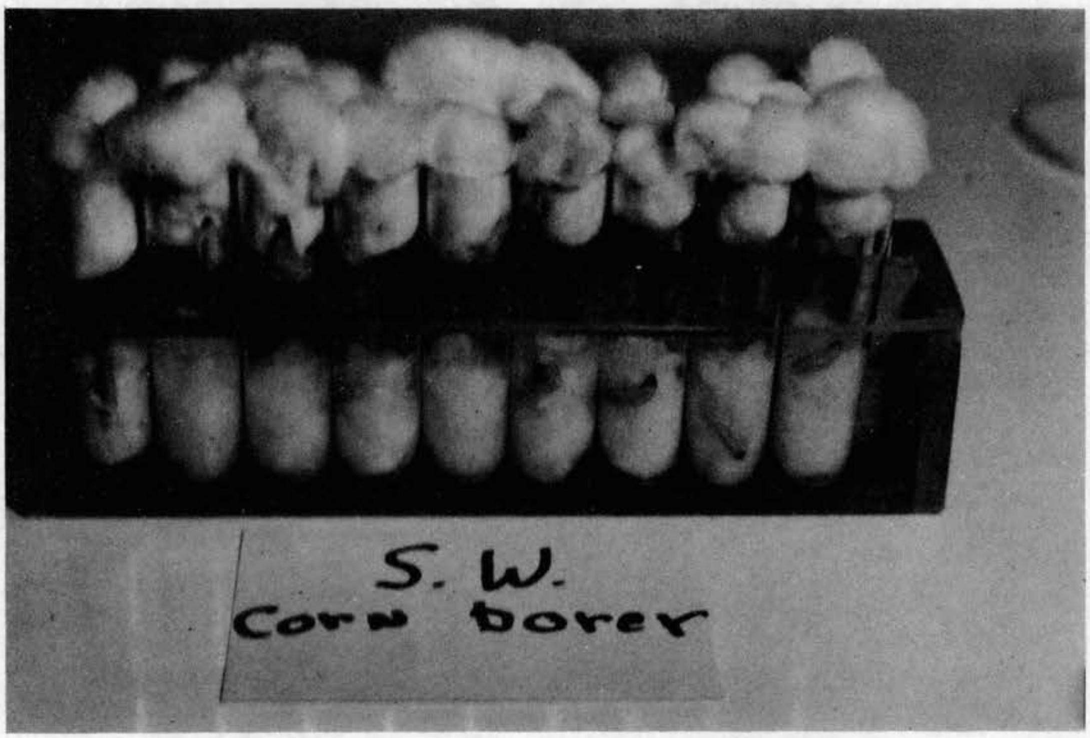

Fig. 6. Glass shell vials and cotton used for inducing pupation of southwestern corn borer larvae.

days after the start of the experiment. Highest pupation percentages in the petri dishes and glass shell vials occurred in 36 days and in 42 days in the medicine cups. Pupation induction was successful by use of all these types of chambers. Although the highest pupation percentage was obtained by use of petri dishes, either type of chamber could be used successfully and more than one larva could be used per chamber.

An experiment was conducted using medicine cups on pupation induction of diapausing southwestern corn borer larvae collected from sorghum plants in the fall. These data are presented in Table 21. First pupation was observed 42 days after the larvae were placed in the medicine cups, and highest pupation percentage was observed 56 days after the start of the experiment. A total of 102 of the 120 larvae, or 85.0 percent, pupated over a period of 28 days. Data obtained in medicine cups using fall-collected diapausing larvae from sorghum plants are about the same as that obtained when overwintering larvae were used.

Data are presented in Table 22 to show that pupation induction of diapausing laboratory-reared larvae could be obtained by use of the technique employing medicine cups. A pupation percentage of 88.3 involving 106 pupae from 120 larvae was obtained. First pupation occurred in 44 days, and maximum pupation occurred in 58 days.

From the data presented in Tables 20, 21, and 22, it is apparent that pupation of diapausing southwestern corn borer larvae can be obtained by use of several types of chambers and that results were similar regardless of the source of diapausing larvae.

Table 21. Observation on pupation induction of diapausing southwestern corn borer larvae, collected from sorghum stalks in the fall, in medicine cups.[1]

Observed pupation date [2]	No. pupae [3]	Percent pupation
December 9	8	6.6
December 16	21	17.5
December 23	62	51.6
December 30	7	5.8
January 6	4	3.3
January 13	0	0.0
Total	102	85.0

[1]One larva per cup.

[2]Experiment started October 28.

[3]Initially 120 larvae

Table 22. Observation on induction of pupation of diapausing laboratory reared southwestern corn borer larvae in medicine cups.[1]

Observed pupation date [2]	No. pupae [3]	Percent pupation
February 28	10	8.3
March 7	34	28.3
March 14	40	33.3
March 21	16	13.3
March 28	6	5.0
Total	106	88.3

[1]One larva per cup.

[2]Experiment started January 15.

[3]Initially 120 larvae.

Induction of Diapause of Southwestern Corn Borer Larvae

By the diapause induction technique employed here, a method of saving southwestern corn borer larvae for future use is available. As shown in Table 23, 383 of 550 or 69.3% of the larvae entered diapause by the end of 36 days. Sixty-five remained in the spotted summer-form, and 74 pupated. It is probable that many of the 65 summer-form larvae would have entered diapause if observations had been continued beyond the 36-day observation date. Figure 7 shows plastic petri dishes and medicine cups containing dry artificial diet used to induce diapause in southwestern corn borer larvae.

Preliminary Screening of Sorghum Varieties and Hybrids for Resistance to Three Lepidopterous Insects

The first screening test to evaluate 144 sorghum varieties for resistance to the corn earworm and fall armyworm and 75 varieties for resistance to the southwestern corn borer gave some satisfactory results. Evaluation of varying degrees of resistance was based on two criteria: (1) leaf injury rating and (2) head injury rating.

Resistance to Corn Earworm (Field Infestation)

The data on resistance obtained from field tests were difficult to evaluate by statistical analysis. However, differences in reaction of the entries to infestation are shown by use of frequency histograms of the data on the two criteria, as shown in Figs. 8 and 9. Because there was no information on resistance of sorghums to the corn earworm, no resistant check could be used in the tests. Therefore, based on observed damage to the plants, arbitrary selections of plants for resistance in the two rating categories were as follows: (1) leaf injury, 0-2; and (2) head injury, 0-2. On this basis, 40 of 144 entries were

Table 23. Diapause induction of laboratory-reared southwestern corn borer larvae observed at end of 36-day period.

Test no.	No. larvae started	Diapausing larvae No.	Diapausing larvae Percent	No. dead larvae	No. larvae pupating	No. larvae unchanged
1	200	132	66.0	16	28	24
2	150	110	73.3	9	11	20
3	200	141	70.5	3	35	21
Totals and Averages	550	383	69.6	28	74	65

Fig. 7. Plastic petri dishes and medicine cups with dry artificial diet used to induce diapause in southwestern corn borer larvae.

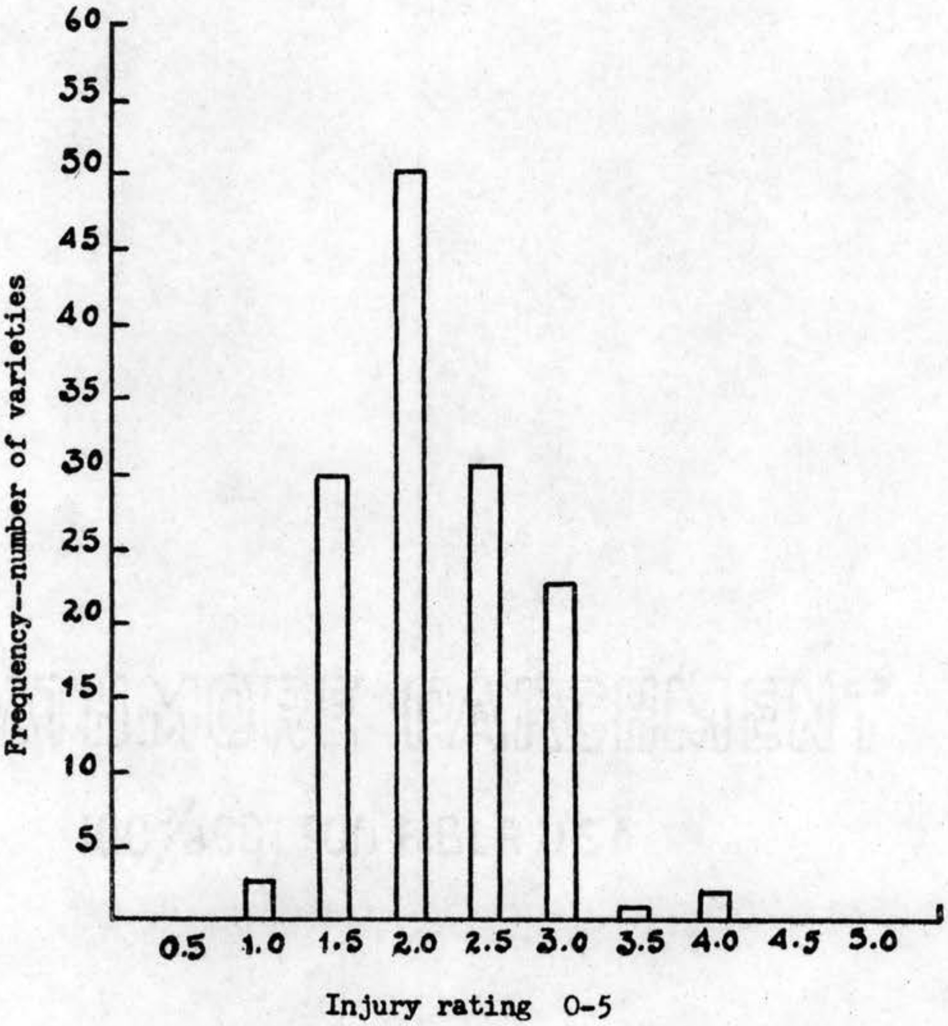

Fig. 8. Injury rating of sorghum leaves damaged by the corn earworm 14 days after initial whorl infestation.

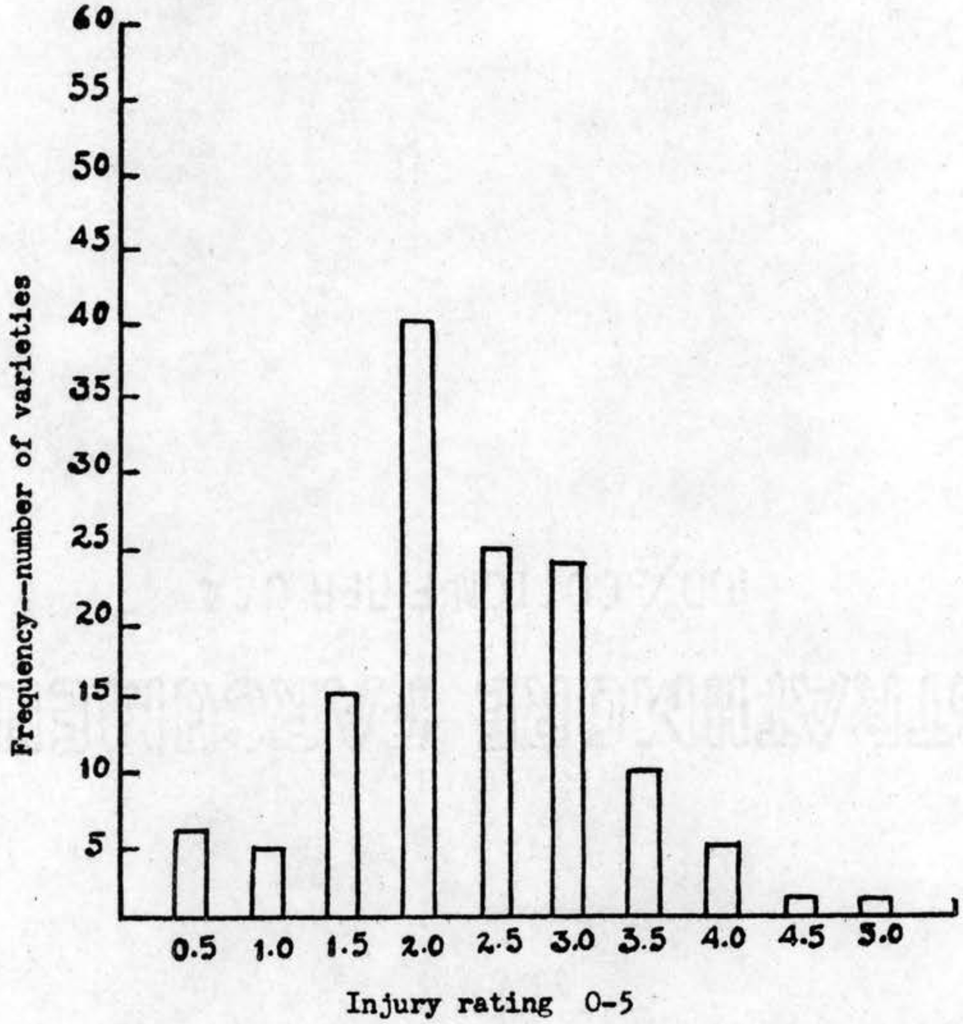

Fig. 9. Injury rating of sorghum heads damaged by the corn earworm 20 days after initial head infestation.

selected as showing resistance to the corn earworm (Table 24). Entries with damage ratings higher than 2.0 were considered susceptible and were discarded.

From the data presented in Table 23 it is apparent that some degree of resistance is shown among the 144 entries tested. However, because little information was available on field manual infestation, definite conclusions regarding resistance could not be drawn from the data presented. The plants were in the late whorl stage of development when they were infested with larvae, and more recent data show that infestation with "black-head" stage eggs at an earlier stage of plant development results in better establishment of larvae on the plants.

Resistance to Corn Earworm (Greenhouse Infestation)

A test designed to obtain data on stage of development of sorghum plants which would be most attractive for feeding and establishment by first-instar corn earworm larvae was conducted in the greenhouse. Sweet Milo, Corneous Durra, and Chiltex No. 874 were planted in 8-inch pots (3 plants per pot), and there were six replicates of each. There were 4 planting dates at 10-day intervals, so that plants were 10, 20, 30, and 40 days old when infested. Each plant in each of the age groups was infested with 5 first-instar larvae when the youngest plants were 10 days old. Ratings for leaf injury and numbers of leaves damaged were made 3 weeks after infestation. Data in this regard are presented in Table 25. On the basis of amount of feeding and injury, it is indicated that plants 20-30 days old are more satisfactory for larval establishment and development.

The above information is of importance in connection with resistance studies. Preliminary data had shown that corn earworms would not

Table 24. Reaction of 40 manually infested grain sorghums of 144 tested showing resistance to corn earworm attack.

Entry No.	Variety	Average leaf injury rating-days after infestation			Total no. larvae on 5 heads	Average no. per head
		Whorl		Head		
		7 days	14 days	20 days		
5	Rest. Milo T.S. 338	1.0	1.0	1.4	9	1.8
16	Martin	1.0	1.0	2.0	13	2.6
67	Shantung Kaoling	1.0	1.2	1.0	7	1.4
17	White Martin	1.0	1.2	1.4	8	1.6
91	Hydro Kafir	1.0	1.2	1.4	8	1.6
32	Cache Feterita	1.0	1.2	1.4	3	0.6
22	Quadroon	1.0	1.2	1.6	5	1.0
56	Combine Kafir 60	1.0	1.2	1.6	4	0.8
116	Corneous Sorghum	1.0	1.2	1.8	2	0.4
47	Early Hegari T.S. 252	1.0	1.2	1.8	6	1.2
44	Premo	1.0	1.4	1.2	0	0.0
35	Red Feterita	1.2	1.4	1.6	2	0.4
110	Witch Weed Rest. Kafir	1.0	1.4	1.6	5	1.0
115	Tall White Sorghum	1.2	1.4	1.6	4	0.8
14	Caprock	1.0	1.4	2.0	13	2.6
125	Leoti	1.2	1.6	0.0	0	0.0

Table 24 (Continued)

Entry No.	Variety	Average leaf injury rating-days after infestation			Total no. larvae on 5 heads	Average no. per head
		Whorl		Head		
		7 days	14 days	20 days		
34	White Feterita	1.2	1.4	2.0	5	1.0
43	Chiltex No. 874	1.2	1.6	1.0	0	0.0
64	Schrock DD	1.2	1.6	1.0	4	0.8
51	Kafir No. 812	1.2	1.6	1.6	3	0.6
61	Darset	1.2	1.6	1.8	6	1.2
122	Rancher	1.2	1.8	0.0	1	0.2
124	Red Amber	1.2	1.8	0.0	0	0.0
7	Wheatland	1.4	1.8	1.0	7	1.4
3	DD White Sooner	1.0	1.8	1.2	16	3.2
50	Kafirita No. 811	1.2	1.8	1.2	2	0.4
137	Rox Orange	1.2	1.8	1.2	0	0.0
10	Plainsman	1.6	1.8	1.6	13	2.6
18	Kafir x Milo 8-28	1.4	1.8	1.8	0	0.0
19	Edwards Combine	1.4	1.8	1.8	12	2.4
25	Kalo No. 902	1.4	1.8	1.8	4	0.8
93	Pearl Kafir	1.2	1.8	1.8	11	2.2
117	Grahoma	1.2	1.8	1.8	19	3.8

Table 24 (Continued)

Entry No.	Variety	Average leaf injury rating-days after infestation			Total no. larvae on 5 heads	Average no. per head
		Whorl		Head		
		7 days	14 days	20 days		
29	Dwarf Feterita 3-1	1.2	1.8	2.0	5	1.0
107	Red Kafir	1.2	1.8	2.0	0	0.0
48	Hegari No. 750	1.0	2.0	1.2	5	1.0
134	Sumac 1712	1.2	2.0	1.2	0	0.0
133	Sumac 63715	1.0	1.0	1.6	0	0.0
74	Wonder Club	1.4	2.0	2.0	6	1.2
140	Tan Sugar Drip	1.4	2.0	2.0	0	0.0

Table 25. Effect of stage of sorghum plant development on survival and establishment of corn earworm infestation.

Variety	Age of plants when infested (days)							
	10		20		30		40	
	Avg. injury rating	No. of damaged leaves	Avg. injury rating	No. of damaged leaves	Avg. injury rating	No. of damaged leaves	Avg. injury rating	No. of damaged leaves
Sweet Milo	2.42	4.17	1.58	3.83	2.58	5.58	1.25	2.25
Corneous Durra	1.88	4.77	3.00	6.00	2.20	6.20	1.23	2.00
Chiltex No. 874	2.25	4.16	2.33	5.16	2.78	5.73	1.41	2.58
Total	6.54	13.10	6.91	14.99	7.56	17.51	3.88	6.83
Average	2.18	4.31	2.30	4.99	2.52	5.84	1.29	2.27

survive on seedling plants (up to 7 days old), and plants approaching head exsertion, likewise, were not attractive for leaf feeding.

Resistance to Fall Armyworm (Field Infestation)

Differences in reaction of sorghum entries to fall armyworm infestation were observed under field infestation conditions, but for the same reason discussed above for corn earworm infestations, the results were difficult to evaluate. Data in this regard on 34 entries showing resistance out of the 144 tested, are presented in Table 25. The remainder of the entries were discarded as being susceptible. Data on the 144 entries with respect to leaf injury ratings are presented in Fig. 10, and for head injury in Fig. 11.

It is apparent from the data in Table 26 and Figs. 9 and 10 that there were differences in reaction to fall armyworm infestation. However, as was the case with the corn earworm infestations, further studies are necessary on technique before definite conclusions with respect to resistance and susceptibility can be drawn.

Resistance to Fall Armyworm (Greenhouse Infestation)

First-instar fall armyworm larvae fed and became established on seedling sorghum test plants about 7 days old (3-5 leaf stage of growth) in the greenhouse. Apparently, growth inhibitors or unsatisfactory nutrients in the young plants that prevented feeding on seedling plants by the corn earworm and southwestern corn borer did not affect the fall armyworm larvae. Data on the reaction of 46 entries of 144 tested having leaf injury ratings of 4.00 or less, 3 and 5 days after infestation, (based on rating scale of 0-10) are presented in Table 27. The data on reaction of the 144 entries, 3 and 5 days after infestation

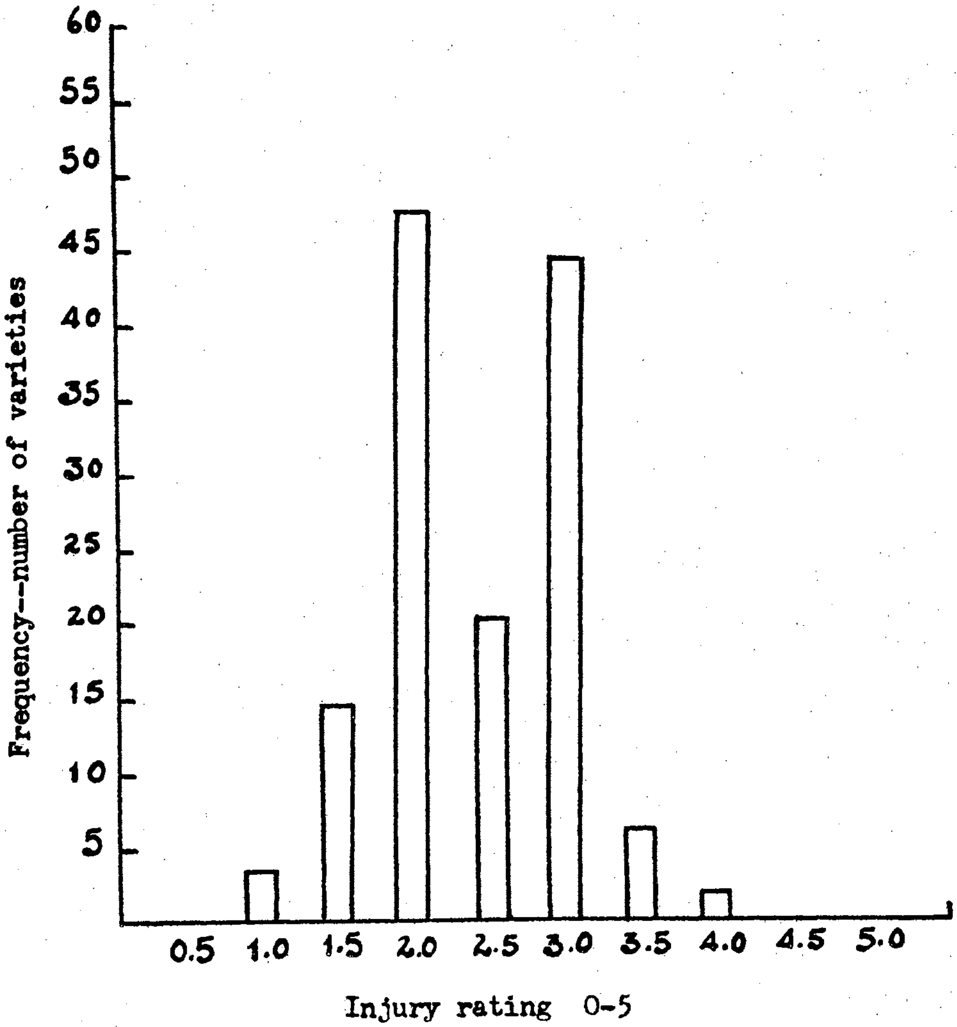

Fig. 10. Injury rating of sorghum leaves damaged by the fall armyworm 14 days after initial whorl infestation.

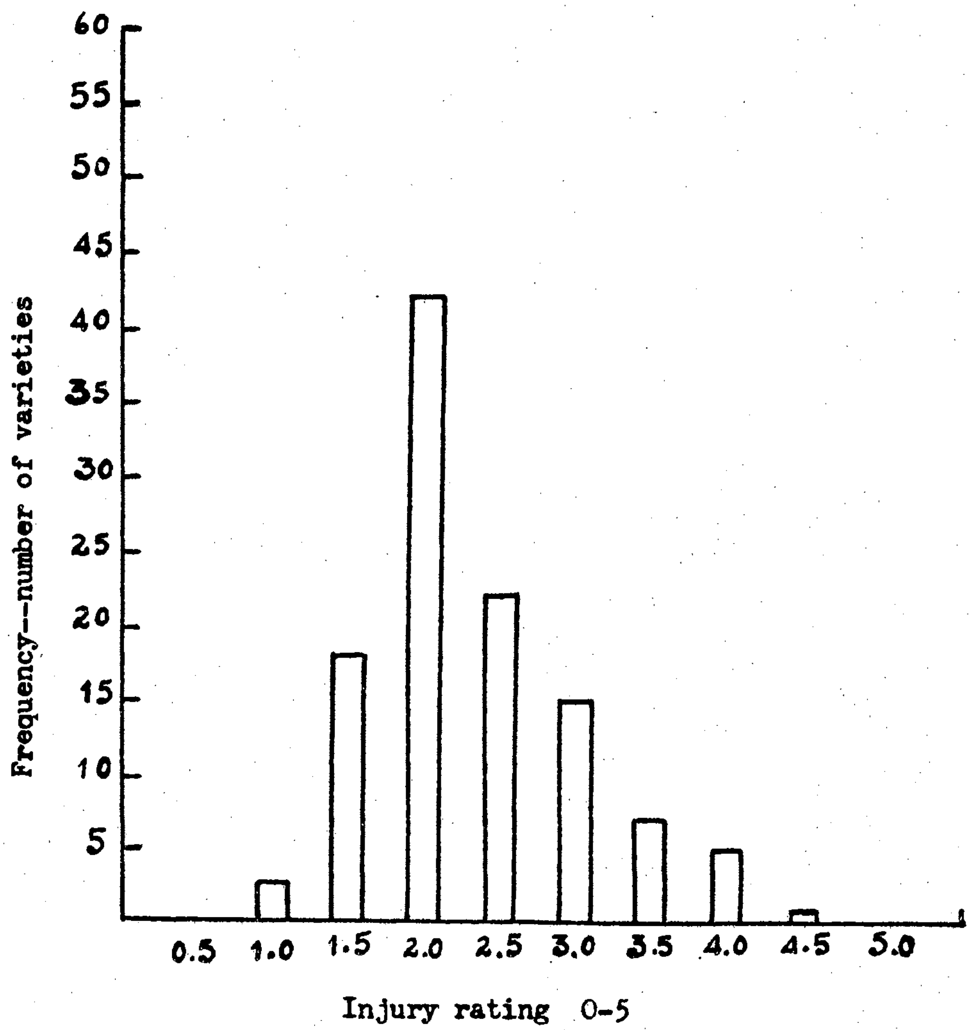

Fig. 11. Injury rating of sorghum heads damaged by the fall armyworm 20 days after initial head infestation.

Table 26. Reaction of 34 manually infested grain sorghums of 144 tested, showing resistance to fall armyworm attack.

Entry No.	Variety	Average leaf injury rating-days after infestation			No. larvae on 5 heads	Average no. larvae per head
		Whorl		Head		
		7 days	14 days	20 days		
33	Feterita No. 693	1.0	1.0	1.6	5	1.0
14	Caprock	1.2	1.2	1.4	5	1.4
12	Redbine	1.2	1.2	2.0	7	1.4
90	Reed Kafir	1.0	1.2	2.0	1	0.2
138	White African	1.2	1.4	1.4	2	0.4
82	Weskan 1117	1.2	1.4	1.6	4	0.8
104	Early Dwarf Red Kafir	1.0	1.4	1.6	0	0.0
109	Tall Red Kafir 7	1.0	1.4	1.6	12	2.4
16	Martin	1.4	1.4	2.0	6	1.2
110	Witch Weed Rest. Kafir	1.2	1.4	2.0	3	0.6
121	Early Amber	1.0	1.6	1.0	2	0.4
134	Sumac 1712	1.6	1.6	1.2	5	1.0
131	Red X	1.4	1.6	1.2	4	0.8
112	Greeley Kafir	1.2	1.6	1.6	0	0.0
34	White Feterita	1.4	1.6	2.0	11	2.2
17	White Martin	1.4	1.6	2.0	6	1.2
113	Tricher	1.0	1.8	1.8	13	2.6

Table 26 (Continued)

Entry No.	Variety	Average leaf injury rating-days after infestation			No. larvae on 5 heads	Average no. larvae per head
		Whorl		Head		
		7 days	14 days	20 days		
125	Leoti	1.8	1.8	1.8	4	0.8
46	Bontia	1.6	2.0	1.0	5	1.0
50	Kafirita No. 811	1.0	2.0	1.0	6	1.2
39	Banar Durra	1.0	2.0	1.2	1	0.2
51	Kafir No. 812	1.2	2.0	1.2	6	1.2
63	Sagrain	1.2	2.0	1.2	11	2.2
124	Red Amber	1.6	2.0	1.2	8	1.6
4	Ryer Milo No. 15	1.0	2.0	1.4	6	1.2
37	Red Durra	1.6	2.0	1.4	7	1.4
15	Midland	1.0	2.0	1.6	10	2.0
102	Pink Kafir	1.0	2.0	1.6	3	0.6
2	DD Yellow Sooner	1.0	2.0	1.8	16	3.2
94	Corneous Kafir	1.6	2.0	1.8	11	2.2
115	Tall White Sorghum	1.0	2.0	1.8	7	1.4
10	Plainsman	1.2	2.0	2.0	11	2.2
29	Dwarf Feterita 3-1	1.0	2.0	2.0	10	2.0
132	Sumac 35038	1.6	2.0	2.0	2	0.4

Table 27. Reactions of 46 sorghums to manual infestation with first-instar larvae in the seedling stage out of 144 tested in the greenhouse.

Entry No.	Variety	Average leaf injury rating-days after infestation		Average no. larvae per plant after 5 days	Average larval weight after 5 days (mg)
		3 days	5 days		
42	Corneous Durra	2.00	3.00	0.28	0.00140
102	Pink Kafir	2.50	3.10	0.40	.00080
43	Chiltex #874	2.69	3.15	0.23	.00056
15	Midland	2.33	3.18	1.00	.00136
18	Kafir x Milo 8-28	2.63	3.18	0.72	.00097
2	DD Yellow Sooner	2.08	3.25	1.16	.00158
60	Darso OK #1	2.55	3.30	1.00	.00280
45	Ajax	2.30	3.38	0.30	.00140
76	Cody	2.40	3.40	0.50	.00060
6	Bonar x Day 4	2.50	3.41	1.41	.00066
61	Darset	2.55	3.44	1.00	.00097
59	Darso 615	2.60	3.45	1.00	.00079
21	Norghum	2.60	3.45	0.41	.00134
11	Redbine 66	2.46	3.54	1.15	.00268
3	DD White Sooner	2.90	3.60	1.40	.00134
80	Stand Bkhl Kafir #71	2.54	3.63	0.63	.00110
81	Stand Bkhl Kafir #204	2.90	3.63	1.18	.00092

Table 27 (Continued)

Entry No.	Variety	Average leaf injury rating-days after infestation		Average no. larvae per plant after 5 days	Average larval weight after 5 days (mg)
		3 days	5 days		
101	White African 665	2.66	3.66	1.00	.00084
110	Witch Weed Rest. Kafir	2.91	3.66	1.60	.00077
8	White Wheatland	2.00	3.67	1.00	.00220
113	Tricher	2.84	3.69	0.76	.00097
23	Sweet Milo	2.28	3.71	0.71	.00188
7	Wheatland	2.00	3.75	0.66	.00201
56	Combine Kafir 60	3.54	3.75	0.83	.00122
58	White Darso	3.16	3.75	0.80	.00122
104	Early Dwf. Red Kafir	2.62	3.75	0.75	.00155
117	Grahoma	3.00	3.75	0.58	.00114
121	Early Amber	3.41	3.75	0.25	.00110
44	Premo	2.84	3.76	0.30	.00040
16	Martin	2.09	3.82	0.90	.00112
57	Dwarf Kafir 44-14	3.09	3.85	0.61	.00126
85	Sharon Kafir	2.88	3.88	1.44	.00022
86	Santa Fe Kafir	3.10	3.90	1.10	.00113
78	Dawn-Ko XM	2.58	3.91	0.83	.00051
122	Rancher	3.25	3.91	0.50	.00057

Table 27 (Continued)

Entry No.	Variety	Average leaf injury rating-days after infestation		Average no. larvae per plant after 5 days	Average larval weight after 5 days (mg)
		3 days	5 days		
5	Rest. Milo ts 338	2.66	4.00	1.20	.00145
10	Plainsman	2.63	4.00	0.41	.00214
50	Kafirita #811	2.76	4.00	0.61	.00173
63	Sagrain	3.54	4.00	0.16	.00065
65	Tecti x Feterita	3.83	4.00	0.25	.00143
75	Club Kafir	3.09	4.00	1.00	.00124
77	Pierce Kafir	3.00	4.00	1.09	.00096
91	Hydro Kafir	3.00	4.00	1.09	.00123
97	Atlas	3.16	4.00	0.66	.00062
116	Corneous Sorghum	2.91	4.00	0.66	.00110
123	Black Amber	2.41	4.00	0.50	.00075

are presented in Fig. 12 and 5 days after in Fig. 13.

Based on the data presented in Table 27 and Figs. 12 and 13, rather marked resistance to the fall armyworm is shown. Entries with leaf injury rating of not more than 4 and with not more than two larvae per plant 5 days after infestations were considered as having a high degree of resistance. The remainder of the 144 entries were discarded as being susceptible. Average larval weights (Table 27) also may be used as a measure of resistance. Generally, plants rated as having the highest degree of resistance had the lowest number of surviving larvae and lower larval weights. Many of the susceptible plants had larger numbers of larvae due to migration from resistant plants, and they were almost completely destroyed.

From the data presented, it is evident that screening for resistance to the fall armyworm can be done on plants in the seedling stage infested with first-instar larvae. This technique will be employed in future tests for resistance of sorghums to the fall armyworm.

Resistance to Southwestern Corn Borer (Field Infestation)

Although sorghum is not a major host of the southwestern corn borer, infestations do occur occasionally, and damage is sometimes severe. The borer life cycle on sorghum is usually longer than on corn. Rolston (1955) found that at 80^{o} F the larval period on corn and sorghum plants was 36 and 49 days, respectively. Painter (1951) stated that the southwestern corn borer could develop on sorghums in Kansas, but that the larvae averaged only about one-half the size of those developing on corn. Gifford et al. (1961) reported that borer survival on sorghums was never higher than 26.5%, but that they could complete their life cycle on sorghum. Since the borer does infest

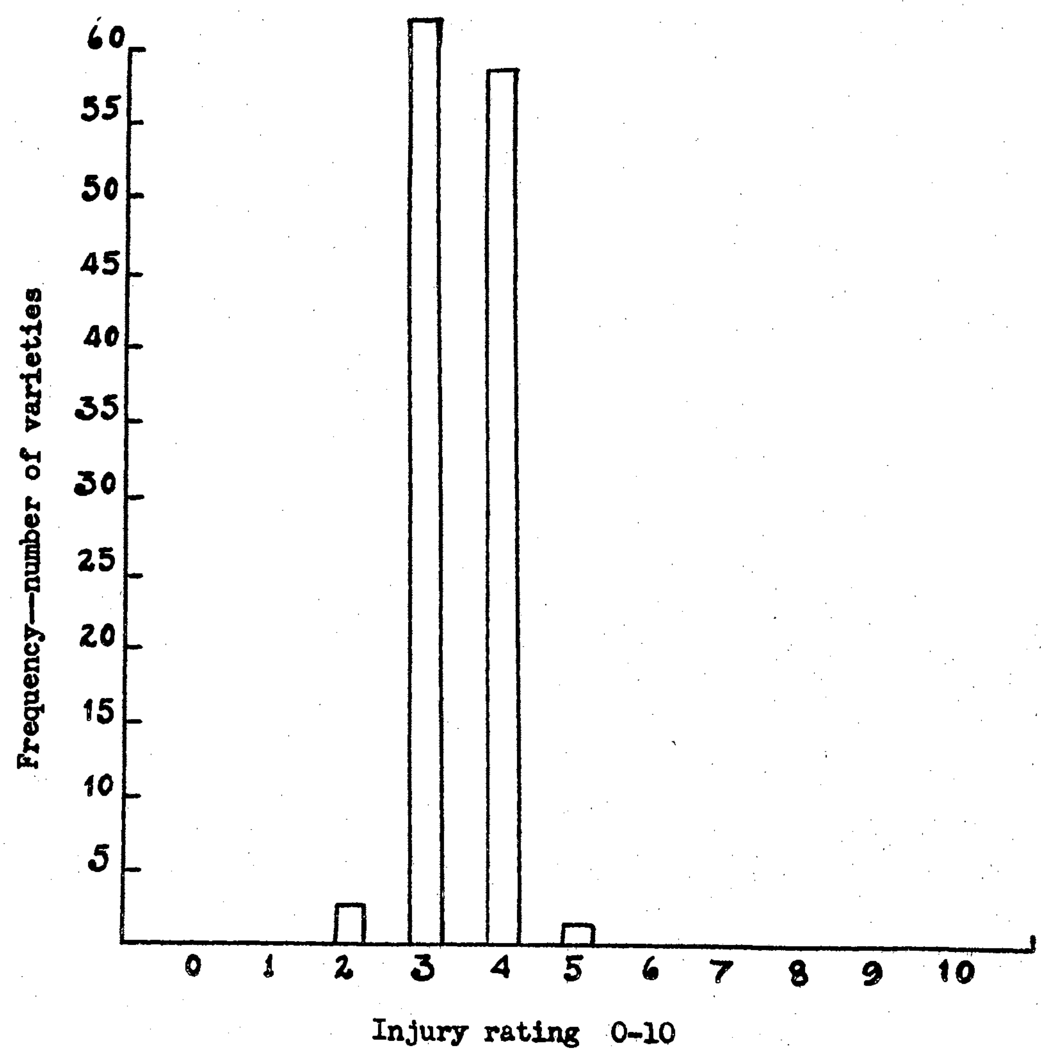

Fig. 12. Injury rating of sorghum seedlings at 3 days after infestation by first instar larvae of the fall armyworm.

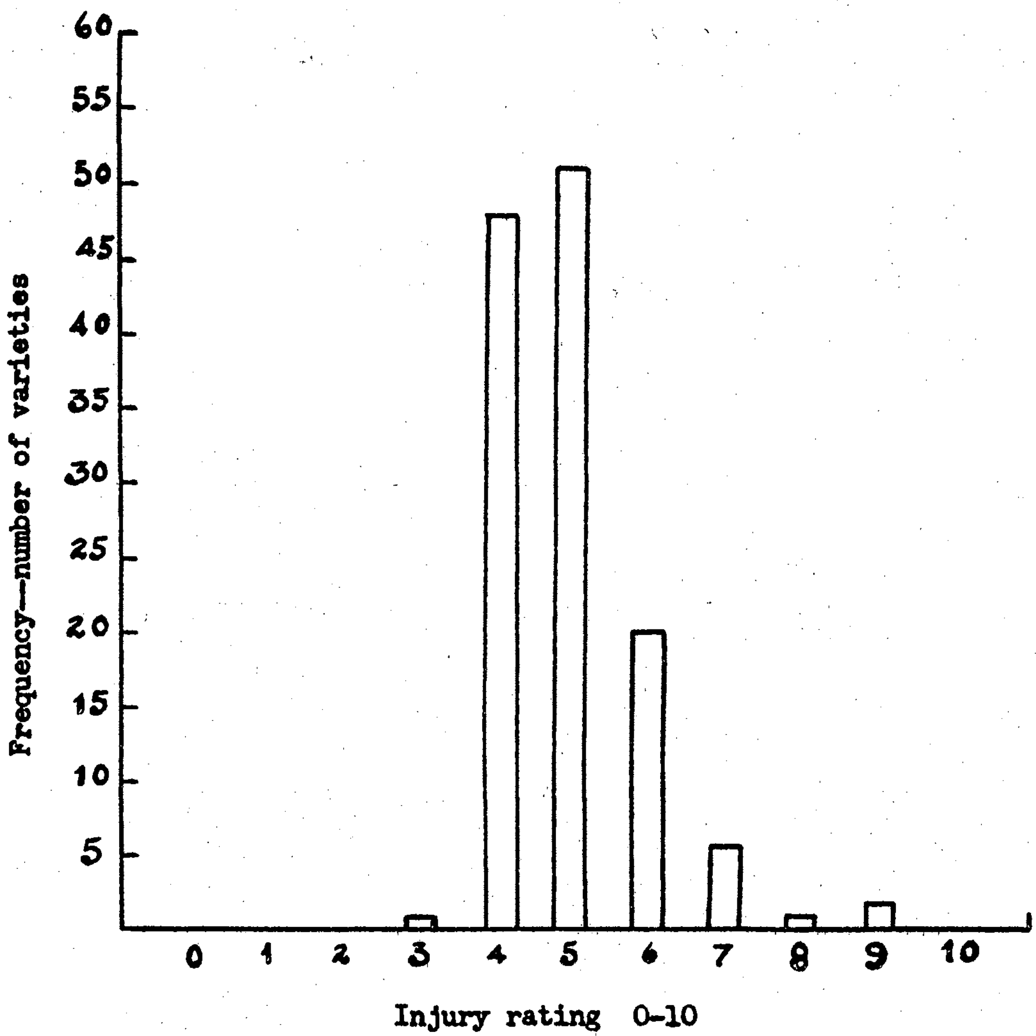

Fig. 13. Injury rating of sorghum seedlings at 5 days after infestation by first instar larvae of the fall armyworm.

sorghums occasionally, an attempt was made to test 75 varieties for resistance by applying "black-head" stage eggs on waxed paper to whorls at the rate of 25 per plant.

Leaf injury ratings were made 7 and 14 days after infestation. On the basis of a rating scale of 0-5, lines having a rating of no more than 1.5 or 1.6 were considered as showing resistance. There were 20 of the 75 tested in this category, as shown in Table 28. Data in this regard are also presented in Fig. 14.

As a result of the manual infestation with "black-head" stage eggs, all infested plants showed leaf feeding, and most of them were girdled in the fall. When plant dissections were made in October, 47% of the stalks were found having larvae in the immaculate form.

The above data show that manually infesting sorghums in the whorl stage of plant growth with "black-head" stage eggs is a satisfactory technique for testing for resistance to the southwestern corn borer.

Resistance to Southwestern Corn Borer (Greenhouse Infestation)

A test designed to obtain data on the stages of sorghum plant development which would be most attractive for feeding and establishment of southwestern corn borers was conducted in the greenhouse. The technique was the same as that previously described for the corn earworm greenhouse infestation test. The result of the test involving infesting 10-, 20-, 30-, and 40-day-old plants are presented in Table 29. Thirty-day old plants had the highest average leaf injury rating and the highest average number of damaged leaves. These data show that in testing sorghum lines for resistance to the southwestern corn borer, best results can be obtained by infesting the whorls of plants when they are between 20 and 30 days old.

Table 28. Reaction of 20 manually infested grain sorghums of 75 tested, showing resistance to southwestern corn borer attack.

Entry No.	Variety	Average leaf injury rating-days after infestation		Total no. immaculate larvae	No. of 5 examined stems showing damage
		7 days	14 days		
33	Feterita	1.0	1.0	0	0
34	White Feterita	1.0	1.0	1	2
81	Standard Bkhl Kafir No. 204	1.0	1.0	0	1
130	African Millet Sorgo	1.0	1.0	1	1
132	Sumac 35038	1.0	1.0	3	3
95	Dawn Kafir	1.0	1.2	3	3
100	White Kafir	1.0	1.2	2	2
107	Red Kafir	1.0	1.4	2	4
113	Tricher	1.0	1.4	4	4
88	Lowe's Bkhl Kafir	1.0	1.4	1	1
94	Corneous Kafir	1.0	1.4	4	4
102	Pink Kafir	1.0	1.4	5	5
108	Red Kafir C.I. 34	1.0	1.6	3	3
43	Chiltex No. 874	1.0	1.6	2	4
84	Texas Bkhl Kafir	1.0	1.6	2	3
62	Combine Sagrain	1.0	1.6	2	3
99	Ellis	1.0	1.6	3	3
55	Dwarf Kafir 24-43	1.0	1.6	4	5
117	Grahoma	1.0	1.6	1	3
65	Tecti x Feterita	1.4	1.6	0	0

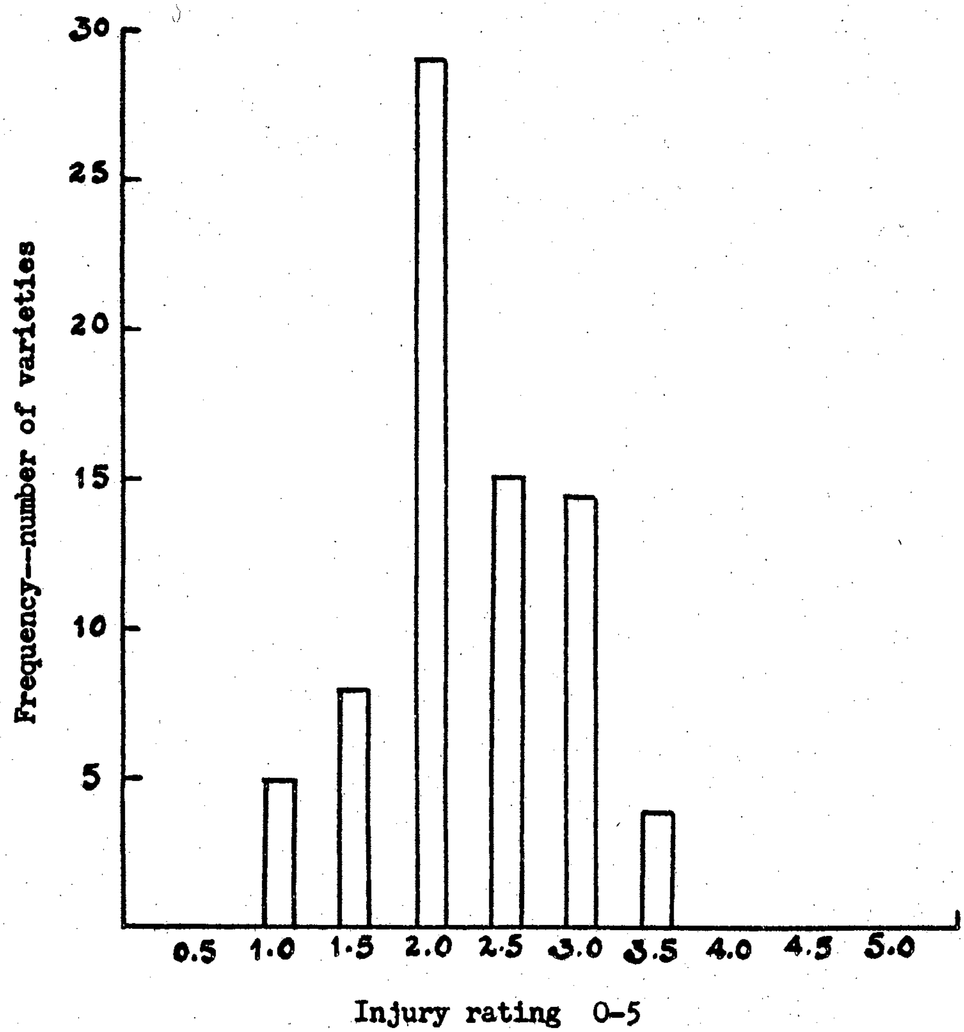

Fig. 14. Injury rating of sorghum leaves damaged by the southwestern corn borer 14 days after initial whorl infestation.

Table 29. Effect of stage of sorghum plant development on survival and establishment of southwestern corn borer.

Variety	Age of plants when infested (days)							
	10		20		30		40	
	Avg. leaf injury rating	No. damaged leaves	Avg. leaf injury rating	No. damaged leaves	Avg. leaf injury rating	No. damaged leaves	Avg. leaf injury rating	No. damaged leaves
Sweet Milo	0.86	1.26	1.50	2.05	1.50	1.75	0.87	0.87
Corneous Durra	2.25	2.90	1.90	3.27	2.33	3.83	1.20	2.00
Chiltex no. 874	0.93	1.80	1.00	1.27	2.33	3.40	2.16	3.33
Total	4.04	5.96	4.40	6.59	6.16	8.98	4.23	6.20
Average	1.35	1.99	1.46	2.19	2.05	2.99	1.41	2.06

SUMMARY AND CONCLUSIONS

The studies showed that the corn earworm, fall armyworm, and southwestern corn borer could be produced successfully in mass numbers on an artificial diet in the laboratory. Paper strips measuring 1½ by 2 inches laid on top of the screen-top oviposition chamber were satisfactory as an oviposition site for the corn earworm. Waxed paper was used for fall armyworm and southwestern corn borer oviposition. Egg production by the three species of moths was as follows: corn earworm - 50 moths produced 16,610 eggs over a 5-day observation period, an average of 332 per female; fall armyworm - 803 egg masses were produced by 82 females over a 5-day observation period, an average of 9.8 masses per female, or an average of 133.8 first-instar larvae per mass. A total of 107,441 first-instar larvae were produced by 82 females in this test; southwestern corn borer - 72 females produced 12,179 eggs, an average of 169 eggs per female.

Based on previous biological studies on oviposition and development of the larvae of the three species, studies were conducted in the laboratory on calculated and actual pupation and moth emergence dates. The calculated pupation and moth emergence dates were quite similar to the actual dates. It is now possible to plan an experiment so that eggs and larvae will be available when needed for manual plant infestations.

Studies conducted on rearing chambers showed that 1-ounce medicine cups containing artificial diet were satisfactory for rearing larvae of the corn earworm, fall armyworm, and southwestern corn borer. Numbers of first-instar larvae started per cup giving satisfactory results were: corn earworm, 1; fall armyworm, 3; and southwestern corn borer, 2.

The standard wheat germ larval diet contains a commercially prepared vitamin complex fortification mixture which is expensive (currently $9.10 per pound). Tests showed that substitution of relatively inexpensive brewer's yeast (currently 63¢ per pound), which contains most of the vitamins found in the vitamin complex fortification mixture, but in different amounts, might be practical. The highest rate of brewer's yeast tested (72 g per 3600 g wheat germ diet) produced 52.5 percent pupation of corn earworm larvae, whereas the standard diet produced 74 percent pupation. Pupation percentages and pupal weights increased as the amount of brewer's yeast in the diet was increased. On this basis, it is possible that further tests using larger amounts of brewer's yeast would result in a relatively inexpensive, satisfactory diet.

In tests using 5 ml propionic acid per 3600 g of wheat germ diet as an antimicrobial agent to control contaminating microorganisms, satisfactory pupation of corn earworm larvae was obtained. Pupal weights were reduced slightly, but development was not affected. Similar results were obtained with the fall armyworm. However, addition to the diet of more than 5 ml was found to be toxic to both insects.

The larval period of the corn earworm and fall armyworm was prolonged to 98 days by use of fluctuating low storage temperatures, whereas, at room temperatures it was 18 to 21 days. This technique is useful for holding larvae until needed at a later date.

Pupation of diapausing southwestern corn borer larvae was obtained in the laboratory by placing them on wet absorbent cotton in several types of containers such as petri-dishes, medicine cups, and glass shell vials. Pupation percentages in the respective containers were 97.2, 91.6, and 86.1.

Diapause of southwestern corn borer larvae was induced by keeping them at room temperature on a diet containing 90, instead of 80, grams of agar and at low relative humidity in either petri dishes or medicine cups. About 70 percent of the larvae entered diapause after 36 days under these conditions.

In tests designed to determine developmental stage of the insect to use for obtaining maximum infestation of sorghum plants in connection with manual infestation studies, it was found that "black-head" stage eggs (shortly before hatching) gave the best results for the corn earworm, fall armyworm, and southwestern corn borer.

Tests were also conducted in the greenhouse involving infestation with first-instar larvae of the three species to determine stage of plant development to obtain optimum infestation. First-instar larvae of the fall armyworm became established and survived on seedling sorghum plants, but those of the corn earworm and southwestern corn borer did not survive on seedling plants. Sorghum plants 20 to 30 days old were required for establishment and survival of the corn earworm and southwestern corn borer.

Manual infestation of 144 sorghum varieties and hybrids with "black-head" stage eggs of corn earworm and fall armyworm and 75 varieties with the southwestern corn borer eggs produced some differences in reaction among the entries, but definite conclusions regarding resistance could not be drawn from the data presented. These plants were infested in the late-whorl stage of development, and later greenhouse studies showed that better larval establishment and development would have been obtained if infestations had been made in the early-whorl stage. However, the small amount of damage from corn earworm shown by 40 of the 144 entries was considered an indication of resistance in those lines. There were 34 of the 144 entries showing resistance to the fall armyworm, and 20 of 75 showing resistance to the southwestern corn borer.

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