# DEVELOPMENT OF TECHNIQUES FOR MASS PRODUCTION OF LEPIDOPTEROUS LARVAE FOR USE IN CONNECTION WITH SORGHUM INSECT RESISTANCE STUDIES

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# PREFACE

Several artificial diets for rearing insects have entered the entomological picture in the past several years. Very little is known, however, about the effects that would result from rearing insects on these synthetic media.

After conferring with Dr. Harvey L. Chada, and other staff members of the Entomology Department, it was decided that information should be obtained on the development of mass rearing techniques of some lepidopterous sorghum insects, and a comparison made of their biology on natural and artificial diets.

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## INTRODUCTION

A great deal of effort has been exerted in the last few years in breeding crops that are resistant to insects. Due to the low incidence of insect populations during some years, and the fact that this type of work can only be done during the summer months in the field, considerable research along this line is carried on in the greenhouse under controlled conditions. Consequently, it is necessary to maintain cultures of test insects so the work can be carried on throughout the year.

An artificial diet for rearing phytophagous insects is very advantageous. One advantage is the reduced labor and space requirement, since there is no need to grow food plants in a greenhouse for a constant food supply. Also, the artificial diet does not need to be replaced at frequent intervals, as does fresh plant material, due to spoilage. Another advantage is the lesser incidence of undesirable insect pathogens which may be more readily introduced by the use of natural food. Still another important advantage of using artificial diets is that they can be important tools in studying the nutritional requirements of certain insects, since each constituent of the diet may be defined. Data in this regard could enable the plant breeder to breed plants lacking in one or more of the necessary requirements of certain insect species.

Although several artificial diets have been developed for rearing phytophagous insects, there is very little information comparing the biology of insects reared on artificial diets and on natural food. This study was conducted to determine if there were significant differences in the biology of three species of lepidopterous insects that attack grain sorghums when reared on an artificial diet as compared with sorghum as food, and to develop a satisfactory method for laboratory rearing. The test species used were the corn earworm, Heliothis zea (Boddie), the fall armyworm, Laphygma frugiperda (J. E. Smith), and the southwestern corn borer, Zeadiatraea grandiosella (Dyar).

The detailed study of the three species included (1) the most desirable artificial diet for both larvae and adults, (2) the number of larvae that could be reared per container, (3) growth rate, (4) mortality rate, (5) duration of each life stage, (6) reproductive potential of insects reared on the diets, (7) the effects of changing the larval diet at various stages in the larval cycle, and (8) the number of generations that could be reared.

Preliminary tests with artificial diets and sorghum were also conducted on two other lepidopterous species, the tobacco budworm, Heliothis virescens (F.), and the sorghum webworm, Celama sorghiella (Riley). Due to the difficulty in the taxonomic separation of Heliothis virescens and Heliothis zea larvae, it was believed that the possibility existed that H. virescens may attack grain sorghums, and could be mistaken for H. zea in the field. A study was conducted in the greenhouse to determine if H. virescens would become established on sorghum in a test in which H. zea was used as a check.

Field-collected, late-instar larvae of the sorghum webworm fed on an artificial diet, completed pupation, and emerged as adults, but they failed to oviposit in the laboratory. Because of this failure to reproduce, it was not feasible to conduct a comparative study of this insect species.

Because the corn earworm, fall armyworm, and southwestern corn borer are destructive pests of sorghum, the development of varieties, lines, and hybrids having resistance to these pests appears to be the best approach to control and reduction of crop losses. Since sorghums are relatively low value-per-acre crops, insect control with chemicals is not always practical because of the costs of such control practices. Also, sorghum seed and forage are used as food for humans and domestic animals, and toxic residues following the use of chemicals for insect control might be harmful.

Therefore, the research data resulting from the experiments described herein are deemed of importance in the development of a sorghum insect resistance studies program. Results of this research should provide techniques for rearing several lepidopterous larvae in mass numbers for use in screening sorghum varieties, lines, and hybrids for insect resistant germ plasm, which is not now available. Plant breeders provided with such resistant germ plasm, could then make crosses which could result in the development of insect resistant hybrids.

## REVIEW OF LITERATURE

### Rearing insects on plant material

Entomologists have been rearing phytophagous insects for one kind of study or another for many years. The early methods of rearing employed living plants or freshly-cut plant material as food.

Ellisor (1935) reared corn earworms during the winter on corn and rape in a greenhouse. Barber (1936 a) describes a method for rearing corn earworm larvae. He removed young ears of corn from the plants, hung them in a screen cage, and infested each ear with one or two newly-hatched larvae. The larvae developed as well on the cut ears as they did in the field. This method required a great deal of labor, was quite expensive, and the number of insects that could be reared was limited. Callahan (1956, 1962) used metal "egg-crate" type rearing trays containing small sections of corn ears as the larval food. A greater number of larvae could be reared by this method than could be reared using whole ears of corn. Hensley (1960) used greenhouse-grown corn to rear larvae of three species of the *Diatraea* complex in the laboratory. McEwen and Hervey (1960) developed a method for mass rearing the cabbage looper, *Trichoplusia ni* (Hbn.), in the laboratory using broccoli foliage as food.

### Development of artificial diets

More knowledge of the nutritional requirements of phytophagous insects led to the development of artificial diets. Trager (1947), Friend (1958), and House (1961) give very good summaries of those requirements. The first artificial food for rearing larvae of a phytophagous insect was developed by Bottger (1942) for the European corn borer, Ostrinia nubilalis (Hbn.) (formerly Pyrausta nubilalis (Hbn.)). After conducting preliminary tests (Bottger, 1940) and reviewing the literature on the chemical constituents of corn, Bottger (1942) formulated 20 diets containing varying amounts of different materials known to contain these constituents, and various carriers for them. The best results were obtained by using casein as a protein source, glucose and sucrose for the carbohydrate requirements, corn oil for fat, inorganic salts to buffer the pH, agar as a carrier, and vitamins A, $B_1$, and E. Although this was a major development in the field of entomology, the diet needed to be improved, since only 36% of the larvae started on the diet were able to survive to maturity. Those reaching maturity appeared to be normal, but no eggs were deposited by the female moths. This was believed to be due to the small number of adults obtained and the lack of synchronization of adult emergence, which prevented mating.

Beck et al. (1949) developed a semi-synthetic larval diet for the European corn borer which resulted in a higher survival rate. The only plant material it contained was dry powdered corn leaves. Other constituents were glucose, casein, cholesterol, corn oil, Wesson's salts, yeast powder, choline, agar, and cellulose. They reported that corn leaves contained an unknown factor essential to larval growth. Later, studies by Beck and Stauffer (1950) and Beck (1950, 1953) were conducted to develop an aseptic method for rearing the larvae, to determine the effects of omitting single constituents from the diet, and to determine the nature of the corn leaf factor in the diet.

After the successful development of these first diets, diets for other plant feeding insects were developed by other workers. Beckman et al. (1953) developed a chemically defined diet for the pink bollworm, Pectinophora gossypiella (Saund.). The diet contained no plant tissue, making it possible to determine the chemical constituents quite accurately. Although some of the larvae reached the adult stage and appeared normal, the mortality rate was high. Vanderzant and Reiser (1956 b) formulated a purified casein diet for the pink bollworm which resulted in up to 100% pupation of all larvae started. A wheat germ diet was developed by Adkisson et al. (1960 a) which promoted normal growth and development of the pink bollworm. An average of 81.5% of the larvae placed on the diet became adults.

Five generations of boll weevils, Anthonomus grandis Boh., were reared in the laboratory on a semi-synthetic diet developed by Vanderzant and Davich (1958). Adults would feed on the diet, but would not oviposit unless cotton plant extracts were added. Vanderzant (1959) found inositol to be the necessary factor in the boll weevil diet and she was able to rear 16 generations when it was added to various diet mixtures, including the pink bollworm wheat germ medium.

The wheat germ diet developed for the pink bollworm by Adkisson et al. (1960 a) was modified by Vanderzant et al. (1962) to rear larvae of the bollworm, Heliothis zea (Boddie). It was necessary to omit sodium alginate and add ascorbic acid to the diet for normal development of this insect. Four generations of the bollworm were reared during the winter. By adding dry powdered cotton leaves to the bollworm diet, Ignoffo (1963) was able to rear large numbers of the cabbage looper. Pan and Long (1961) mass-reared the sugarcane borer, Diatraea saccharalis (F.) on a semi-synthetic diet containing an acetone extract from corn plants.

There have been several phytophagous insects successfully reared on artificial media since such diets were initiated. George et al. (1960), who reared several lepidopterous corn insects on one diet, stated that "modifications in the physical consistency of the medium or addition of attractants may make it possible to rear a number of different species on essentially the same medium".

Requirements and problems in insect rearing

The primary requirement for rearing insects successfully is providing them with a diet that satisfies their nutritive requirements and is of a consistency that initiates feeding. Essential nutritive requirements can be incorporated into the diet by adding purified chemicals which are found in the host plant. Beck et al. (1949), Vanderzant (1959), and Ignoffo (1963) supplimented artificial diets with plant extracts or dried plant parts to promote normal growth or reproduction. The correct consistency of the medium can be controlled by adding varying amounts of a carrier such as agar.

Quaintance and Brues (1905) reported that cotton bollworm moths did not begin to oviposit until they had fed. Lukefahr and Griffin (1956) stated that food containing sugars induced pink bollworm moths to lay about twice as many eggs as those receiving only water. Ellisor (1935) fed corn earworm moths on a 10% sucrose solution, and Callahan (1962) fed them a 10% honey solution. Both methods provided good results. Shorey (1963) said that fecundity of adult cabbage loopers was directly related to the concentration of their food.

Several environmental factors such as light, temperature, and humidity are important in insect rearing. Some diffused light is necessary for activity of corn earworm moths, but it should be at low intensity of one foot candle or below (Callahan, 1956, 1958, 1962). Callahan (1962) reported 73 to 80 F as the optimum temperature for rearing corn earworms. McEwen and Hervey (1960) found the optimum temperature for rearing cabbage loopers to be 73 to 77 F. Vanderzant and Reiser (1956 a) reared pink bollworms at 84 F. Maximum numbers of eggs are deposited by corn earworm moths when the relative humidity is around 90% (Callahan, 1962). Ditman et al. (1940) stated that weight loss of corn earworm pupae depends on humidity, ranging from 1.6% loss at 100% to 18.6% loss at 0% relative humidity.

A surface for oviposition is necessary when rearing multiple generations of insects in the laboratory. Hensley (1960) used a smooth-surface waxed paper as an oviposition surface for three species of the Diatraea complex. Callahan (1956) said that a villous surface was necessary for oviposition by the corn earworm. He used cloth for both egg deposition and for adults to cling to after emergence, thus allowing for wing expansion. Ignoffo (1963) provided paper toweling on which the cabbage looper moths deposited eggs. The rough surface of the towel presented small depressions in which the eggs were placed. The boll weevel oviposited directly on an artificial diet when plant extracts or pollen were added (Vanderzant and Davich, 1961). The females preferred a curved, rather than a flat, surface for oviposition. Callahan (1957) stated that the oviposition surface for insects should resemble closely the surface of the host where the eggs are deposited. He reported more eggs deposited by corn earworm moths on a villous cloth surface than on corn plants when both were in the same cage.

Isolation into separate rearing cells becomes necessary when rearing cannibalistic species. Barber (1936 b) said that only a single larva of the corn earworm usually survives in one ear due to cannibalism. In fact, he reared individuals of that species from all larval instars to the adult stage with no other food than other earworm larvae. Late instars, however, were more cannibalistic than the early ones. He reported that from 65 to 88% of crowded larvae survived to the third instar. Vanderzant et al. (1956) found pink bollworms to be extremely cannibalistic when reared together. Beck and Stauffer (1950) reared 10 to 15 European corn borer larvae per 125 ml Erlenmeyer flask with very little cannibalism. Pan and Long (1961) reared 10 sugarcane borer larvae per 250 ml Erlenmeyer flask with no cannibalism reported.

McEwen and Hervey (1960) said that strict sanitation is necessary for rearing cabbage loopers to prevent outbreaks of polyhedral virus. If completely aseptic proceedures are not practiced in rearing insects on artificial diets, several fungus and yeast organisms will grow on the medium. If equipment and laboratory facilities are of a nature that sterilization and sanitation are not possible, mold and yeast inhibitors can be incorporated into the medium. Vanderzant et al. (1962) reared four generations of bollworms aseptically without adding inhibitors for microorganisms. They also reared them under non-aseptic conditions by adding para hydroxybenzoate as a mold inhibitor. Ignoffo (1963) used methyl para hydroxybenzoate to inhibit growth of mold, and aureomycin to inhibit yeast growth on the larval medium when rearing cabbage loopers.

Air circulation within the rearing chamber is very important. Mathes (1936) stated that too little air circulation resulted in more favorable conditions for mold growth and greater decomposition, while too much air caused excessive drying of the food. Vanderzant et al. (1962) reported that too much moisture condensed in rearing vials with screw caps due to lack of air circulation within the vials. They used cotton plugs to stopper the vials. Ignoffo (1963) fastened bolting cloth over the larval rearing jars to allow proper air circulation, yet prevent the larvae from escaping.

Comparison of insects reared on natural and artificial diets

A few of the workers who have reared insects artificially have presented data comparing the insects' life cycles on both natural and synthetic diets. This review pertains to literature on individual species under natural and artificial conditions. Cordova (1962) found no significant difference in the life cycle of the corn earworm when reared on corn and an artificial diet. He reared four consecutive generations of this species on each diet before the study was terminated. The insects required about 32 days from egg to egg on both diets. No appreciable differences were noted in the body weights and measurements of individuals from the two populations.

Adkisson et al. (1960 b) stated that growth and development of the pink bollworm reared on an artificial diet approximated that reported for the insect in the field. They reported that pupae from the diet were slightly smaller than those collected from cotton bolls, but female moths reared from the diet laid more eggs than field collected moths (Adkisson et al., 1960 a). This may have been due to the possibility that moths in the field deposited part of their eggs before they were captured. Adkisson (1961) found that pink bollworm moths reared on a

1% cottonseed meal diet laid more eggs than those reared on wheat germ diet, 5% cottonseed meal diet, cotton squares, or cotton bolls. George et al. (1960) found the corn earworm able to complete the larval cycle in 15.5 days when reared on the European corn borer diet developed by Beck et al. (1949), after completing the first instar on corn silk. Vanderzant et al. (1962) reported 12 days in the larval cycle of the corn earworm reared on a wheat germ medium, but Cordova (1962) stated it took 17.4 and 16.5 days, respectively, when reared on wheat germ medium and corn.

Pan and Long (1961) found that sugarcane tops were slightly superior to an artificial diet in rearing the sugarcane borer. Mortality was 40% on the natural diet as compared with 60% on the artificial medium. Larval weights were also slightly greater when reared on natural food. Each larva required approximately 7.5 g of the artificial diet or 10 g of the sugarcane tops to complete development, and larvae began to pupate between 23 and 28 days after being placed on the respective diets. Hensley (1960) found that an average of 25.2 days was required for the sugarcane borer to complete the larval cycle when reared on sections of corn plants in the laboratory.

An average of 9.2 days was required for the cabbage looper to complete the larval stage when reared on an artificial diet, according to Ignoffo (1963). McEwen and Hervey (1960) reported that 9 to 11 days were needed for completion of the larval stage when the loopers were fed broccoli foliage in the laboratory. The broccoli-reared loopers had slightly greater head capsule measurements than those reared on the artificial medium, except for the first instar, but pupal weights were greater for those reared on the artificial diet.

Lukefahr and Martin (1964) reported that female bollworm moths reared on an artificial medium and fed a 50% sucrose solution deposited an average of 934 eggs. When corn and cotton squares were used as the larval diet, and sucrose was fed as the adult diet, the moths laid an average of 429 and 465 eggs, respectively. Moths reared from the artificial diet and fed the sucrose solution laid more viable eggs and lived longer than those reared from corn or cotton squares and fed on either sucrose or water alone. Sucrose-fed adults of the tobacco budworm, *Heliothis virescens* (F.), and the cotton leafworm, *Alabama argillacea* (Hbn.), laid more eggs than those fed only water. Cordova (1962) reported the average pupal weights for corn earworms reared on an artificial diet to be 585.6 mg, and on corn, 595.5 mg. Lukefahr and Martin (1964) found that pupae of bollworms (corn earworms) reared on artificial diet or corn averaged less than 500 mg.

## Numbers of generations reared artificially

Laboratory-reared cabbage loopers fed broccoli (McEwen and Hervey, 1960) were produced at the rate of 13 generations per year. Ignoffo (1963) reared six generations of the looper on an artificial diet before publishing his data, but if more generations had been possible at the rate of 23 days each (as reported for the six generations), almost 16 generations could have been produced per year. Vanderzant and Davich (1958) reared five generations of boll weevils on an artificial diet. The next year Vanderzant (1959) reared 16 generations of this same species. Vanderzant and Reiser (1956 a) produced three generations of the pink bollworm artificially and stated that many more probably could have been possible.

Cordova (1962) and Vanderzant et al. (1962) reared four consecutive generations of the corn earworm (cotton bollworm) on an artificial diet. Callahan (1962) stated: "...not more than five successive generations of the corn earworm may be produced by using harvested food under laboratory conditions. A larger number of successive generations can be reared only on living plants." He also said: "It is apparent that the main difficulties in rearing the corn earworm involve its unpredictable mating habits in the laboratory." However, Ellisor (1935) reported that second generation corn earworm moths reared on living plants in a greenhouse were weak and their eggs infertile. Even though problems are involved in rearing insects artificially, Dougherty (1959) said that insects are the only invertebrate metazoa that have been reared axenically (purely) on chemically defined diets.

# METHODS AND MATERIALS

## A test with tobacco budworm

### Establishment and development on grain sorghum

RS 610 hybrid grain sorghum was planted in the greenhouse for this test. When the plants were in the early whorl stage, 5, 10, or 20 first instar larvae of the tobacco budworm were placed on each of ten plants. No sign of establishment of the larvae could be observed by daily checks. Further attempts were made to infest the plants at the same rate in the late whorl stage and when they began to head. No establishment was observed in either attempt.

In order to eliminate the possibility of other factors being involved in the failure of larvae to become established on the plants, another test as a check was conducted including the corn earworm, at the same infestation rates. The same plants were used as were previously, and at the time of infestation the heads were in the early milk stage.

At the end of six days all larvae of both species on infested heads were counted. At the end of 12 days all the larvae were removed from the heads and isolated individually with a small piece of sorghum head in 3 1/2-oz ice cream cups. Records were taken on the infestation rate, number of larvae living after 6 and 12 days, and the number that pupated.

## Tests with corn earworm, fall armyworm, and southwestern corn borer

Extensive rearing tests were conducted in the laboratory with the corn earworm, fall armyworm, and southwestern corn borer on an artificial diet, and on grain sorghum as a natural diet. All the work was done in an insectary room 12 by 14 ft during the September 1962-April 1964 period. A relatively constant temperature of 80 F was maintained throughout the year by means of a thermostatically controlled refrigerated window airconditioner during warm weather and by furnace heat supplemented by a thermostatically controlled electric heater during cold weather. During the day the lighting in the room was by four 40-watt fluorescent tubes and two windows, 42 by 30 and 30 by 19 inches. At night diffused light was provided by means of a 60-watt incandescent bulb in a lamp placed on the floor under a table and directed toward the wall. The relative humidity in the rearing room was uncontrolled, but it seemed to be satisfactory for rearing all stages of the insects involved.

### Test with various diets

Before extensive tests could be initiated on rearing the insects in the laboratory, a suitable larval medium had to be adopted for each of the species involved. An ideal diet would be one that would suffice in rearing all three species. This would eliminate the problem of maintaining more than one diet for the larvae.

In reviewing the literature it was found that a wheat germ medium had been successfully used in rearing the corn earworm. Another diet, containing dry powdered corn leaves, had been used in rearing the

European corn borer. These two artificial diets, and two which were slight modifications of the wheat germ diet, were tested on the corn earworm, fall armyworm, and southwestern corn borer. The constituents of the four diets were as follows:

Diet Number 1 (Wheat germ diet)

| | |
|---|---|
| Agar | 90 g |
| Casein | 126 g |
| Sucrose | 126 g |
| Wesson's salts | 36 g |
| Alphacel | 18 g |
| Wheat germ | 108 g |
| Ascorbic acid | 15 g |
| Methyl para hydroxybenzoate (15% solution W/V in ethyl alcohol) | 36 ml |
| Formaldehyde (0.1 g/ml in water) | 18 ml |
| Choline chloride (0.1 g/ml in water) | 36 ml |
| Potassium hydroxide (4 M) | 18 ml |
| Vitamin stock solution | 6 ml |
| Vitamin E and corn oil (1 ml alpha tocopherol in 99 ml corn oil) | 10 ml |
| Water | 3100 ml |

Diet Number 2 (Corn meal diet)

This diet was the same as number 1, except 108 g of corn meal were substituted for 108 g of wheat germ.

Diet Number 3 (Powdered corn leaf diet)

This diet was the same as number 1, except 108 g of dry powdered corn leaves were substituted for 108 g of wheat germ.

Diet Number 4 (European corn borer diet)

| | |
|---|---|
| Agar | 27.3 g |
| Glucose | 8.5 g |
| Casein | 42.8 g |
| Cholesterol | 3.4 g |
| Wesson's salts | 5.2 g |
| Brewer's yeast | 34.3 g |
| Corn leaf powder | 27.6 g |
| Corn tassel powder | 27.6 g |
| Choline chloride | 0.5 g |
| Cellulose | 13.7 g |

| | |
|---|---|
| Mold inhibitor<br>(170 ml 95% alcohol, 15 g methyl hydroxy-<br>benzoate, 20 g sorbic acid) | 20.0 ml |
| Vitamin E and corn oil<br>(1 ml alpha tocopherol in 99 ml corn oil) | 1.8 ml |
| Water | 1050.0 ml |

Newly-hatched larvae of the corn earworm, fall armyworm, and southwestern corn borer were placed individually in 3 1/2-oz ice cream cups with a small amount of one of the four diets. Three hundred larvae of each species were fed on each diet, and the diet was replaced at weekly intervals to provide a fresh food supply. The number of days required for completion of the larval stage and the rate of pupation on the various diets were recorded for each species.

### Test on number of larvae per rearing carton

After a suitable diet was adopted, a test was conducted to determine if several larvae could be reared in one chamber without extensive cannibalism. This procedure would greatly reduce the labor and cost of materials. One-half pint ice cream cartons were used as rearing chambers. The cartons were infested at the rates of 10, 20, 30, 40, and 50 newly-hatched larvae, with ten replications for each infestation level for each species. The diet was replaced at weekly intervals to provide fresh food. A record was kept on the number of larvae pupating in each carton. The data were analyzed as a randomized block experiment following the procedures outlined by Steel and Torrie (1960).

### Adult food test

Corn earworm and fall armyworm adults feed, but the southwestern corn borer moths do not. A test was conducted to determine the most satisfactory adult diet for the corn earworm and fall armyworm to insure maximum egg production.

After pupation the pupae were sexed and placed on wet cotton inside 3 1/2-oz ice cream cups. These cups were placed inside separate oviposition cages constructed of 1/4-in. hardware cloth forming a cylinder nine inches high and seven inches in diameter. The cages had a top and bottom made of 1/8-in. hardware cloth, the top being constructed for opening to gain entry to the cage. Paper toweling was taped around the outside of the cage as a surface for corn earworm moth oviposition, and since fall armyworm moths prefer to oviposit on smooth surfaces, heavy-duty waxed paper was used for them.

The moths were fed by means of glass shell vials containing various solutions, stoppered with a small piece of sponge. These vials were inverted through holes in the tops of the cages. The sponge absorbed the solution and kept a constant food supply available to the moths.

The treatments in the adult food test were: no food, 10% sucrose, 50% sucrose, 10% honey, and 50% honey solutions. Ten male and ten female pupae were placed in each cage, and each treatment was replicated three times. After moths emerged and oviposition began, the oviposition surface was replaced daily and the number of eggs recorded. Egg counts were made by gridding the oviposition surface, before placing it on the cage, and using a dissecting microscope to count the eggs in each section. The data were analyzed as a randomized block design, according to the methods described by Steel and Torrie (1960).

Oviposition test

In order to determine the number of insects that should be reared in order to provide a given number of eggs for various tests, it was necessary to conduct an oviposition study on each species. A test was set up using oviposition cages as described previously. Heavy-duty waxed paper was used as an oviposition surface for the fall armyworm and southwestern corn borer, and paper toweling was used for the corn earworm.

Ten male and ten female moths of each species were placed in a cage and four replications were used. The corn earworm and fall armyworm moths were fed a 50% solution of sucrose in distilled water. After oviposition began, the oviposition surface was replaced daily and the number of eggs laid per day was recorded. A standard deviation test was conducted on the data, following the procedures by Steel and Torrie (1960).

Head capsule measurement study

A test was conducted to determine if any overlap occurred in the head capsule measurements of different larval instars of each species reared on the artificial diet and on grain sorghum. The head capsules of 20 newly-hatched larvae were measured and recorded in each test. To obtain a more accurate measurement it was necessary to kill the larvae by cutting them transversely at the prothorax. "Brood mates" of the first instar corn earworm and fall armyworm larvae were placed in 3 1/2-oz ice cream cups and the southwestern corn borer larvae in 1 by 3 3/4-in. glass shell vials, half of them on artificial medium and the other half on grain sorghum parts. Small branches of sorghum heads were fed to the corn earworm, and succulent pieces of the whorl were provided for the fall armyworm and southwestern corn borer. The natural food was changed at two-day intervals to prevent spoilage. Although it would have been unnecessary to change the artificial diet so often, it was also replaced at two-day intervals to make the test uniform. After each larval molt, 20 more larvae from each diet were killed and the head capsule measured and recorded. Only those larvae whose cast skins could be found in the containers were used. This procedure was followed throughout all six instars of each species. The procedures outlined by Steel and Torrie (1960) were used to conduct a standard deviation test on the data.

Comparison of life cycle on sorghum and artificial medium

In order to determine if the life cycle of those insects reared on the artificial diet was similar to those reared on grain sorghum, it was necessary to conduct a detailed study on larval development, duration of each life stage, and adult fecundity on the two diets. Twenty-five newly-hatched larvae of the corn earworm and of the fall armyworm were weighed and isolated on each diet in separate cells of polyethylene ice cube trays. Each tray had 14 cells with dimensions of 1 3/4 by 1 1/4 by 1 in. deep. The trays were covered with 1/4-in. plywood cut to the size of the tray. The plywood had a 1/2-in. layer of absorbant cotton covered with unbleached cotton muslin stapled to it to allow air circulation within the cells, yet keep the larvae confined. The covers were held tightly in place by large rubber bands. The same number of southwestern corn borer larvae were confined in 1 by 3 3/4-in. glass shell vials, stoppered with cotton plugs.

The natural diet of the corn earworms consisted of small branches of newly-emerged sorghum heads, and that of the fall armyworms and southwestern corn borers, succulent pieces of sorghum whorls. The wheat germ diet described previously was fed to the other test larvae.

Larval weights were recorded each day. Instars were determined by the size of the head capsule and recorded at the time of weighing. With some experience it was quite easy to determine the larval instar without magnification. After all larvae were weighed and the instar determined, they were placed in clean rearing chambers containing fresh food.

Upon pupation the pupae were weighed and sexed. Pupal weights were recorded each day until emergence to determine weight changes during the pupal stage. After emergence, the adults were weighed, and ten males and ten females placed in oviposition cages. The total number of eggs laid in each cage was divided by the number of female moths (10) to determine the average number of eggs laid per female.

The test was analyzed as a paired experiment, as outlined by Steel and Torrie (1960), using pupal weights on the day of pupation as the criterion for comparison.

### The effect of diet change

It was considered important to know what effect a change in the diet at various intervals during the larval stage would have on the growth and development of the insect. The criterion for this determination was the length of the larval cycle and the weight of the pupae at the time of pupation as a result of the different treatments. The test conducted on the corn earworm and fall armyworm employed 250 larvae of each species divided into ten treatments of 25 larvae each. The ten treatments were further divided into two groups of five treatments each. One group of larvae was isolated in individual cells of polyethylene ice cube trays with artificial medium as the initial food. The other group was isolated in similar trays with sorghum as the initial food.

The food was changed at three-day intervals, and the alternate diet was substituted for the initial diet as the treatment called for it. The test on the southwestern corn borer was conducted in essentially the same manner in 1 by 3 3/4-in. glass shell vials. However, due to the longer larval cycle of the southwestern corn borer, it was necessary to add an additional treatment of 25 larvae to each of the two groups. The treatments for the test are outlined below.

Group 1

| | Days fed on: | |
|---|---|---|
| | Initial diet (Sorghum) | Alternate diet (Artificial) |
| a) | Entire larval stage | - |
| b) | 3 | Remainder |
| c) | 6 | Remainder |
| d) | 9 | Remainder |
| e) | 12 | Remainder |
| f) | 15* | Remainder |

Group 2

| | Days fed on: | |
|---|---|---|
| | Initial diet (Artificial) | Alternate diet (Sorghum) |
| a) | Entire larval stage | - |
| b) | 3 | Remainder |
| c) | 6 | Remainder |
| d) | 9 | Remainder |
| e) | 12 | Remainder |
| f) | 15* | Remainder |

* Involved only in the test with the southwestern corn borer.

Ten additional larvae, not involved in the test, were maintained on each of the treatments and substituted into the test at random in the event of death of one of the test larvae. On the day of pupation, each pupa was weighed and the duration of the larval stage was recorded. A standard deviation test, as described by Steel and Torrie (1960), was conducted on the data.

Mass rearing

Great care had to be taken when handling individual newly-hatched larvae of the test species in order to prevent injury. Cautious handling was very time consuming, thus a more practical approach needed to be worked out. A previous test described under "test on number of larvae per rearing carton" showed that it was practical to rear several southwestern corn borer larvae per container, because they are not cannibalistic, thus individual handling was not necessary. The corn earworm and fall armyworm, on the other hand, are cannibalistic and must be isolated. As stated in the literature review, the corn earworm is not very cannibalistic during the first three instars, and can be reared in crowded conditions with little mortality. The same situation was suspected for the fall armyworm.

A test was conducted to determine the optimum number of larvae that could be successfully reared in one container through the first week of the larval cycle. Plastic sandwich trays, 4.5 by 4.5 by 1.5 in. deep, were used for rearing chambers. A square hole was cut in each lid and a piece of unbleached cotton muslin glued in place over the hole to allow air circulation within the tray. The trays were approximately half filled with liquid diet medium which was allowed to stand overnight to harden.

The treatments in the test consisted of five different infestation rates per tray. They were 100, 200, 300, 400, and 500 larvae per tray, and each treatment was replicated six times. The larvae were obtained from four oviposition cages for each species, each containing a large colony of moths. The eggs were allowed to hatch on the oviposition surface. The larvae, upon hatching, suspended themselves from the oviposition surface by a fine thread, and the large numbers so hanging provided an ample supply for test purposes. A camel's hair brush was used to transfer the larvae to the rearing trays. The brush was inserted just below the oviposition surface, and upon contact with the thread, the suspended larvae were transferred to the brush. Several larvae could be picked up at the same time by this method and easily counted while hanging from the brush. They were then placed in the rearing trays, at the various rates of infestation, without being touched. At the end of seven days the number of larvae still alive in each tray was recorded. The data were analyzed as a randomized block design as described by Steel and Torrie (1960).

The larvae that were still alive in the six replicates of the 100-larvae-per-carton treatment were then isolated in individual cells of polyethylene ice cube trays for the remainder of the larval cycle. The number pupating in each replicate was then recorded.

Generation study

A test was initiated at the beginning of this study to determine the number of consecutive generations of each species that could be maintained in the laboratory on an artificial diet. Two hundred larvae from each generation of all three species were isolated in individual rearing chambers. They were used for no other test but the generation study.

The first two generations of the corn earworm were reared in 3 1/2-oz ice cream cups. The next three generations were reared in 1 by 3 3/4-in. glass shell vials. The sixth and all succeeding generations were reared in cells of polyethylene ice cube trays.

The first six generations of the fall armyworm were reared in 3 1/2-oz ice cream cups, the next three in 1 by 3 3/4-in. glass shell vials, and the remainder in polyethylene ice cube trays.

The southwestern corn borer was reared for three generations in 1 by 3 3/4-in. glass shell vials. Ice cream cups were used for the fourth generation. The vials were again used to rear the fifth and sixth generations. Polyethylene ice cube trays were used for the seventh, and plastic sandwich boxes, 4.5 by 4.5 by 1.5 in. deep, were used for the eighth and succeeding generations.

Information recorded for the generation study was: the number of the generation, number of larvae started, percent survival, average number of days in the larval stage, and type of rearing chamber.

## A TEST WITH TOBACCO BUDWORM

### Establishment and development on grain sorghum

<u>Results and discussion</u>

Data showing the results of the tobacco budworm test are presented in Table 1. Observations at 6- and 12-day intervals showed that the

Table 1. Survival of tobacco budworm and corn earworm larvae on grain sorghum heads in the early milk stage.

| Infestation rate | No. plants | No. larvae | No. living after 6 days | No. living after 12 days | Pupation Number | Pupation Percent |
|---|---|---|---|---|---|---|
| 5/plant | | | | | | |
| Budworm | 10 | 50 | 0 | 0 | 0 | 0 |
| Earworm | 10 | 50 | 26 | 12 | 12 | 24 |
| 10/plant | | | | | | |
| Budworm | 10 | 100 | 0 | 0 | 0 | 0 |
| Earworm | 10 | 100 | 63 | 19 | 14 | 14 |
| 20/plant | | | | | | |
| Budworm | 10 | 200 | 0 | 0 | 0 | 0 |
| Earworm | 10 | 200 | 100 | 26 | 20 | 10 |

tobacco budworm had not established itself on the sorghum heads, but the corn earworm, which was used as a check, had definitely become established. Twenty-four, 14, and 10% of the corn earworm larvae survived to pupation at the 5-, 10-, and 20-larvae-per-plant infestation rates, respectively.

Although *Heliothis virescens* is closely related to *Heliothis zea*, and both species can develop on certain species of plants, this test shows that *H. virescens* could not survive on grain sorghum plants of the variety used.

## TESTS WITH CORN EARWORM

### Test on various diets

Results

The wheat germ diet was far superior to the other three diets for rearing corn earworms, as shown in Table 2. Of the 300 larvae started

Table 2. Effects of various larval diets on corn earworm larval development and pupation.

| Diet used | No. larvae | Pupation Number | Pupation Percent | No. days in larval stage |
|---|---|---|---|---|
| Wheat germ | 300 | 228 | 76 | 15.3 |
| Corn meal | 300 | 63 | 21 | 18.8 |
| European corn borer | 300 | 60 | 20 | 24.9 |
| Powdered corn leaf | 300 | 31 | 10 | 25.0 |

on the wheat germ diet, 76% survived to pupation and required an average of 15.3 days to complete the larval cycle. The earworm was able to develop on the other diets, but pupation percentages were greatly reduced, and it took considerably more time for the larvae to complete the larval stage. The least desirable diet was the one containing powdered corn leaves, with pupation reduced to only ten percent, and the length of the larval period increased to nearly ten days more than was required on the wheat germ diet.

Discussion

Most of the larval mortality on all four diets occurred during the first week. The food in each cup was replaced at weekly intervals, and the cups not containing living larvae were discarded. Most of the larvae reaching the third instar pupated, as will be demonstrated more clearly in a later test.

The physical consistency of the four diets was very similar, which indicates that an inability to feed on the corn meal, powdered corn leaf, and European corn borer diets was probably not the problem, since the larvae fed readily on the wheat germ diet. It was more likely that some nutritional factor or factors were lacking in the diets.

## Test on number of larvae per rearing carton

Results

The results of the test to determine if several corn earworm larvae could be successfully reared in one container are presented in Figure 1. An average of 1.3 larvae reached the pupal stage in rearing chambers infested with ten larvae each. Only a small increase in the number reaching pupation was observed in containers infested with 20, 30, 40, and 50 larvae each, with respective pupation of 1.8, 1.9, 2.1, and 2.2 pupae per carton. The theoretical mean, based on the 10-larvae-per-carton infestation, would have been only 6.5 pupae for each group of 50 larvae started.

The analysis of variance showed that there were no significant differences in the number of pupae per carton at the .01 level of probability when 10, 20, 30, 40, and 50 larvae were reared per carton.

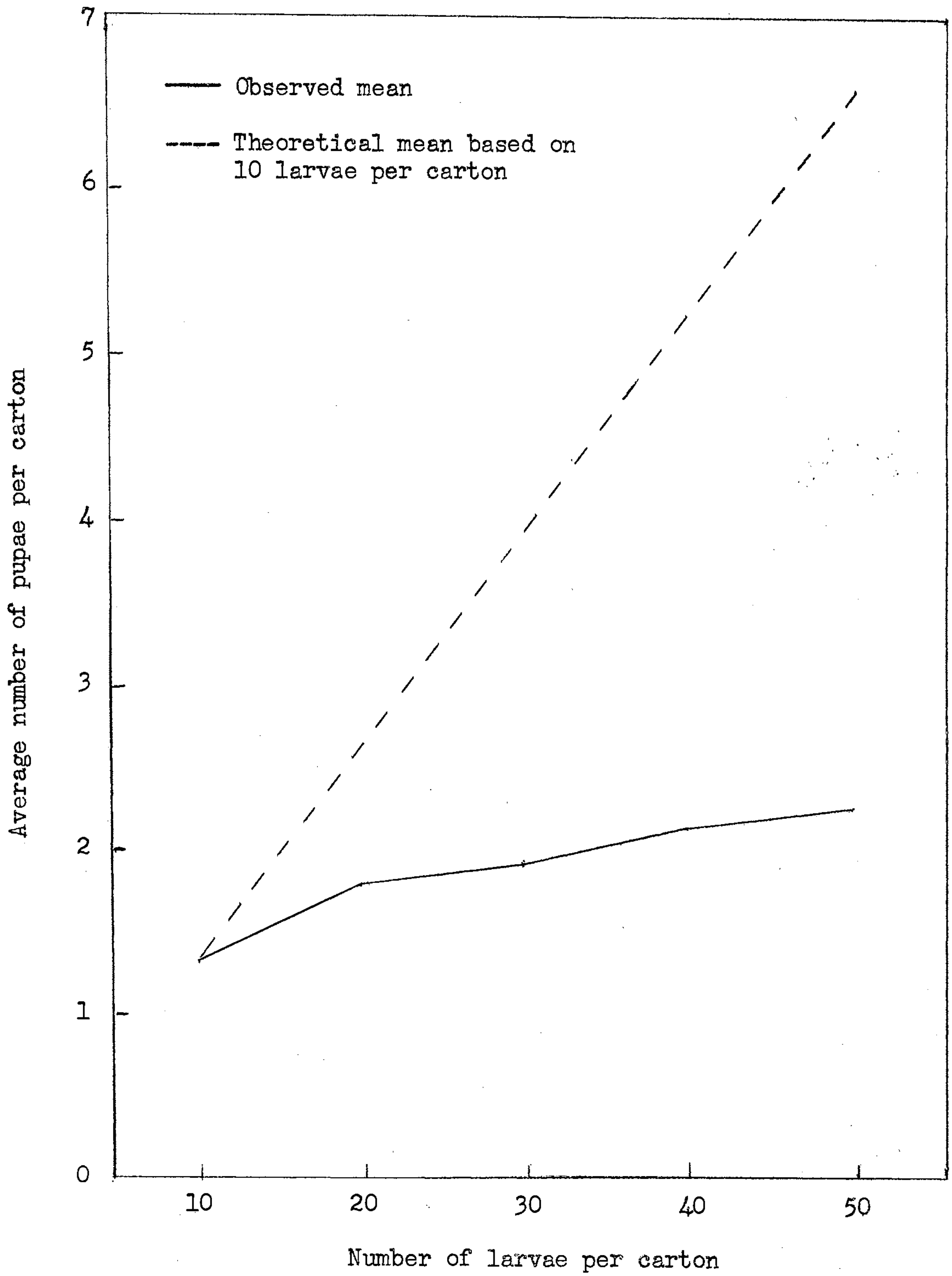


Figure 1. Corn earworm pupation as related to numbers of larvae per carton when reared on artificial (wheat germ) diet.

Discussion

The results of this test showed that it was impractical to rear several corn earworm larvae throughout the larval cycle in the same container. Due to the aggressive nature of the larvae, a high degree of cannibalism occurred. This resulted in little more than one or two larvae per carton reaching the pupal stage, regardless of the infestation rate. These results indicate that corn earworm larvae should be isolated, at least during part of their larval cycle, which will be discussed more fully in a later test.

## Adult food test

Results

Although a few eggs were deposited by moths receiving no food, the average number per female was only four eggs. The analysis of variance for the test showed a highly significant difference between treatments. As shown by Duncan's new multiple range test (Table 3), where moths received no food oviposition was significantly reduced, as compared to all other treatments. Those moths receiving 50% sucrose solution laid

Table 3. The effects of varying adult food concentrations on corn earworm oviposition (Duncan's new multiple range test).

| | Adult diet | | | | |
|---|---|---|---|---|---|
| | No food | 10% honey | 10% sucrose | 50% honey | 50% sucrose |
| Average no. eggs per female | 4 | 406 | 456 | 503 | 545 |

Those mean numbers not connected by the same line are significantly different at the .01 level of probability.

a greater number of eggs (545 per female), but that treatment was not significantly different from the 50% honey (503 per female) or 10% sucrose (456 per female) treatments. However, the mean was significantly greater than for the 10% honey treatment (406 per female). No significant differences were shown between the 10% honey, 10% sucrose, and 50% honey treatments.

## Discussion

Although the number of eggs laid by moths feeding on 50% sucrose was not significantly higher than on the 10% sucrose or 50% honey treatments, it was chosen as the adult diet for later tests. The average number of eggs per female was 42 more than the average on 50% honey, and 89 more than 10% sucrose. Sucrose was less expensive, and honey was more difficult to measure and tended to crystalize after extended storage.

# Oviposition test

## Results

Data on the daily and accumulative oviposition rates of female corn earworm moths are presented in Figure 2. No eggs were deposited on the first and eighth days of the adult cycle, and averages of only two and nine were laid on the second and seventh days, respectively. Peak egg production was observed on the fourth day after emergence, when an average of 219 per female was laid. Then oviposition declined toward the end of the adult cycle. By the fifth day after adult emergence, 90% oviposition was recorded.

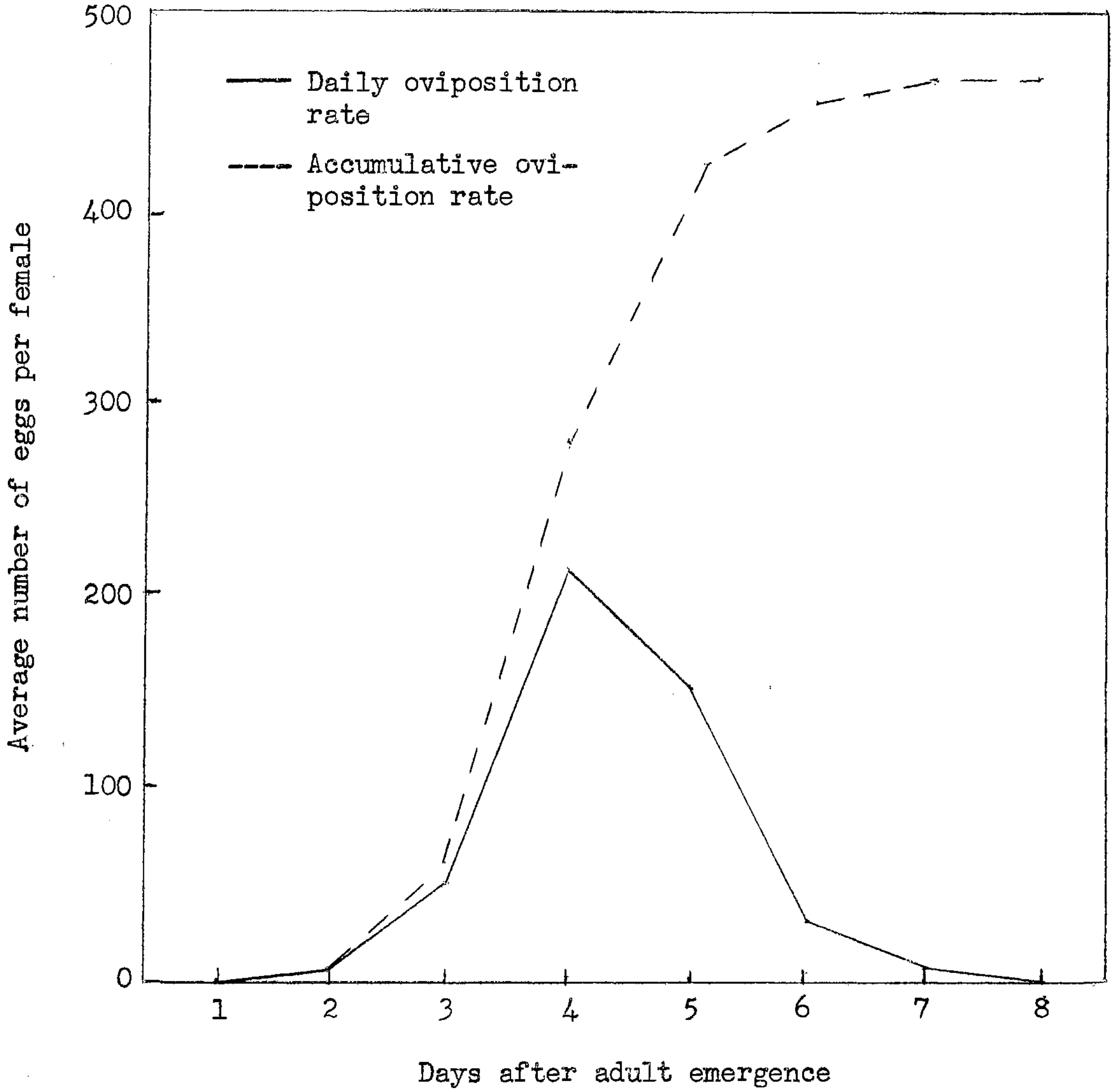


Figure 2. Daily and accumulative oviposition rate per female corn earworm moth during the adult stage.

<u>Discussion</u>

Most mating was observed on the first and second night after adult emergence, but some mating occurred on the third night. Most of the eggs were deposited on the paper on the side of the cage that was exposed more directly to the low-intensity light source provided for the moths.

As shown in Figure 2, large numbers of corn earworm eggs were obtained with the type oviposition cage described previously, and it was used in all subsequent tests with the corn earworm.

Head capsule measurement study

Results

Data comparing the head capsule measurements of corn earworm larvae reared on grain sorghum and on an artificial diet are presented in Figure 3. Little difference was observed between the average head capsule width of given instars reared on the two diets. The average capsule width and standard deviation is shown above each bar on the graph for the given instar and larval food.

Discussion

No overlap was observed in the head capsule width of consecutive instars on either diet. The instars could be identified by naked-eye observation after some experience. The growth progression in capsule width followed essentially a straight line progression when plotted on semi-logarithmic paper, which supports Dyar's rule (Dyar, 1890) for head capsule progression of lepidopterous larvae. On the basis of these data, it is evident that the artificial diet is satisfactory for growth and development of corn earworm larvae.

Comparison of life cycle on
sorghum and artificial medium

Results

The results of the test comparing the life cycle of the corn earworm

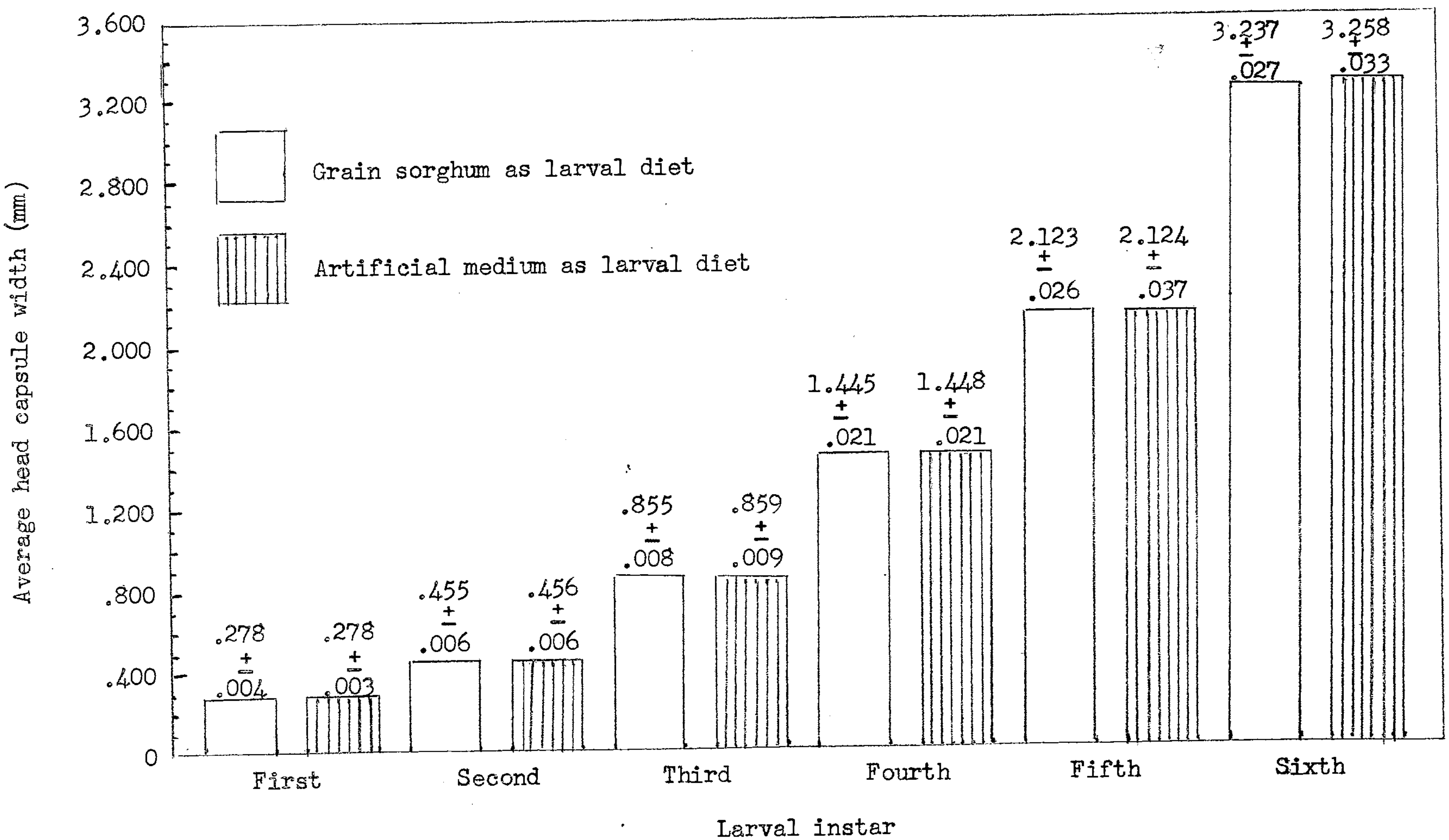


Figure 3. Comparison of head capsule widths (mm) and standard deviations for corn earworm larvae reared on natural (grain sorghum) and artificial (wheat germ) diets.

on grain sorghum and artificial diet is shown in Table 4. Data are presented on the weight gained or lost, maximum weight attained, and the

Table 4. Corn earworm development on natural (grain sorghum) and artificial (wheat germ) diets.

| Life stage | Average weight gained (g) | | Average maximum weight (g) | | Average days in life stage | |
|---|---|---|---|---|---|---|
| | Nat. | Art. | Nat. | Art. | Nat. | Art. |
| Larval instar | | | | | | |
| 1 | .00080 | .00082 | .00085 | .00087 | 4.0 | 4.0 |
| 2 | .00499 | .00512 | .00584 | .00600 | 2.3 | 2.2 |
| 3 | .01537 | .01460 | .02121 | .02061 | 2.0 | 2.0 |
| 4 | .05287 | .05399 | .07408 | .07460 | 2.0 | 2.0 |
| 5 | .21453 | .21423 | .28860 | .28883 | 2.0 | 2.0 |
| 6 | .30353 | .32395 | .59214 | .61278 | 3.0 | 3.1 |
| Pupa | -.19595 | -.21598 | .39619 | .39680 | 10.3 | 10.2 |
| Adult | -.07826 | -.07629 | .31793 | .32051 | 8.0 | 8.4 |
| Egg | - | - | .00005* | .00005** | 3.0 | 3.0 |
| Total | - | - | - | - | 36.6 | 36.9 |

* An average of 627 eggs laid per female with 88% hatch.
** An average of 668 eggs laid per female with 87% hatch.

number of days required for completion of each life stage. The larvae gained steadily until about midway in the sixth instar, then began to lose weight in preparation for pupation. More weight was gained in the sixth instar than was gained in the first five instars combined. The insect lost about as much weight in the change from larva to pupa as it gained during the fifth instar. The change from pupa to adult resulted in even more weight loss. Statistical analysis as a paired experiment showed no significant difference in pupal weights, at the .01 level of probability, when reared on sorghum or artificial medium.

The duration of each life stage was very similar on both diets. The larvae required 15.3 days on each diet, and the pupae 10.3 days on sorghum and 10.2 days on the artificial medium. The adults reared from sorghum lived 8.0 days and those from artificial medium 8.4 days. Eggs obtained from both groups took 3.0 days to hatch. The total number of days required for completion of the life cycle on sorghum was 36.6 days and for the artificial diet 36.9 days.

Moths reared from sorghum laid an average of 627 eggs per female with 88% of them hatching. Those females reared from the artificial diet laid an average of 668 eggs with an 87% hatch.

## Discussion

The life cycle of the corn earworm reared on an artificial diet was found to be very similar to the life cycle of those fed grain sorghum, when both groups were reared in the laboratory. Assuming that the insects would react similarly when placed on grain sorghums in the field or in a greenhouse, larval rearing of the corn earworm on artificial medium in the laboratory would be a satisfactory method of obtaining large numbers of this species for various tests on plant resistance, chemical control, or even possibly insect sterilization studies.

Effect of diet change

Results

Data showing the effects of a change of diet from sorghum to artificial medium and from artificial medium to sorghum at various intervals in the larval cycle are given in Table 5. The pupal weights for

Table 5. The effects of changing the larval food of the corn earworm from natural (sorghum) to artificial (wheat germ), and visa versa, at various intervals during the larval stage.

| Days fed on | | Pupal weight (mg) | Days in larval stage |
|---|---|---|---|
| **Group 1** | | | |
| Nat. | Art. | | |
| All | - | 396 ± 2 | 15.2 ± 0.1 |
| 3 | Remainder | 398 ± 2 | 15.2 ± 0.1 |
| 6 | Remainder | 397 ± 2 | 15.2 ± 0.1 |
| 9 | Remainder | 396 ± 2 | 15.2 ± 0.1 |
| 12 | Remainder | 397 ± 2 | 15.3 ± 0.1 |
| **Group 2** | | | |
| Days fed on | | | |
| Art. | Nat. | | |
| All | - | 396 ± 2 | 15.2 ± 0.1 |
| 3 | Remainder | 397 ± 2 | 15.3 ± 0.1 |
| 6 | Remainder | 396 ± 2 | 15.4 ± 0.1 |
| 9 | Remainder | 397 ± 2 | 15.2 ± 0.1 |
| 12 | Remainder | 396 ± 2 | 15.2 ± 0.1 |

all treatments were very similar, ranging from 396 ± 2 to 398 ± 2 mg. The length of the larval cycle was also similar for all treatments, with a range of 15.2 ± 0.1 to 15.4 ± 0.1 days.

Discussion

This test, along with the test comparing the life cycle on the two diets, indicates that corn earworms can be reared satisfactorily in the laboratory on artificial diets for use in conducting various experiments. Also, the indication that the artificial diet can be substituted for natural food, and visa versa, shows its practicability. This development makes possible the rearing of this insect for test purposes throughout the year.

Pupal weights on the day of pupation were chosen as a criterion for the comparison, because pupae are easy to handle, weight change is slight because they do not feed or deficate, and they represent a definite stage in the life cycle of the insect. These factors made it possible for a more uniform comparison.

## Mass rearing

Results

The survival of corn earworm larvae after seven days on artificial medium as affected by numbers per rearing carton is presented in Figure 4. The analysis of variance showed a significant difference among treatments at the .01 level of probability. The 100-, 200, and 300-larvae-per-carton infestation levels were each significantly different from all other treatments when analyzed by the Duncan's new multiple range test. The 400- and 500-larvae-per-carton levels were not sig-

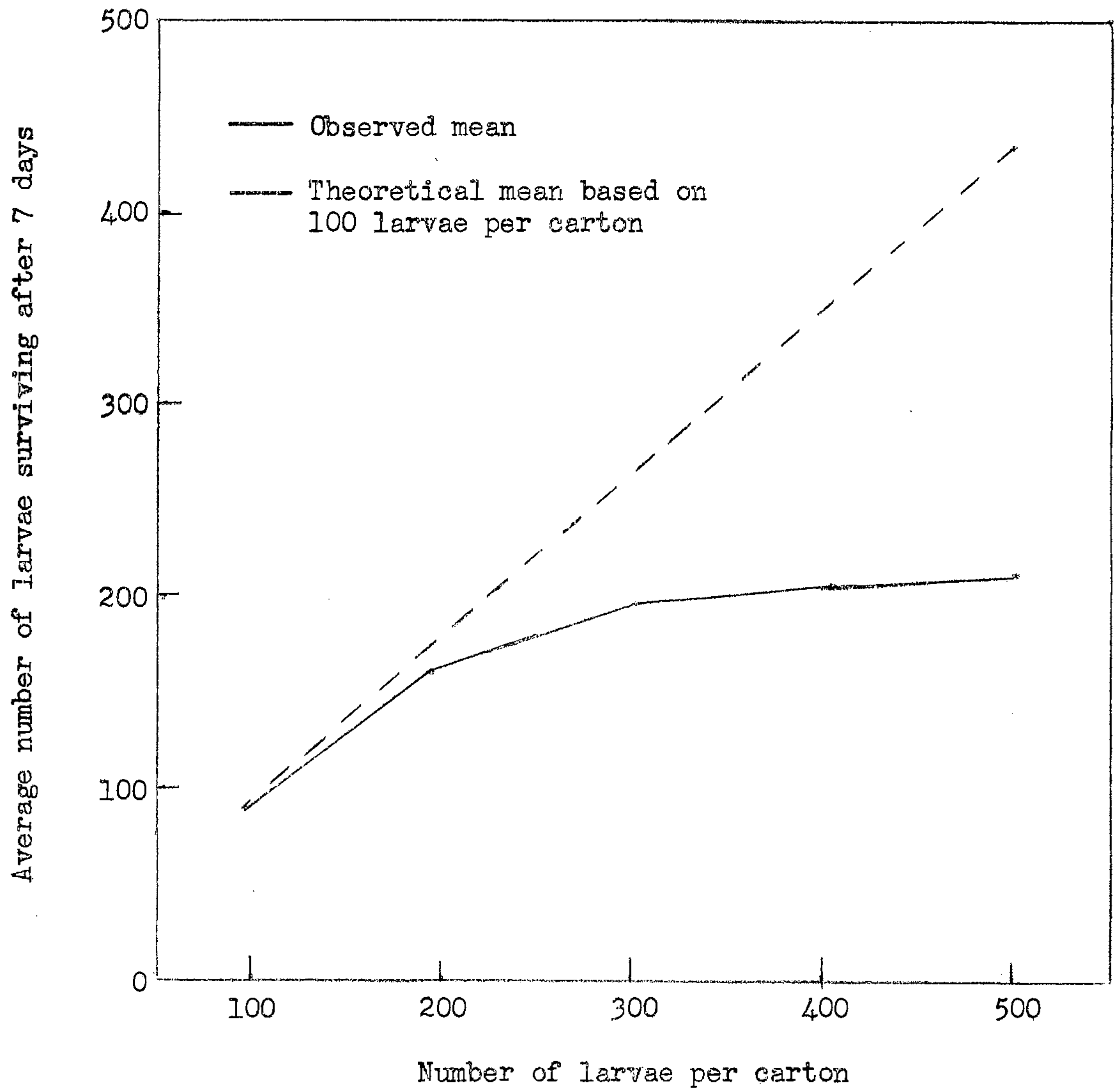


Figure 4. Survival of corn earworm larvae after seven days on artificial (wheat germ) diet at various levels of infestation.

nificantly different, but both were significant from all others. In Figure 4, the mean survival at the 200 infestation level is very near the theoretical mean based on the 100 level of infestation. The means for the higher levels of infestation drop more and more below the theoretical mean. At the 100 infestation level, an average of 88 larvae per carton was alive after seven days. The survival rate had dropped to slightly more than half the total larvae started at the 400-larvae-per-carton level, and less than half at the 500 level.

Of those larvae living after one week at the 100-larvae-per-carton level, and isolated in individual chambers, 97.5% pupated.

Discussion

It is hard to say which infestation level would be most desirable in rearing corn earworm larvae in large numbers. The mean survival at the 400 and 500 levels is not enough greater than the 300 level to be practical. A higher percentage survived at the 100-larvae level of infestation, however, the percentage at the 200 level was not a great deal smaller.

If space is limited and egg production unlimited, the 300-larvae-per-carton level would probably be most desirable, since the survival at that infestation level was significantly higher than the 200 level. On the other hand, if plenty of space is available for rearing chambers, and egg production is somewhat limited, the 200-larvae level may be more satisfactory.

The lack of significance between the survival rate at the 400- and 500-larvae levels indicates that a plateau is reached at about 400 larvae per carton. Because of cannibalism, approximately the same number of larvae should survive for one week at any infestation level above 400.

## Generation study

Results

The results of the study of consecutive generations of the corn earworm on an artificial diet are presented in Table 6. This species was successfully reared through 14 consecutive generations in the laboratory on artificial medium with no apparent loss in vigor or signs of other detrimental effects. An average of 72.5% of all larvae started on the artificial diet throughout the study survived through

Table 6. Results of the consecutive generation study of the corn earworm on artificial (wheat germ) diet.

| Generation | Number started | Percent survival | Days in larval stage | Rearing chamber |
|---|---|---|---|---|
| 1 | 200 | 71.0 | 17.7 | Ice cream cups |
| 2 | 200 | 73.0 | 17.6 | Ice cream cups |
| 3 | 200 | 78.5 | 16.1 | Shell vials |
| 4 | 200 | 74.5 | 16.2 | Shell vials |
| 5 | 200 | 65.0 | 15.9 | Shell vials |
| 6 | 200 | 74.5 | 15.5 | Ice trays |
| 7 | 200 | 51.0* | 17.0 | Ice trays |
| 8 | 200 | 46.5* | 16.9 | Ice trays |
| 9 | 200 | 79.5 | 15.7 | Ice trays |
| 10 | 200 | 74.0 | 15.3 | Ice trays |
| 11 | 200 | 91.0 | 15.6 | Ice trays |
| 12 | 200 | 77.0 | 15.2 | Ice trays |
| 13 | 200 | 85.0 | 15.5 | Ice trays |
| 14 | 200 | 76.0 | 15.8 | Ice trays |
| Average | 200 | 72.5 | 16.1 | - |

* Excessive yeast growth on artificial medium in rearing trays.

pupation. The average duration of the larval cycle was 16.1 days. A sharp reduction in the percent survival occurred in the seventh and eighth generations, dropping below 50% in the latter group. A survival rate of 91.0% occurred in the eleventh generation, which was the highest throughout the study.

Discussion

Little difference was found in the survival rate and length of the larval cycle when the corn earworm was reared in 3 1/2-oz ice cream cups, glass shell vials, or polyethylene ice trays. The ice cream cups were used to rear two generations, but were found to be undesirable, because the larval food had to be replaced weekly. Drying of the medium made it undesirable for the larvae. The shell vials, stoppered with cotton, were then used to rear three generations. The medium did not have to be replaced during the larval cycle, but much labor was involved in cleaning and sterilizing the vials. Also it was difficult to remove the pupae from the vials without injury. Polyethylene ice trays were used to rear the remaining generations. Excessive drying of the medium was prevented by their use, and they were easy to clean. Pupae were easily removed from the individual cells of the trays.

The reduced pupation rate in the seventh and eighth generations was due to excessive yeast growth on the artificial medium in the rearing trays. Beginning with the ninth generation, 100 g of agar, instead of 90 g, and 2900 ml of water, rather than 3100 ml, were used in making the medium. This resulted in a somewhat drier, more solid, medium which seemed to be unfavorable for growth of yeast organisms. The survival rate increased to normal after these modifications were made.

## TESTS WITH FALL ARMYWORM

### Test on various diets

Results

The results of tests to determine the most desirable diet for rearing fall armyworm larvae in the laboratory are presented in Table 7. Of those larvae fed the wheat germ diet, 84% survived through

Table 7. Effects of various larval diets on fall armyworm larval development and pupation.

| Diet used | No. larvae | Pupation | | No. days in larval stage |
|---|---|---|---|---|
| | | Number | Percent | |
| Wheat germ | 300 | 251 | 84 | 15.0 |
| Corn meal | 300 | 76 | 25 | 18.2 |
| European corn borer | 300 | 33 | 11 | 21.3 |
| Powdered corn leaf | 300 | 6 | 2 | 24.1 |

pupation after spending an average of 15.0 days in the larval stage. That was undoubtedly the most satisfactory diet used, since the next highest survival rate was 25% on the corn meal diet. Some larvae were able to develop on the European corn borer and powdered corn leaf diets, but the survival rates were very low, being 11 and 2%, respectively. Also, the duration of the larval stage on the corn meal, European corn borer, and powdered corn leaf diets increased by 3.2, 6.3, and 9.1 days, respectively, over those reared on the wheat germ diet.

Discussion

Some nutritional factor necessary for fall armyworm larval development seemed to be lacking in the corn meal, European corn borer, and powdered corn leaf diets. The larvae seemed to feed on the diets, but most of the mortality occurred during the first week. Several of the larvae died during the molting process and never completely shed the skin.

Test on number of larvae per rearing carton

Results

Figure 5 shows the results of the test to determine if several fall armyworm larvae could be reared in one rearing chamber without extensive cannibalism. A very low survival rate occurred for all treatments, ranging from 1.2 larvae in those cartons infested with ten larvae each to 2.0 in those containing 50 larvae. Even the theoretical mean, based on ten larvae per carton, is low, being only 6.0 larvae for the 50-larvae infestation level.

The analysis of variance for the test showed that there was no significant difference between any of the treatments at the .01 level of probability.

Discussion

This test showed that several fall armyworm larvae should not be reared in the same rearing chamber throughout the larval cycle. Low

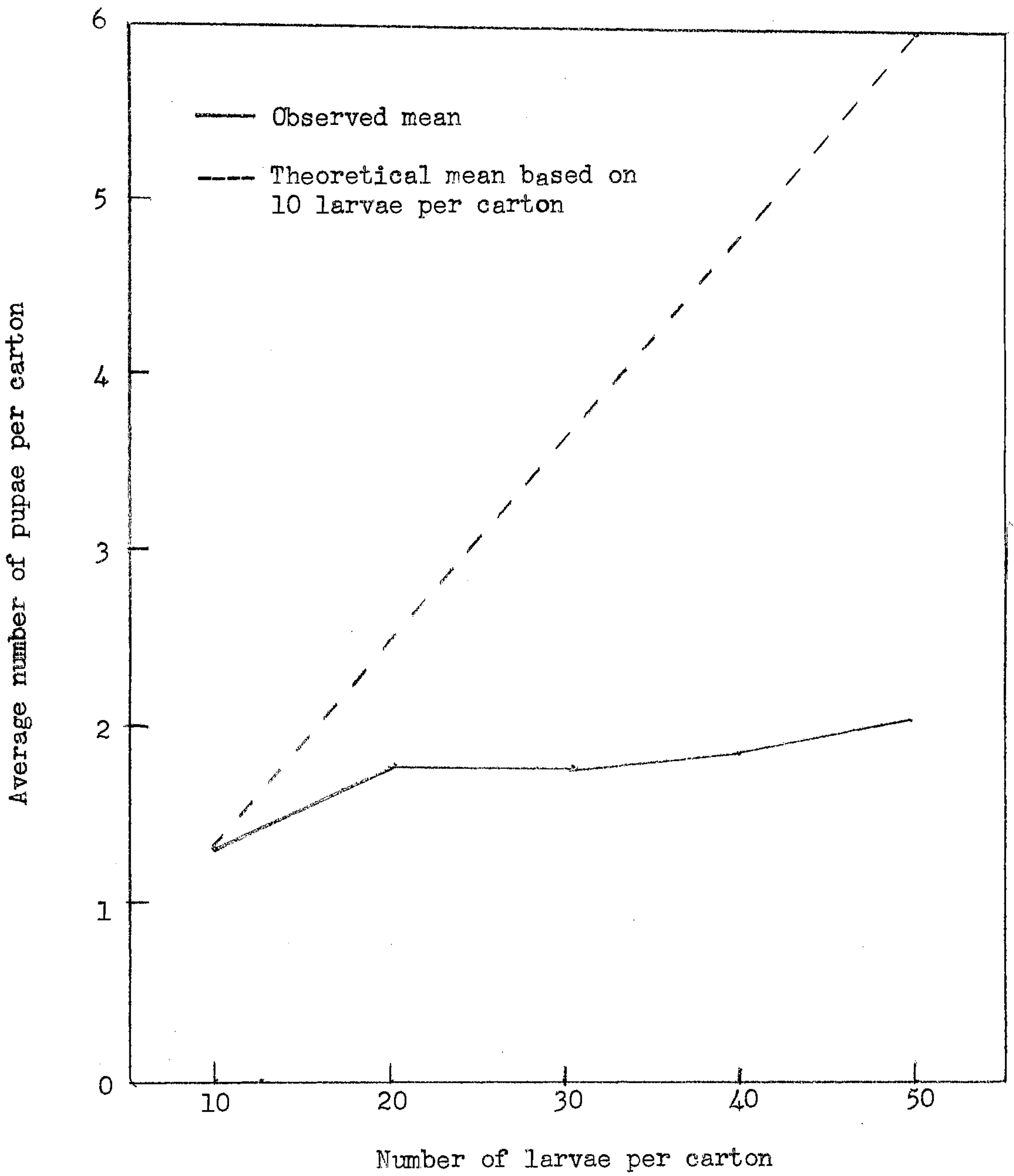


Figure 5. Fall armyworm pupation as related to numbers of larvae per carton when reared on artificial (wheat germ) diet.

survival occurred due to the cannibalistic habits of the larvae. Observation of the larvae under crowded conditions showed that they would attack and devour each other without provocation as if their sole purpose was to obtain food. This also occurred when a generous supply of the artificial diet was available to several larvae in one container. It appears that, regardless of the infestation level, an average of only one or two larvae will survive in one container.

## Adult food test

### Results

Some oviposition occurred where the moths received no food, but an average of only nine eggs per female was laid. The analysis of variance showed a significant difference at the .01 level of probability in the number of eggs laid per moth when fed on the various diets. The Duncan's new multiple range test (Table 8) shows that

Table 8. The effects of varying adult food concentrations on fall armyworm oviposition (Duncan's new multiple range test).

| | Adult diet | | | | |
|---|---|---|---|---|---|
| | No food | 10% sucrose | 10% honey | 50% honey | 50% sucrose |
| Average no. eggs per female | 9 | 547 | 591 | 592 | 632 |

Those mean numbers not connected by the same line are significantly different at the .01 level of probability.

average to be significantly lower than the averages for all other treatments. The means for the 10% sucrose, 10% honey, 50% honey, and 50% sucrose treatments were 547, 591, 592, and 632 eggs per female, respectively. Table 8 shows there was no significant difference in any of those four treatment means.

Discussion

The 50% sucrose solution was chosen as the adult diet for future tests, since sucrose is less expensive than honey, and its use resulted in an average of 85 more eggs per female than the 10% solution, which was almost significant at the .01 level of probability. Also, honey is more difficult to handle, and has a tendency to crystalize after being stored for extended periods.

## Oviposition test

Results

Data showing the results of the test to determine the daily and accumulative oviposition rates of female fall armyworm moths are given in Figure 6. No eggs were deposited on the first and eighth days of the adults' life. Averages of only one and nine eggs were laid per female on the second and seventh days, respectively. Moths laid more eggs on the fourth day than on any other, with an average of 309 per female. Egg production then began to decrease during the last half of the adult cycle. On the fifth day after adult emergence, 93% of the total eggs had been laid.

Discussion

By using this method of egg production, large quantities of fall armyworms could be produced in the laboratory for various tests. The moths mate freely in the cages, and mating was observed several times at night.

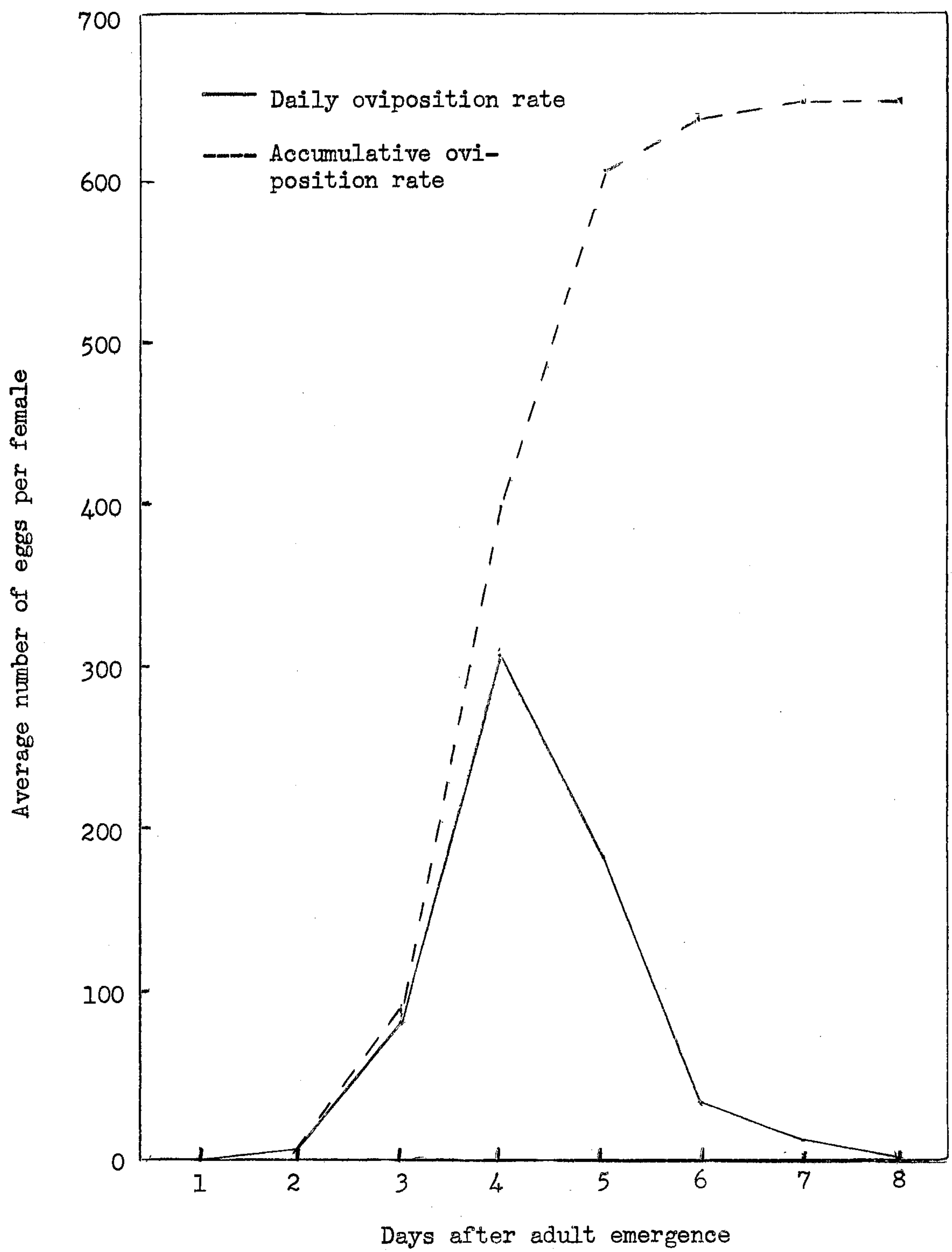


Figure 6. Daily and accumulative oviposition rate per female fall armyworm moth during the adult stage.

## Head capsule measurement study

### Results

A comparison of head capsule measurements of fall armyworm larvae reared on grain sorghum and artificial medium is given in Figure 7. Only slight differences occurred between the average head capsule measurements of each larval instar reared on the two diets. Each bar on the graph has the average head capsule width and standard deviation shown above it for the instar and food represented.

### Discussion

No overlap in head capsule width of consecutive instars occurred when fed either sorghum or the artificial diet. After some experience, the instars could be identified without the aid of magnification. The progression in head capsule width followed essentially a straight line, when plotted on semi-logarithmic paper, which helps support Dyar's rule (Dyar 1890).

## Comparison of life cycle on sorghum and artificial medium

### Results

Data comparing the life cycle of the fall armyworm on grain sorghum and artificial medium diets are presented in Table 9. On both diets the sixth instar larvae gained more weight than the combined weight gain of the first five instars. The larvae gained weight until the sixth instar larvae began to prepare for pupation. The maximum weight for the pupae on both diets was slightly less

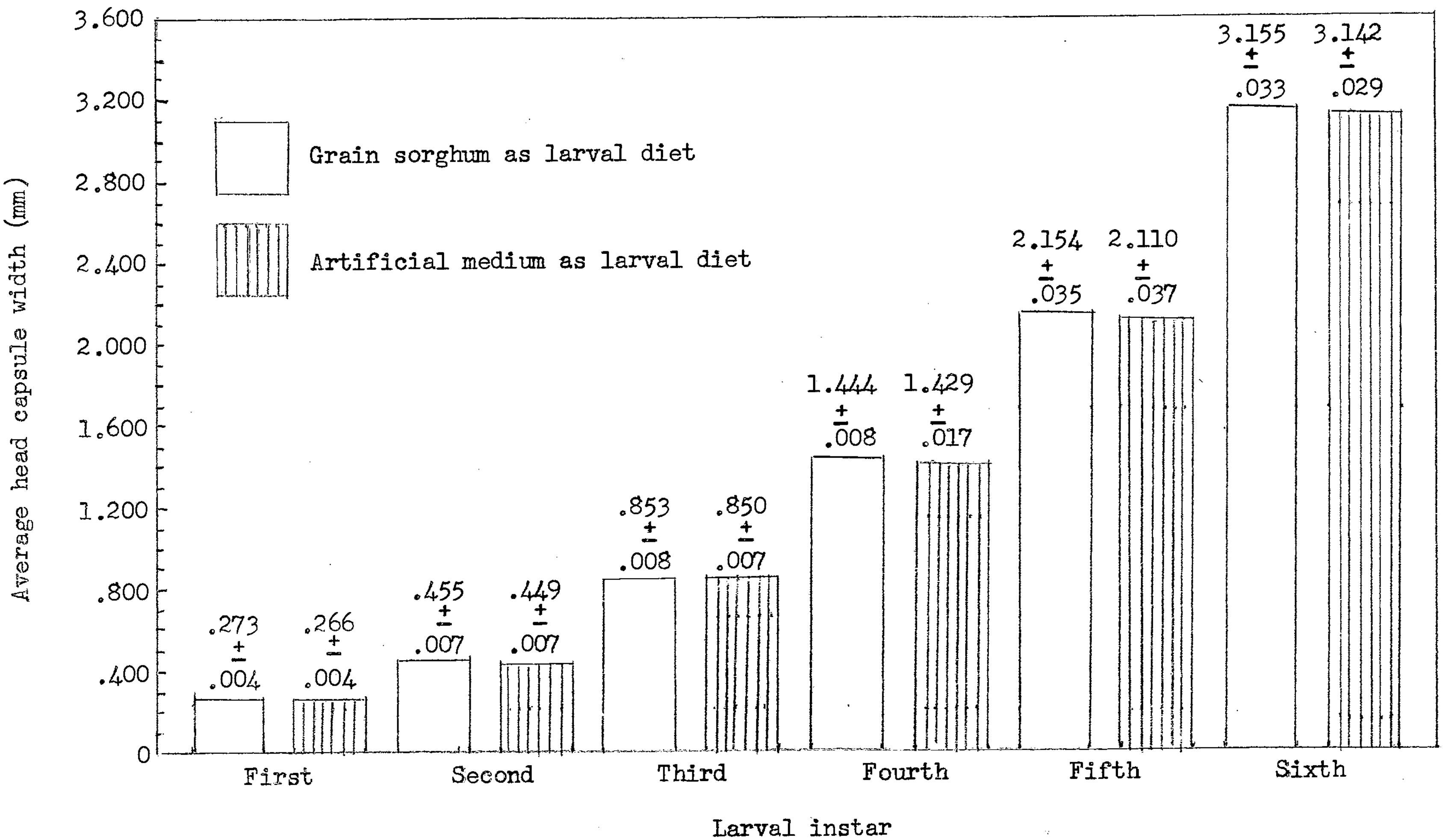


Figure 7. Comparison of head capsule widths (mm) and standard deviations for fall armyworm larvae reared on natural (grain sorghum) and artificial (wheat germ) diets.

Table 9. Fall armyworm development on natural (grain sorghum) and artificial (wheat germ) diets.

| Life stage | Average weight gained (g) | | Average maximum weight (g) | | Average days in life stage | |
|---|---|---|---|---|---|---|
| | Nat. | Art. | Nat. | Art. | Nat. | Art. |
| Larval instar | | | | | | |
| 1 | .00036 | .00033 | .00041 | .00038 | 3.8 | 3.0 |
| 2 | .00250 | .00272 | .00291 | .00310 | 2.0 | 2.2 |
| 3 | .01675 | .00694 | .01966 | .01004 | 1.9 | 1.1 |
| 4 | .03014 | .03931 | .04980 | .04935 | 1.6 | 2.2 |
| 5 | .11324 | .11085 | .16304 | .16020 | 2.0 | 2.0 |
| 6 | .37227 | .35507 | .53531 | .51527 | 3.1 | 4.4 |
| Pupa | -.28721 | -.28063 | .24810 | .23464 | 10.1 | 10.2 |
| Adult | -.03519 | -.03805 | .21291 | .19659 | 7.5 | 7.5 |
| Egg | - | - | .00005* | .00005** | 3.0 | 3.0 |
| Total | - | - | - | - | 35.0 | 35.6 |

* An average of 849 eggs laid per female with 83% hatch.
** An average of 868 eggs laid per female with 81% hatch.

than half the maximum weight attained by the sixth instar larvae. Additional weight was lost in the change from pupa to adult.

The length of time spent in each life stage was very similar on both diets. The average duration of each life stage in days on sorghum and medium, respectively, was: larva, 14.4 and 14.9; pupa, 10.1 and 10.2; adult, 7.5 and 7.5; and egg, 3.0 and 3.0. The average total number of days in the life cycle was 35.0 on sorghum and 35.6 on the artificial diet.

Moths reared from sorghum laid an average of 849 eggs per female and 83% of them hatched. The artificial diet-reared moths laid an average of 868 eggs each, with an 81% hatch.

The pupal weights on the day of pupation were analyzed as a paired experiment. The analysis showed no significant difference at the .01 level of probability in the weights of pupae reared on the two diets.

## Discussion

The sorghum-fed and artificial diet-fed fall armyworm had similar life cycles. If the insect would react similarly when placed on field- or greenhouse-grown sorghum, the artificial method of larval rearing would be satisfactory for producing them in large numbers for various tests.

Pupal weights were used as the criterion for statistical analysis for the same reasons given for the corn earworm.

### Effect of diet change

## Results

Data on the effects of changing the larval food from sorghum to artificial diet and from the artificial diet to sorghum is given in Table 10. There was little difference in the average pupal weights from all the various treatments. The lowest mean was 243 $\pm$ 2 mg, and the highest was 246 $\pm$ 2 mg. The average duration of the larval cycle was also very similar, ranging from 14.8 $\pm$ 0.1 to 15.0 $\pm$ 0.1 days.

## Discussion

The results of this and the test comparing the life cycles on the

Table 10. The effects of changing the larval food of the fall armyworm from natural (sorghum) to artificial (wheat germ), and *visa versa*, at various intervals during the larval stage.

**Group 1**

| Days fed on Nat. | Days fed on Art. | Pupal weight (mg) | Days in larval stage |
|---|---|---|---|
| All | - | 243 ± 2 | 14.9 ± 0.1 |
| 3 | Remainder | 244 ± 2 | 14.8 ± 0.1 |
| 6 | Remainder | 246 ± 2 | 15.0 ± 0.1 |
| 9 | Remainder | 246 ± 2 | 14.9 ± 0.1 |
| 12 | Remainder | 246 ± 2 | 14.9 ± 0.1 |

**Group 2**

| Days fed on Art. | Days fed on Nat. | Pupal weight (mg) | Days in larval stage |
|---|---|---|---|
| All | - | 244 ± 2 | 15.0 ± 0.1 |
| 3 | Remainder | 244 ± 2 | 15.0 ± 0.1 |
| 6 | Remainder | 244 ± 2 | 15.0 ± 0.1 |
| 9 | Remainder | 245 ± 2 | 14.9 ± 0.1 |
| 12 | Remainder | 245 ± 2 | 14.9 ± 0.1 |

two diets indicates that rearing the fall armyworm on an artificial medium would be an acceptable method of obtaining large numbers of this species for various studies. The larvae could be transferred to sorghum from the artificial diet at any time during the larval cycle without affecting the life cycle.

Mass rearing

## Results

Data showing the survival of fall armyworm larvae after one week on artificial medium as affected by numbers per rearing carton are presented in Figure 8. The analysis of variance showed that there was a significant difference among treatments at the .01 level of probability.

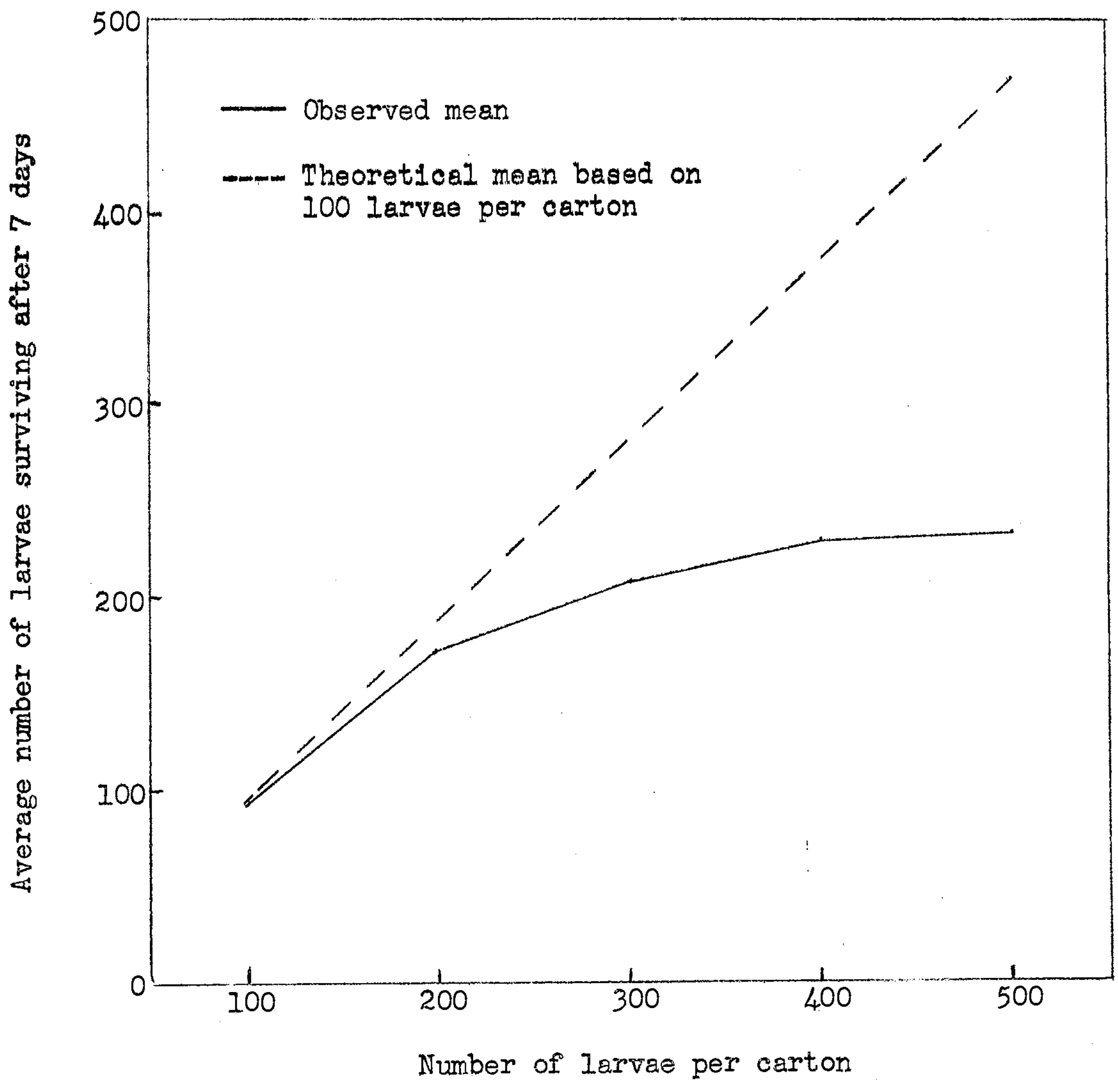


Figure 8. Survival of fall armyworm larvae after seven days on artificial (wheat germ) diet at various levels of infestation.

The Duncan's new multiple range test showed that the 100-, 200-, and 300-larvae-per-carton levels were each significantly different from other treatments. The 400- and 500-larvae levels were not significantly different from each other, but both were significantly different from the other three.

The theoretical mean (Figure 8), based on the 100-larvae treatment, is only slightly higher than the observed mean at the 200-larvae level. This indicates that the percent survival at that infestation level is almost as great as that for the 100-larvae level. The observed means at the 300, 400, and 500 levels are much lower than the theoretical means. At the 100-larvae-per-carton level, an average of 92 of the 100 larvae started in each container survived for one week, for a 92% survival. At the 500-larvae-per-carton infestation level, 230 of the 500 started in each carton survived, for a survival rate of only 46%.

Of those larvae living after one week at the 100-larvae-per-carton level and isolated in individual chambers, 98% pupated.

## Discussion

The infestation level to use in the mass production of the fall armyworm would depend on several factors. The 400 and 500 levels should definitely not be used because of the low survival percentages. Of those started at the 100-larvae level, 92% survived, but 85% survival occurred at the 200 level, which is not a great reduction in survival rate. If egg production is high, the 300-larvae level may be more economical, because an average of 37 more larvae were produced per carton above the 200 level, and shelf space was saved. However, if egg production is limited, it may be more desirable to use an infestation level that will give a higher survival percentage.

The number of larvae surviving in one carton began to level off at the 400-larvae infestation level. This indicates that crowding prevents little more than a certain number to survive when the infestation rate is above the 400-larvae level.

Generation study

Results

Data on the consecutive number of generations of the fall armyworm reared on an artificial diet are presented in Table 11. Seventeen consecutive generations were reared in the laboratory, with an average of 82.5% of all larvae started surviving through pupation. The larvae required an average of 15.1 days to complete the larval cycle.

Excessive yeast growth occurred on the artificial medium in the rearing trays during the eleventh and twelfth generations. However, this did not affect the survival percentage. In fact, the highest survival rate during the entire study (93.0%) occurred in the twelfth generation.

Discussion

The type of rearing chamber used for fall armyworm larvae seemed to have little or no effect on the survival rate or length of the life cycle. For rearing the first six generations, 3 1/2-oz ice cream cups were used. These were found to be undesirable because the larval medium dried and had to be replaced at weekly intervals. This resulted in a great amount of handling of larvae. Also, the cups cost approximately one cent each and had to be discarded after each larval cycle

Table 11. Results of the consecutive generation study of the fall armyworm on artificial (wheat germ) diet.

| Generation | Number started | Percent survival | Days in larval stage | Rearing chamber |
|---|---|---|---|---|
| 1 | 200 | 80.0 | 15.5 | Ice cream cups |
| 2 | 200 | 70.0 | 15.7 | Ice cream cups |
| 3 | 200 | 78.0 | 15.4 | Ice cream cups |
| 4 | 200 | 73.5 | 15.5 | Ice cream cups |
| 5 | 200 | 76.0 | 14.9 | Ice cream cups |
| 6 | 200 | 74.5 | 14.8 | Ice cream cups |
| 7 | 200 | 82.5 | 14.9 | Shell vials |
| 8 | 200 | 84.5 | 15.0 | Shell vials |
| 9 | 200 | 85.0 | 15.1 | Shell vials |
| 10 | 200 | 89.0 | 14.9 | Ice trays |
| 11 | 200 | 86.5* | 15.3 | Ice trays |
| 12 | 200 | 93.0* | 15.6 | Ice trays |
| 13 | 200 | 86.0 | 14.8 | Ice trays |
| 14 | 200 | 82.0 | 14.9 | Ice trays |
| 15 | 200 | 90.0 | 14.9 | Ice trays |
| 16 | 200 | 87.5 | 15.0 | Ice trays |
| 17 | 200 | 81.5 | 15.0 | Ice trays |
| Average | 200 | 82.5 | 15.1 | - |

* Excessive yeast growth on artificial medium in rearing trays.

was completed to prevent contamination. The seventh, eighth, and ninth generations were reared in glass shell vials. It was not necessary to replace the medium in these vials during the larval cycle, but cleaning and sterilizing the vials involved a great deal of labor. Pupae were also difficult to remove from the vials without injury. Polyethylene ice cube trays were used to rear generations 10 through 17. Excessive drying of the medium was prevented by their use. The ice trays were easy to clean and sterilize, and it was possible to remove the pupae from the individual cells with ease.

Although yeast growth did not affect development of the fall armyworm, 100 g of agar, rather than 90 g, and 2900 ml of water, rather than 3100 ml, were used in the diet. This alteration in the medium made it drier and more solid, which reduced yeast growth. Since it was necessary to use the modified diet for the corn earworm, which was affected by yeast, and in order to prevent the making of two different diets, the modified diet was also used for the fall armyworm. The drier diet did not appear to affect the insects' development.

## TESTS WITH SOUTHWESTERN CORN BORER

### Test on various diets

Results

Table 12 shows the results of the test to determine the most suitable diet for rearing larvae of the southwestern corn borer. The highest survival rate and the shortest larval cycle were obtained by feeding the wheat germ diet. An average of 79% of these larvae survived

Table 12. Effects of various larval diets on southwestern corn borer larval development and pupation.

| Diet used | No. larvae | Pupation Number | Pupation Percent | No. days in larval stage |
|---|---|---|---|---|
| Wheat germ | 300 | 236 | 79 | 19.3 |
| European corn borer | 300 | 201 | 67 | 20.6 |
| Powdered corn leaf | 300 | 52 | 17 | 24.9 |
| Corn meal | 300 | 12 | 4 | 26.0 |

through pupation after spending an average of 19.3 days in the larval stage. The European corn borer diet, with 67% survival and 20.6 days spent as larvae, also, was fairly satisfactory. Survival on the powdered corn leaf and corn meal diets was low, and the larval periods were increased in length by almost a week over those fed wheat germ medium.

## Discussion

Although the European corn borer diet was not satisfactory for rearing corn earworm and fall armyworm larvae, it proved to be fairly desirable as a larval medium for the southwestern corn borer. The corn meal diet was least satisfactory, which did not agree with the results obtained in similar tests on corn earworm and fall armyworm. In this latter case, the powdered corn leaf medium gave the poorest results. Most of the southwestern corn borer larvae fed the corn meal and powdered corn leaf diets died during the first week of the larval stage.

### Test on number of larvae per rearing carton

## Results

Data on the determination of the number of southwestern corn borer larvae that could be reared per carton is presented in Figure 9. The analysis of variance showed a significant difference in survival rate between different treatments at the .01 level of probability.

The Duncan's new multiple range test showed that each of the 10-, 20-, and 30-larvae-per-carton infestation levels was significantly different from all other treatments. The 40- and 50-larvae levels were not significantly different from each other, but each is significantly different from the other three.

The observed mean survival at the 20- and 30-larvae levels were only slightly below the theoretical mean based on the 10-larvae-per-carton level, but the 40- and 50- larvae levels began to fall farther below the theoretical mean.

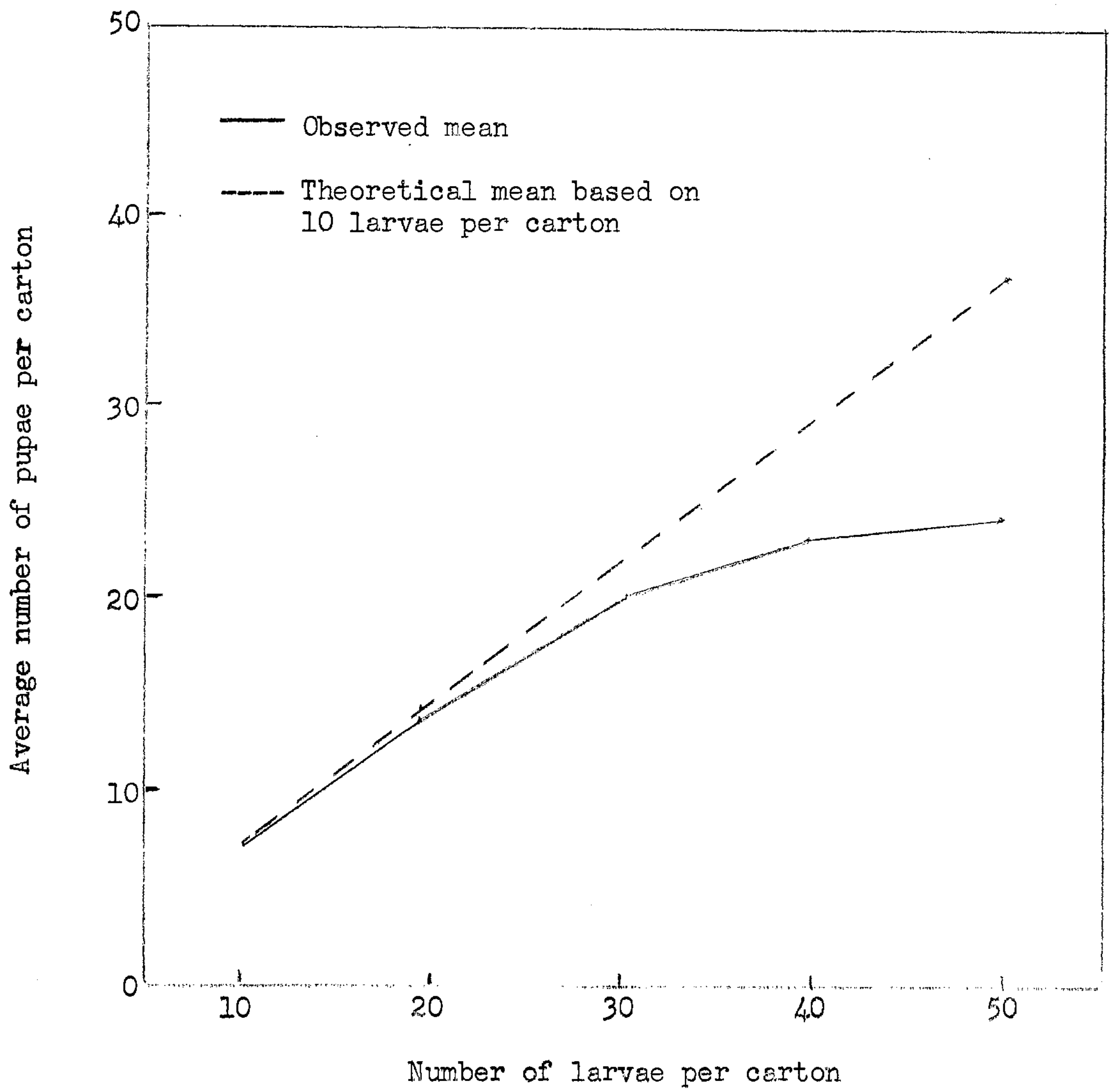


Figure 9. Southwestern corn borer pupation as related to numbers of larvae per carton when reared on artificial (wheat germ) diet.

## Discussion

Due to their lack of cannibalistic habits, it was possible to rear southwestern corn borer larvae throughout their larval cycle with several in each rearing container. The most desirable level for mass rearing purposes would probably be somewhere between 20 and 30 larvae per carton, since the observed mean was very close to the theoretical mean at those levels.

Oviposition test

Results

Data on the daily and accumulative oviposition rates for the southwestern corn borer are presented in Figure 10. This species did not begin to oviposit until the second day after emergence, when an average of only six eggs each were laid. Peak egg production was reached on the fourth day, when 95 eggs per female were laid. The accumulative egg production by the fourth day was 75% of the total production of 195 eggs per female at the end of six days.

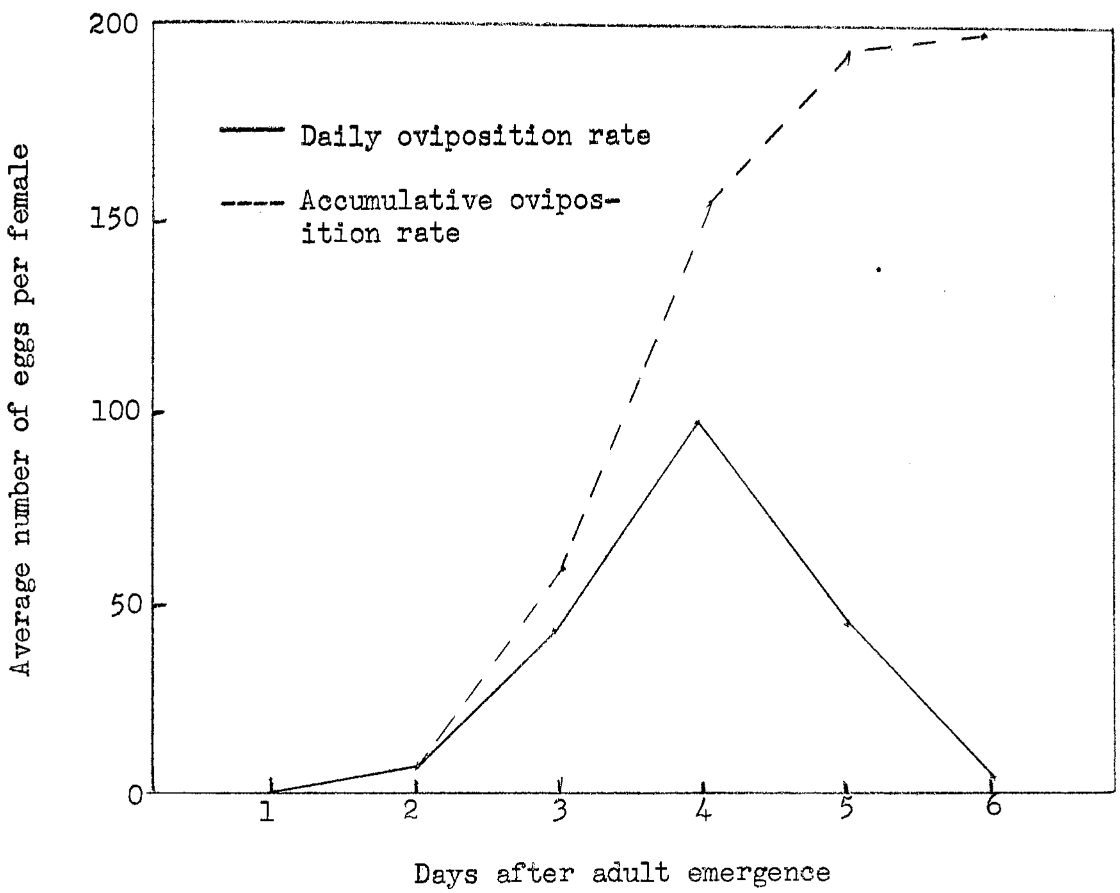


Figure 10. Daily and accumulative oviposition rate per female southwestern corn borer moth during the adult stage.

Discussion

The test showed that large numbers of southwestern corn borer eggs can be obtained by using this rearing method. The oviposition cages, as described in the methods and materials section, were satisfactory, since the oviposition surface could be removed from the outside of the cage without the moths escaping.

Head capsule measurement study

Results

Data on the comparison of head capsule width of southwestern corn borer larvae fed on sorghum and artificial diets are presented in Figure 11. Very little difference occurred between the head capsule measurements of any larval instar fed either of the two diets. The average head capsule measurement and standard deviation are shown above the bar corresponding to each larval instar and diet.

Discussion

There was no overlap observed in the head capsule width of consecutive instars when fed either diet. The instars could even be separated without magnification after some experience. The growth progression in head capsule width was linear when plotted on semi-log paper, which helps support Dyar's rule (Dyar, 1890).

Comparison of life cycle on
sorghum and artificial medium

Results

Data on the life cycle of the southwestern corn borer reared on

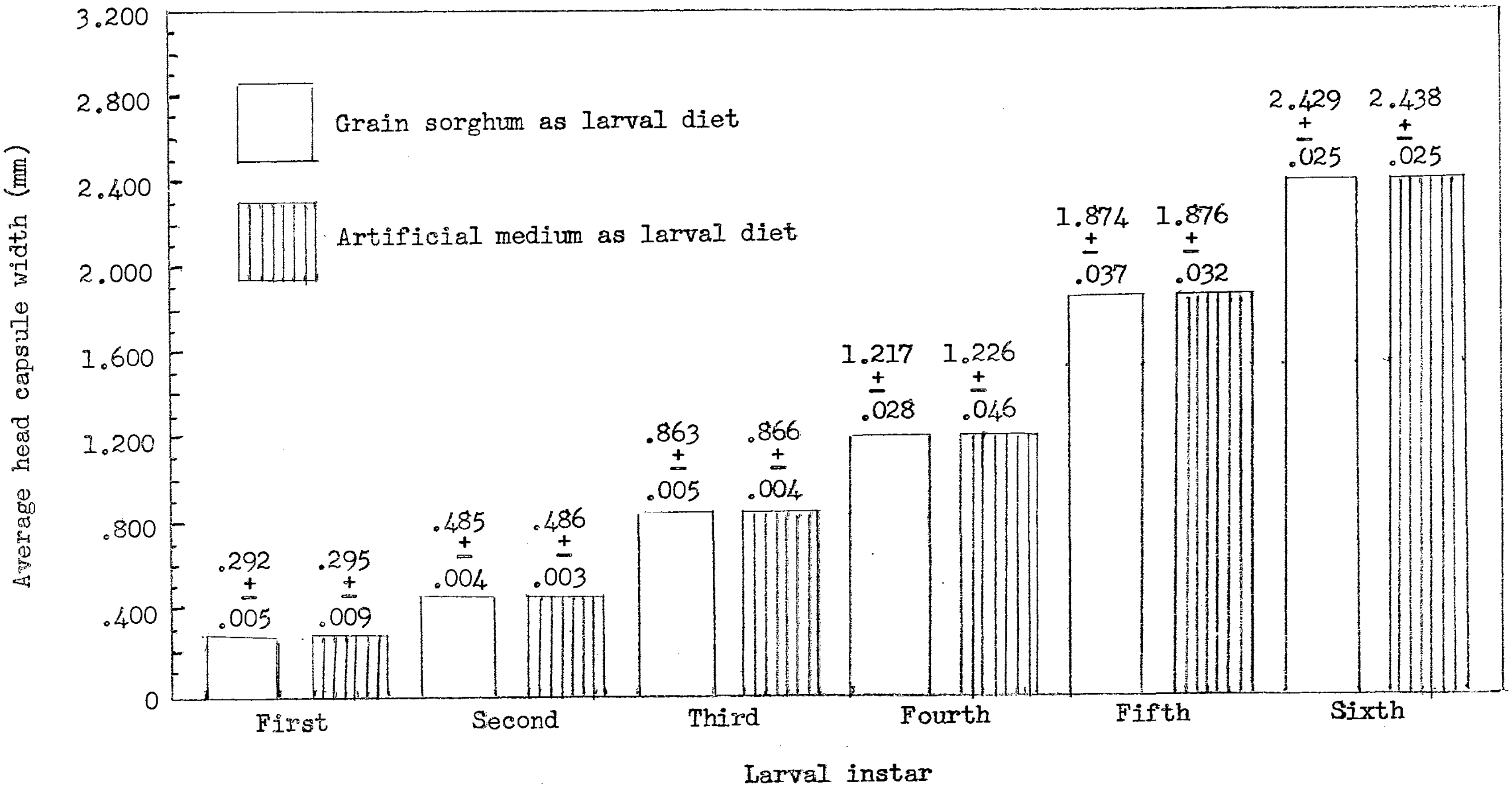


Figure 11. Comparison of head capsule widths (mm) and standard deviations for southwestern corn borer larvae reared on natural (grain sorghum) and artificial (wheat germ) diets.

sorghum and artificial medium are presented in Table 13. Most of the larval weight was gained in the fourth and fifth instars. The larvae became somewhat less active after molting to the sixth instar, and almost all of them began to lose weight the second day after reaching that stage. The average maximum weight attained in the sixth instar was .43800 g on sorghum and .44107 g on the artificial diet. The average pupal weights were similar, being .22821 g and .22919 g on sorghum and artificial diet, respectively. The pupal weight on

Table 13. Southwestern corn borer development on natural (grain sorghum) and artificial (wheat germ) diets.

| Life stage | Average weight gained (g) | | Average maximum weight (g) | | Average days in life stage | |
|---|---|---|---|---|---|---|
| | Nat. | Art. | Nat. | Art. | Nat. | Art. |
| Larval instar | | | | | | |
| 1 | .00028 | .00030 | .00033 | .00035 | 3.5 | 3.4 |
| 2 | .00588 | .00611 | .00621 | .00646 | 3.0 | 3.0 |
| 3 | .04506 | .04490 | .05127 | .05136 | 3.0 | 3.0 |
| 4 | .19972 | .20026 | .25099 | .25162 | 3.0 | 3.0 |
| 5 | .14799 | .14937 | .39898 | .40099 | 3.0 | 3.0 |
| 6 | .08902 | .04008 | .43800 | .44107 | 3.9 | 3.9 |
| Pupa | -.20979 | -.21188 | .22821 | .22919 | 9.5 | 10.1 |
| Adult | -.02273 | -.02204 | .20548 | .20715 | 5.8 | 6.0 |
| Egg | - | - | .00005* | .00005** | 5.0 | 5.0 |
| Total | - | - | - | - | 39.7 | 40.4 |

* An average of 218 eggs laid per female with 90% hatch.
** An average of 229 eggs laid per female with 89% hatch.

the day of pupation was the criterion for statistical analysis. The analysis was made as a paired experiment, and no significant difference was observed at the .01 level of probability.

The average number of days spent in each life stage was very similar on sorghum and medium, being respectively: larva, 19.4 and 19.3; pupa, 9.5 and 10.1; adult, 5.8 and 6.0; and egg, 5.0 and 5.0. The total length of the life cycle on sorghum was 39.7 days, and for the artificial diet 40.4 days.

Moths reared from sorghum laid an average of 218 eggs each with 90% of them hatching. Those reared from the artificial diet laid an average of 229 each, with an 89% hatch.

Discussion

The results indicate that the artificial-diet method of rearing southwestern corn borer larvae would be satisfactory for obtaining large numbers of larvae. They reacted so similarly on the two diets that both seem to contain all the needed requirements for growth and reproduction.

Effect of diet change

Results

Data showing the results of the test to determine the effects of substituting sorghum for artificial medium and artificial medium for sorghum as food for southwestern corn borer larvae at various intervals during the larval cycle are presented in Table 14. The average pupal weights were very similar on the various treatments. They ranged from 227 ± 1 mg to 229 ± 1 mg. Larvae from all treatments spent

Table 14. The effects of changing the larval food of the southwestern corn borer from natural (sorghum) to artificial (wheat germ), and *visa versa*, at various intervals during the larval stage.

**Group 1**

| Days fed on: Nat. | Days fed on: Art. | Pupal weight (mg) | Days in larval stage |
|---|---|---|---|
| All | - | 228 ± 1 | 18.0 ± 0.1 |
| 3 | Remainder | 228 ± 1 | 18.9 ± 0.1 |
| 6 | Remainder | 229 ± 1 | 19.0 ± 0.1 |
| 9 | Remainder | 228 ± 2 | 19.1 ± 0.1 |
| 12 | Remainder | 228 ± 1 | 19.0 ± 0.1 |
| 15 | Remainder | 229 ± 1 | 19.1 ± 0.1 |

**Group 2**

| Days fed on: Art. | Days fed on: Nat. | Pupal weight (mg) | Days in larval stage |
|---|---|---|---|
| All | - | 228 ± 1 | 19.0 ± 0.1 |
| 3 | Remainder | 227 ± 1 | 19.2 ± 0.2 |
| 6 | Remainder | 228 ± 1 | 19.1 ± 0.2 |
| 9 | Remainder | 228 ± 1 | 19.0 ± 0.2 |
| 12 | Remainder | 228 ± 2 | 18.9 ± 0.2 |
| 15 | Remainder | 229 ± 1 | 19.0 ± 0.1 |

approximately the same time in the larval stage. The average larval cycle ranged from 18.9 ± 0.2 to 19.2 ± 0.2 days.

## Discussion

This test and the one comparing the entire life cycle on the two diets indicate that artificially reared southwestern corn borers react similarly to those reared on sorghum. This would make it possible to produce large numbers of that species on artificial medium for various studies. The pupal weights were used for comparison because of uniformity and ease of handling.

## Generation study

### Results

Data on the consecutive generations of the southwestern corn borer reared on an artificial diet in various rearing chambers is presented in Table 15. This species was reared for 12 consecutive generations on artificial medium. There was only 50.5% survival in the twelfth generation, and no fertile eggs were laid by the females.

An average of 68.5% of all larvae started on the diet survived through pupation after spending an average of 19.7 days in the larval stage. The low survival rate in the fourth and seventh generations was partly due to the fact that some larvae chewed through the walls of the ice cream cups and plastic ice trays which were tried as rearing chambers in place of the glass shell vials. The low survival and lack of fertile eggs in the twelfth generation cannot be explained. Otherwise, the survival rate and length of the larval periods were similar in other generations.

### Discussion

Glass shell vials were used first to rear southwestern corn borer larvae. They were hard to clean, so ice cream cups were tried during the fourth generation. Several larvae chewed through the cups and escaped. Shell vials again were used for the fifth and sixth generations.

Table 15. Results of the consecutive generation study of the southwestern corn borer on artificial (wheat germ) diet.

| Generation | Number started | Percent survival | Days in larval stage | Rearing chamber |
|---|---|---|---|---|
| 1 | 200 | 70.0 | 19.9 | Shell vials |
| 2 | 200 | 76.0 | 19.4 | Shell vials |
| 3 | 200 | 74.5 | 19.6 | Shell vials |
| 4 | 200 | 48.0* | 19.1 | Ice cream cups |
| 5 | 200 | 79.5 | 20.0 | Shell vials |
| 6 | 200 | 81.5 | 19.4 | Shell vials |
| 7 | 200 | 53.0* | 19.4 | Ice trays |
| 8 | 200 | 59.0** | 19.6 | Sandwich boxes |
| 9 | 200 | 76.0 | 19.3 | Sandwich boxes |
| 10 | 200 | 85.5 | 19.9 | Sandwich boxes |
| 11 | 200 | 67.0 | 20.3 | Sandwich boxes |
| 12 | 200 | 50.5*** | 20.9 | Sandwich boxes |
| Average | 200 | 68.5 | 19.7 | - |

* Several larvae chewed out of the rearing chamber.
** Excessive yeast growth on artificial medium in rearing chambers.
*** No fertile eggs received from moths.

Polyethylene ice cube trays were tried for the larvae in the seventh generation, but several chewed out of them, also. The larvae from the eighth through the twelfth generations were reared in plastic sandwich boxes with 20 larvae started in each box. The low survival rate noted in the eighth generation, which was reared in sandwich boxes, was due to excessive yeast growth on the medium. The agar in the medium was increased from 90 to 100 g, and water was reduced from 3100 to 2900 ml. This resulted in a dryer medium which seemed to be undesirable for yeast growth. Survival rate increased back to normal in the following generations after the diet was altered.

It is apparent from these data that the southwestern corn borer can be reared satisfactorily using the techniques described previously.

## GENERAL SUMMARY AND CONCLUSIONS

The tobacco budworm, Heliothis virescens (F.), failed to develop on grain sorghum when sorghum plants in the greenhouse were manually infested at levels of 5, 10, and 20 larvae per plant. These data indicate that there is little liklihood that this species occurs as a pest on sorghum and could be confused with the corn earworm.

An artificial diet containing wheat germ was found to be the most desirable larval medium for the corn earworm, Heliothis zea (Boddie), the fall armyworm, Laphygma frugiperda (J. E. Smith), and the southwestern corn borer, Zeadiatraea grandiosella (Dyar), when compared with a diet containing corn meal and two diets containing dry powdered corn leaves.

It was found impractical to rear corn earworm and fall armyworm larvae with several larvae in a container due to extensive cannibalism. An average of only one or two larvae survived through pupation at infestation levels of 10, 20, 30, 40, and 50 larvae per 1/2-pint ice cream carton for both species. On the other hand, several southwestern corn borer larvae were reared in the same rearing chamber. An average of 20 larvae pupated in each 1/2-pint ice cream carton at a 30-larvae-per-carton infestation level.

Adult food was found to be necessary for satisfactory egg production of the corn earworm and fall armyworm moths. The number of eggs laid by females of both species was significantly higher when moths were fed either 10 or 50% honey, or 10 or 50% sucrose solutions. The highest numbers of eggs were obtained from moths fed 50% sucrose, but the mean was not significantly higher than for all other treatments.

The corn earworm, fall armyworm, and southwestern corn borer moths reached a peak in egg production on the fourth day after emergence. All three species laid very few eggs after the fifth day. Adults of the corn earworm and fall armyworm lived an average of eight days, and those of the southwestern corn borer an average of six days.

No significant differences in head capsule widths in the several instars of larvae of the corn earworm, fall armyworm, or the southwestern corn borer were observed when measurements of those reared on sorghum were compared with those reared on the artificial diet. This would indicate the satisfactory nature of the artificial diet.

When corn earworm, fall armyworm, and southwestern corn borer larvae were fed on sorghum or the artificial diet, the diet had little effect upon the life cycles of each of the species. The respective average number of days in the life cycles when fed on sorghum and artificial diet were: corn earworm, 36.6 and 36.9; fall armyworm, 35.0 and 35.6; and southwestern corn borer, 39.7 and 40.4.

It was found, during this study, that a change in the larval diet from sorghum to artificial medium or from artificial medium to sorghum, at various intervals in the larval cycle, had little effect on the pupal weights or duration of the larval cycle of the corn earworm, fall armyworm, or southwestern corn borer.

The corn earworm and fall armyworm larvae could be reared for a period of one week with as many as 200 to 300 per carton without high mortality due to cannibalism. They had to be isolated into individual chambers after that length of time. The southwestern corn borer larvae, because they are not cannibalistic, did not have to be individually isolated.

The corn earworm was reared for 14, and the fall armyworm for 17 consecutive generations in the laboratory on an artificial diet with no apparent detrimental effects. The southwestern corn borer was reared through 12 generations, but for no apparent reason, moths from the last generation did not lay fertile eggs.

On the basis of the data presented herein, artificial diets and techniques have been developed which should have practical use for mass production of eggs, larvae, pupae, and adults of the corn earworm, fall armyworm, and the southwestern corn borer. There is an immediate need for these insects in large numbers in connection with the development of insect resistance in sorghums, but their availability in large numbers for insecticidal and other research is also important.

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