A COMPARATIVE STUDY OF THE IMMATURE STAGES OF THREE SPECIES OF THE *Diatraea* COMPLEX

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A COMPARATIVE STUDY OF THE IMMATURE STAGES OF

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PREFACE

Entomologists have long been intrigued by the apparent similarity of the larvae of Diatraea and Zeadiatraea. The three species of these genera that are of economic importance in the United States are the sugar cane borer, Diatraea saccharalis (Fabr.), the southern corn stalk borer, Diatraea crambidoides (Grote) and the southwestern corn borer, Zeadiatraea grandiosella (Dyar). The taxonomy of adults, seasonal history and biological and chemical control of these species have been investigated rather thoroughly since 1911. However, very little work on larval stages has been accomplished.

The purpose of the present study has been to develop techniques for rearing larvae and to determine the extent of the differences which occur in the morphologies, life cycles and habits of the larvae of the three species reared under the same conditions in the laboratory. Since the rearing work was started during the winter, when the larvae of all three species are normally in diapause or quiescence, a method was developed to induce over-wintering larvae to pupate. The laboratory work has been supplemented with observation and data obtained from study of field populations of Z. grandiosella and D. saccharalis.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure | Page

PART I

INTRODUCTION

The Diatraea complex, which includes the genera Diatraea and Zeadiatraea, contains approximately 45 species of moths indigenous to the Western hemisphere. The larvae of most species are borers of graminaceous or cyperaceous plants. The complex includes a large number of economically important pests of cultivated grasses.

The distribution of Diatraea and Zeadiatraea is primarily neotropical, however six species, D. saccharalis (Fabr.), D. crambidoides (Grote), D. evanescens Dyar, D. lisetta (Dyar), D. venosalis (Dyar), and Z. grandiosella (Dyar) are listed from nearctic regions. D. lisetta and D. venosalis have not been reported since their description (Dyar 1911). D. evanescens is found on native grasses of the genus Paspalum in Louisiana but is not a pest of cultivated crops.

The sugar cane borer, D. saccharalis, is well known as a pest of sugar cane and corn in the Gulf Coast Region of Louisiana and Texas and in the lower half of Florida. It is also a pest of rice in Louisiana and Texas, especially where that crop is grown in close proximity to large acreages of sugar cane or corn.

The southern corn stalk borer, D. crambidoides, is a pest of corn in the southeastern states. Its range extends from Maryland to Florida and westward to Kansas. Since its description by Grote in 1880, it has been reported only twice in Kansas and once in Wisconsin and its present existence in these states is doubtful. States in which it is

reported as an economic pest of corn are Maryland, Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama and Mississippi.

The southwestern corn borer Z. grandiosella is a major pest of corn in the southwestern states. This species was originally described in Diatraea (Dyar 1911) but recently has been assigned to the new genus Zeadiatraea (Box 1955). Its range extends from Texas, northward into Nebraska, westward into Colorado and Arizona and eastward into Louisiana, Arkansas and Missouri. It is also a pest of corn in northern Mexico and is reported to injure sugar cane severely in certain irrigated areas. In the United States the distribution of this species does not include any area where sugar cane is grown, however in 1958, it was collected from corn in Avoyelles Parish in Louisiana at a point only 50 miles from the northern limits of the sugar cane belt.

Another species of Zeadiatraea, the neotropical corn stalk borer, Z. lineolata (Walker) whose distribution is now listed as limited to the southern half of Mexico, has been erroneously reported as occurring in the southwestern states. The insect referred to in those reports was probably Z. grandiosella.

Estimated losses from D. saccharalis damage to sugar cane in Louisiana and Florida range from eight to eleven million dollars annually. In 1951, damage to corn by the southwestern corn borer caused losses estimated at 22 million dollars. Severe losses resulting from damage by D. crambidoides in North Carolina, South Carolina and Virginia were reported prior to 1935. Since these reports, losses have been so small that few attempts have been made to control the pest.

PART II

REVIEW OF THE LITERATURE

History of Diatraea and Zeadiatraea. Considerable confusion exists in the literature relating to species of Diatraea and Zeadiatraea. Until 1911, it was believed that only one species, D. saccharalis, was found in the United States and all papers and records on the southwestern corn borer, southern corn stalk borer and the sugar cane borer were published under that name. In that year Dyar (1911) published a revision of the American species that definitely established the existence of more than one species in this country.

In this revision, Dyar described the southwestern corn borer and the southern corn stalk borer as new species, under the names D. grandiosella and D. zeacolella, respectively, and retained the name D. saccharalis for the sugar cane borer. Although the taxonomic characters used by Dyar were later shown to be unreliable for separating many species (Dyar and Heinrich 1927, Box 1931), his revision was invaluable to economic entomology, because it established as separate species, three of the more important crop pests found in the United States.

Much later, Box (1931) determined that D. zeacolella was synonymous with Chilo crambidoides which had been described by Grote (1880) from a specimen collected in Kansas. Thus the southern corn stalk

borer was assigned the name D. crambidoides under which it is presently known. Recently, Box (1955) placed the southwestern corn borer in a new genus and it is now known as Zeadiatraea grandiosella.

Since 1911, the genus Diatraea has been revised three times, once by Dyar and Heinrich (1927) and twice by Box (1931, 1955). These revisions were based on detailed studies of the genitalia for diagnostic purposes and have resulted in a new and more limited species concept.

Prior to 1911 references to Diatraea in the literature are vague. Fabricius (1794) apparently described the first species as Phalaena saccharalis from specimens collected in the West Indies by a Danish Army officer. Neither the type or specific locality of Fabricius' species is known but several entomologists, Zeller (1881), Howard (1891) and Box (1931) have concluded that this was the species later described by Guilding as D. saccharalis.

Fabricius' note on oviposition, larval habits, pupation and emergence of moths is essentially correct; however, he thought that only one generation occurred each crop season instead of the several generations now known to occur throughout its range. His paper also includes an accurate account of severe damage from borer injury in causing fermentation and loss in juice content of sugar cane.

Thirty years later, Guilding (1828) stated that the sugar cane borer was by far the most common and destructive pest of sugar cane on St. Vincent Island in the West Indies. The sugar cane borer was first recognized as a pest of sugar cane in Louisiana by Avequin (1857). Apparently the first noticeable infestations occurred in 1855 in fields in the Parish of St. John the Baptist.

D. crambidoides was described by Grote (1880) as Chilo crambidoides from specimens reportedly sent to Canada from Kansas. Since that time

there are few records of its occurrence anywhere except in the southeastern states. Leiby (1920) believed that its distribution extended as far westward as Kansas, Oklahoma and Texas, however his paper does not include any reference to it being collected from those states. Dyar and Heinrich (1927) reported it from Kansas as D. zeacolella, and Smith (1942) reported larvae found there in 1941 were possibly D. crambidoides. Drake and Decker (1927) also reported the occurrence of this species, as D. zeacotella (sic), at Racine, Wisconsin. The author believes that the description and reports of D. crambidoides in Kansas and Wisconsin should be regarded critically since at present the species is only found in the southeastern states.

Perhaps the first reference to the southwestern corn borer, Z. grandiosella occurs in a paper by Howard (1891) when he gave an account of larvae of the sugar cane borer infesting corn in New Mexico. Present day distribution records indicate that these larvae could not have been the sugar cane borer, but were probably the southwestern corn borer since it is the only species of the Diatraea complex that is now known to be indigenous to New Mexico.

A technical description of Diatraea and drawings of the genitalia are presented by Dyar and Heinrich (1927). Photomicrographs of the genitalia are given by Box (1931). Technical descriptions and drawings of the genitalia of Zeadiatraea are presented by Box (1955). Methods for separating certain species of Diatraea and Zeadiatraea are presented by Holloway (1916) and Peterson (1948). Box (1955) lists the food plants of Diatraea. The synonymy followed in this paper is that of Box (1931) for Diatraea and the same author (1955) for Zeadiatraea.

Biology and control of D. saccharalis, D. crambidoides and Z. grandiosella. The first work of importance on the biology of the

sugar cane borer in Louisiana was reported by Stubbs and Morgan (1902). Holloway and Loftin (1919) made a more thorough study of its biology, seasonal history and distribution. The bulletin by Holloway et al. (1928) gives a most complete account of its seasonal history, methods of injuring plants, cultural control and distribution in the United States. Since that time,very little work on biology has been reported.

Considerable time and effort has been expended in Louisiana in attempts to control the sugar cane borer by introduction and release of dipterous and hymenopterous parasites of tropical origin (Jaynes 1933, 1939; Ingram et al. 1940). As Clausen (1956) indicated, none has become established, and there appears to be little prospect of the successful utilization in Louisiana of any of the more common parasites of the pest found in the West Indies, Central and South America. Clausen states that there is little likelihood of tropical parasites of D. saccharalis over-wintering in Louisiana because of cool winter temperatures and discontinuous cropping practices, which limits populations of their host. In Florida, where these parasites are effective, sugar cane is a continuous crop as it is in the countries of their origin and provides an adequate host population throughout the year. For many years, attempts have also been made in Louisiana to control D. saccharalis by rearing and releasing the egg parasite Trichogramma minutum Riley (Hinds et al. 1933, Dugas 1943). Jaynes and Bynum (1941) have shown that such releases had no appreciable effect in reducing borer injury to sugar cane. More recently, effective control of this pest has been obtained with the insecticide endrin (Long et al. 1959).

The most important papers on the biology and seasonal history of D. crambidoides are by Leiby (1920), Phillips et al. (1921) and Cartwright

(1934). The only recent work related to this species is presented by Brett (1953) who found that the insecticide endrin was an effective control.

The biology, seasonal history and control of Z. grandiosella has been more thoroughly investigated than that of D. saccharalis and D. crambidoides. Several papers (Todd and Thomas 1930; Thomas and McGregor 1937; Davis et al. 1933; Walton and Bieberdorf 1948; Wilbur et al. 1950, and Rolston 1955 b) relate to the seasonal history and cultural control of the species in various states. Attempts to control the pest with insecticides (Arbuthnot and Walton 1954; Walton et al. 1957; Rolston 1955 a) have shown that several insecticides including endrin are effective, however, the low value of the corn crop in many states prohibits their use.

Burkhardt and Painter (1954) discuss the difference in weight of Z. grandiosella larvae reared on teosinte and on corn. The larval habits of this species were studied by Hensley (1955) and the migration of larvae on corn plants is discussed by Hensley and Arbuthnot (1957). Gifford (1958) investigated the development of larvae on several varieties of grain sorghums. Kevan (1944) has discussed the aspects of diapause in Z. lineolata larvae in Trinidad.

PART III

METHODS AND MATERIALS

Methods for maintaining colonies of moths in the laboratory. In order to obtain eggs for rearing larvae of D. saccharalis, D. crambidoides and Z. grandiosella, moth colonies were established in a laboratory room at a temperature of approximately 22 degrees C. in January 1956. The pupae of each species were placed on damp sand under half gallon ice cream cartons from which the tops and bottoms had been removed. Fine mesh nylon screening served to cover openings in the cages and each cage was lined with waxed paper which was removed daily during periods of oviposition.

The moths emerged and mated and the females deposited eggs on the waxed paper inserts. When an insert bore eggs it was removed and held in a cabinet at a temperature of 27 degrees C. The eggs were examined three days later to determine if they were fertile. Small sections containing eggs in which embryos were developing were clipped from the paper, placed in petri dishes and returned to the cabinet. A few hours prior to eclosure, a section of corn leaf was placed in each petri dish as food for the young larvae and the dish was covered to prevent their escape. Using this technique, 72 first instar larvae of each species were obtained and rearing initiated. The age of larvae of each species

varied no more than 12 hours. The species were reared almost concurrently since rearing of all three was started within a period of eight days.

Laboratory techniques for rearing larvae of D. saccharalis, D. crambidoides and Z. grandiosella. In order to determine the number of instars and to obtain cast head capsules for comparison in size, series of larvae of each species were reared in as uniform conditions as possible in the laboratory. The larvae were reared individually in 15 x 50 mm. shell vials. During the first two instars, cotton plugs were used to confine the larvae to vials, but at the beginning of the third instar these were replaced with caps made from 100 mesh copper screening. Racks for holding vials consisted of small wooden trays capable of holding 36 vials. Except during periods of examination, the larvae were kept in a cabinet in which the temperature was thermostatically controlled at 27 degrees C. and which contained a fan that prevented stratification of air.

Corn plants grown in a greenhouse were used as a source of food for larvae during rearing. It had been determined in previous rearing studies on Z. grandiosella that larvae would feed on freshly cut sections of corn stalks for approximately three days before such material decayed and became unacceptable. A section of stalk measuring 3/8 inch in diameter and one inch long was an adequate three day food supply for one larva. During the first two instars a small section of corn leaf was also placed in each vial since young larvae often feed on leaf tissue in the field. Corn stalks were brought into the laboratory and

cut into one-inch sections. Those sections that were more than 3/8 inch in diameter were split into pieces of appropriate size. The sections were then thoroughly mixed and randomly distributed to vials.

First instar larvae that had hatched the previous night were placed individually in vials containing food. At intervals of three days each larva was removed and placed in a clean vial with a fresh food supply. During the first two instars the larvae were transferred from one vial to another by means of a moistened sable brush. Forceps were used to transfer larger larvae. Each day, the vials were inspected to determine if a molt had occurred. In most instances, the larvae in molting deposited exuviae at one or the other end of the sections of corn stalk, where they could be seen. In a few instances the larva molted within the sections of corn stalk which had to be removed and carefully pulled apart until the exuviae were located. Molts were recorded daily, however, the cast head capsules deposited by molting larvae were removed only on days that food was changed in order to minimize the danger of injury by excessive handling. These head capsules were recorded according to the instar they were associated with and stored in 70 per cent alcohol. They were later measured to determine the variation in width that occurred within and between instars of each species.

Technique for obtaining large numbers of Z. grandiosella eggs. During the summer of 1956, approximately 40,000 fertile eggs were needed to manually infest plants in a study of resistance of varieties of corn to southwestern corn borer attack. It was realized from the outset that individual larval rearing techniques would not be satisfactory, therefore, larvae were allowed to maintain themselves freely on corn plants in the greenhouse or in the field while the newly emerged adults were caged in the laboratory for egg production.

On May 23, 70 corn plants in the late whorl stage of growth in a greenhouse were each manually infested by placing small sections of waxed paper bearing 20 fertile eggs in each plant whorl. Examination of these eggs on May 24 showed that 90 per cent had hatched. The larvae of Z. grandiosella feed for a short time in the whorls and leaf sheaths of corn plants and then tunnel into the stalks, where they remain during the rest of their development. Just prior to pupation a larva constructs an exit tunnel, from the interior of the stalk to the periphery, through which the moth later escapes. The opening of this exit tunnel is easily recognized because the larva in constructing it only partially chews through the epidermis of the stalk, thus forming a small round skeletonized gate which the moth later breaks open while emerging.

On June 20, the first exit tunnels were noticed indicating that the larvae had begun pupating within the stalks. On June 28 the plants were dissected and 206 pupae, 2 pupal cases and 32 late instar larvae were recovered. The pupae were placed in cages, made from cylindrical one-half-gallon food containers, in the laboratory at room temperature. Approximately twenty-five pupae of each sex were placed in each cage. During the period June 28 to July 10, approximately 15,000 eggs were obtained from moths that emerged from these pupae. The procedure was repeated twice during the summer when eggs were needed for resistance work.

Taxonomic methods. During February of 1956, series of photomicrographs of the eggs of the three species were made. Several egg masses of each species that had been deposited the previous night were placed in a cabinet at a controlled temperature of 27 degrees C. and in relative humidities that ranged from 30 to 60 per cent. Each day during incubation one egg mass of each species was photographed.

The same egg mass could not be used throughout the incubation period, because the intense light used in photography appeared to delay embryonic development and in some instances apparently killed the embryo. Since the exact time of deposition was not determined, the data relating to age of eggs presented in the results are approximate and could be in error as much as 12 hours. Each day during incubation, egg masses were observed with the aid of a microscope and changes in color and embryonic development recorded.

The taxonomy of larvae was not studied extensively during the period when they were being reared, but changes in color of head capsules and in pigment of the pinacula, the spots surrounding the primary setae of the abdomen, were recorded as development progressed. The width of cast head capsules was measured by means of an ocular micrometer. Series of preserved larvae were obtained and compared to seek the existence of characters which would allow species differentiation.

Techniques for studying diapause in over-wintering larvae. Mature Z. grandiosella larvae over-winter in cells in the crowns of dead corn stubble. The color of the pinacula of immature, winter-form larvae is dark brown and similar to those of the summer-form, however, as they approach maturity, the color fades and is eventually completely lacking after a late instar molt. The change in coloration in the fall indicated that larvae had ceased feeding and would remain relatively inactive in the crowns of stubble until the spring pupation period.

By periodically wetting over-wintering larvae with water, pupation was induced several months prior to the normal pupation period. Mature winter-form larvae were isolated individually in

15 x 50 mm. shell vials, each of which contained a 1/4 x 1 inch strip of surgical cotton to hold the moisture. Caps made from 100 mesh copper screening were used to confine larvae to vials and the larvae were kept in a cabinet at a temperature of 27 degrees C.

Larvae isolated in this manner usually moved to the bottoms of vials and spun cells in the cotton that were similar to those formed by over-wintering larvae in the crowns of corn stubble, however, a few larvae formed cells near the top of vials. Once a larva had completed a cell, it became inactive unless disturbed.

During the fall and winter of the years 1954-1957, groups of larvae were wetted periodically until pupation or mortality occurred, while comparable groups, which served as checks, received no water. Those larvae that received water were wetted at three day intervals after treatments started. At each wetting, approximately three cubic centimeters of distilled water was placed in each vial. In each case, the cell surrounding the larva was broken open and water was applied directly to the larva and to the cotton in the vial. The vial was then placed open end downward on blotting paper for five minutes to allow the excess water to drain away. Groups of larvae that served as checks were given similar treatment except that they received no water. They were removed from the cabinet whenever those receiving water were removed and the cells surrounding larvae were broken open at three day intervals.

It was not determined that larvae were in continuous contact with water. Observation indicated that the cotton in vials retained some

water for intervals of three days, however, it is not known whether any of this water diffused from the cotton into the cells surrounding the larvae. This method of wetting larvae was used in all of the several experiments reported in this study; however, the time that treatments started varied depending on the purpose of the experiment.

In an attempt to determine the effect of humidity and temperature on pupation of over-wintering larvae, an experiment was conducted during the winter of 1956 and 1957, in which larvae were exposed to various constant humidities at temperatures of 12, 22, and 32 degrees C. The technique used was essentially the same as that described by Peterson (Table 16, 1955). Saturated solutions of several chemicals were used to maintain constant humidities in sealed gallon jars. An amount of reagent grade chemical, in excess to that required for saturation was added to 200 cc. of distilled water in each jar. The chemicals, amounts used and estimates of the humidities established at different temperatures are presented in Table 1.

The relative humidities established at each temperature were not measured during the study. The percentages presented for 22 degrees C. were determined by Peterson (1955) for the same chemicals at that temperature. Those shown for 12 and 32 degrees C. are estimates based on Peterson's data as well as that given by Lange (1956), Stokes and Robinson (1949) and Carr and Harris (1949).

Twenty larvae were individually isolated in shell vials and placed in each gallon jar. A stemmed glass (goblet) seven inches tall and capable of holding six fluid ounces was used to hold the vials. This prevented contact of the larvae with the salt solution, since the bottom of the bowl of the goblet was approximately three inches above the surface of the solution. After the larvae were placed in a jar, it was sealed by screwing the top on firmly and wrapping the cap with electrical tape. In this manner, 20 larvae were held in each combination of humidity and temperature shown in Table 1.

Table 1. Humidities obtained from saturated salt solutions at three temperatures (abridged, Peterson 1955; Lange 1956; Stokes and Robinson 1949; Carr and Harris 1949), Stillwater, Oklahoma, 1956-57.

Chemical	Amt. added to 200 cc. water, grams[1]	Per Cent Relative Humidity		
		12°C	22°C	32°C
Calcium chloride	650	37	33	24
Potassium carbonate	300	47	44	42
Calcium nitrate	450	59	55	46
Ammonium nitrate	650	73	67	61
Sodium chloride	100	78	76	73
Potassium nitrate	100	96	93	91

[1]The amount added was in excess of that required for saturation at any of the three temperatures.

For purposes of comparison, at each temperature, 40 larvae were wetted at three day intervals and forty maintained without water. At

intervals of 30 days after the experiment started the jars were opened and mortality and pupation that had occurred in that interval recorded.

The cabinets in which the three temperatures were maintained each contained 10 cubic feet of space and the temperatures were thermostatically controlled.

PART IV

RESULTS

Comparison of the eggs of Z. grandiosella, D. crambidoides and D. saccharalis. When first deposited, the eggs of these species are very similar in shape, color and external appearance. They are elliptical, flattened and opaque white and are deposited singly or in masses with individual eggs overlapping like shingles. A honeycomb pattern of reticulations is present on the external surface of the egg. This pattern, produced by contact of the chorion with the follicular epithelium of the ovariole during egg formation, appears to be similar in all three species. At this stage of incubation, the only noticeable difference among the three species appears to be variation in size, however as incubation continues, there are distinct differences in color between the eggs of Z. grandiosella and those of the other two species.

At a temperature of 27 degrees C. and in relative humidities ranging from 30 to 60 per cent, the mean number of days to hatch for eggs of Z. grandiosella and D. saccharalis was six days and for D. crambidoides, seven days.

The mean number of eggs per mass deposited by females of each species in the laboratory is presented in Table 2. These data are based on approximately 50 egg masses of each species. The means and ranges in

length and width of eggs of the three species are presented in Table 3. These data are based on approximately 200 eggs of each species.

Table 2. Number of eggs per mass deposited by laboratory caged females of three species of the Diatraea complex, Stillwater, Oklahoma 1956.

Species	Eggs per mass[1]	
	Mean	Range
D. saccharalis	21	1 - 58
Z. grandiosella	9	1 - 26
D. crambidoides	5	1 - 17

[1]These data are based on 50 egg masses of each species.

Table 3. Size in millimeters of eggs deposited in the laboratory by caged females of three species of the Diatraea complex, Stillwater, Oklahoma, 1956.

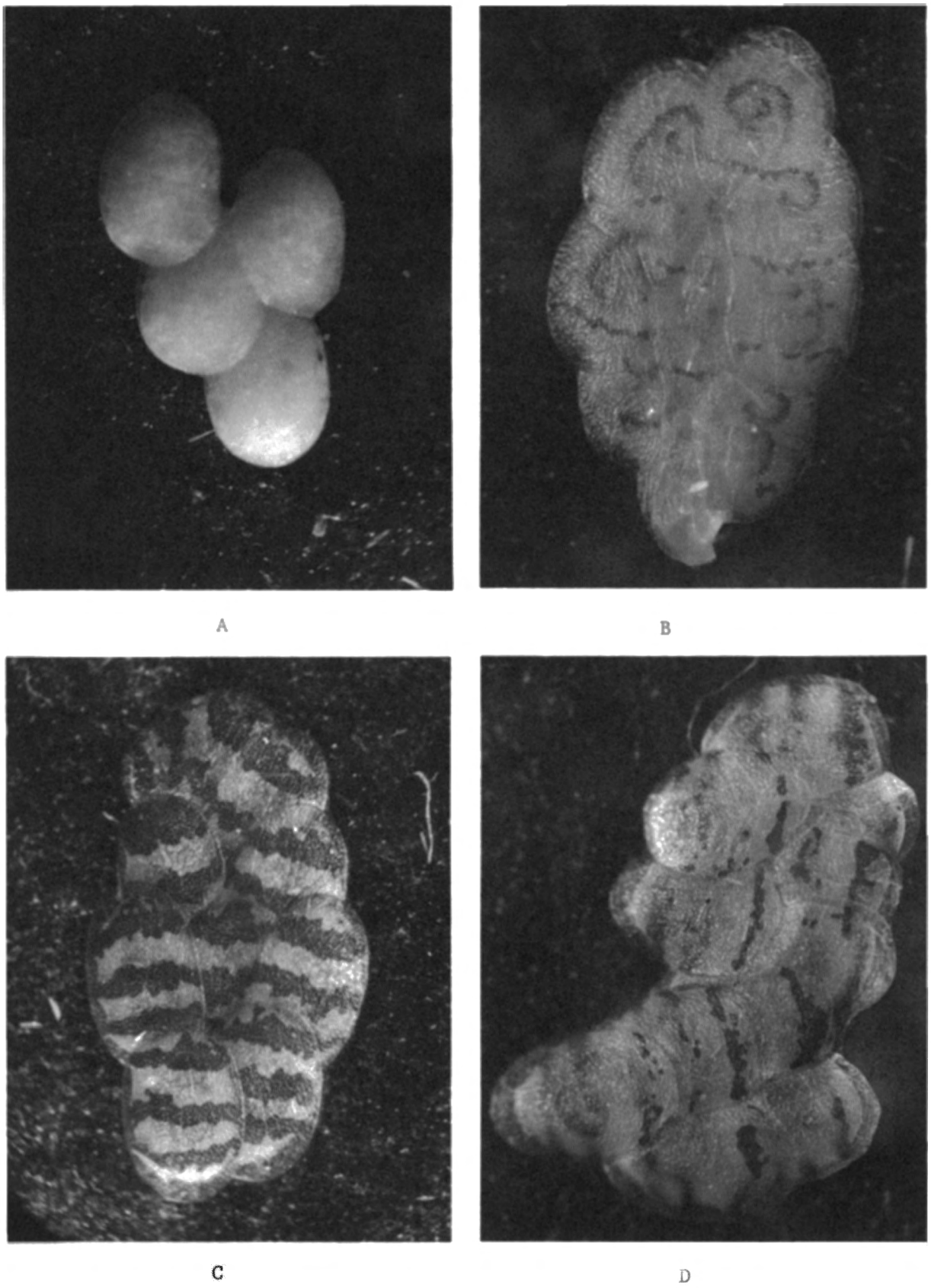
Species	Length		Width	
	Mean	Range	Mean	Range
D. saccharalis	1.20	1.05-1.28	0.79	0.55-0.91
Z. grandiosella	1.31	1.11-1.43	0.81	0.64-1.01
D. crambidoides	1.54	1.21-1.73	0.90	0.72-1.13

Comparable data reported by other authors are: D. saccharalis - length of eggs 1.16 mm., width 0.75 mm. (Holloway et al. 1928); Z. grandiosella - length 1.30 mm., width 0.80 mm. (Davis et al. 1933); and D. crambidoides - length 1.6 mm., width 1.0 mm. (Leiby 1920). Although these last two publications indicate, respectively, that eggs of Z. grandiosella and D. crambidoides are very uniform in size, wide

ranges in both length and width of eggs of each species were noted during this study. Wide variation in size of eggs of these species was not unexpected since the adults, pupae and larvae of each species are extremely variable in size; however length and width are poor criteria for measuring size of elliptical eggs. As incubation progressed, eggs became more flattened. The values for length and width were always smaller for eggs measured a few hours after deposition than those measured just prior to eclosure. Shrinkage occurred in eggs held at temperatures ranging from 30 to 35 degrees C. Under cage conditions, the females of all three species did not always flatten eggs during oviposition as reported by other authors.

Changes in appearance and development of eggs of the three species held at 27 degrees C. and in relative humidities of 30 to 60 per cent are presented in Figures 1-6. The eggs of Z. grandiosella (Figures 1 and 2, A-H) are characterized by the appearance of three transverse red bars in the yolk during the medial period of incubation. Eggs of this species less than one day old (A) appear opaque white and lack visible signs of embryonic development. When two days old (B), the embryo is visible as a dark curled mass, ventrally located near the center of each egg. The three irregular bars begin to form in the yolk above each embryo. The color of these bars and the embryo is dark red in contrast to the white of the remainder of the yolk cytoplasm. Bars are completely formed three days after oviposition (C) and begin to disintegrate in four days old eggs (D). Eventually they diffuse as particles and strands in the surrounding yolk material. During the medial period of

Figure 1. Eggs of Zeadiatraea grandiosella (Dyar) at post-oviposition intervals of (A) one day, (B) two days, (C) three days, (D) four days. Magnification: 20X.



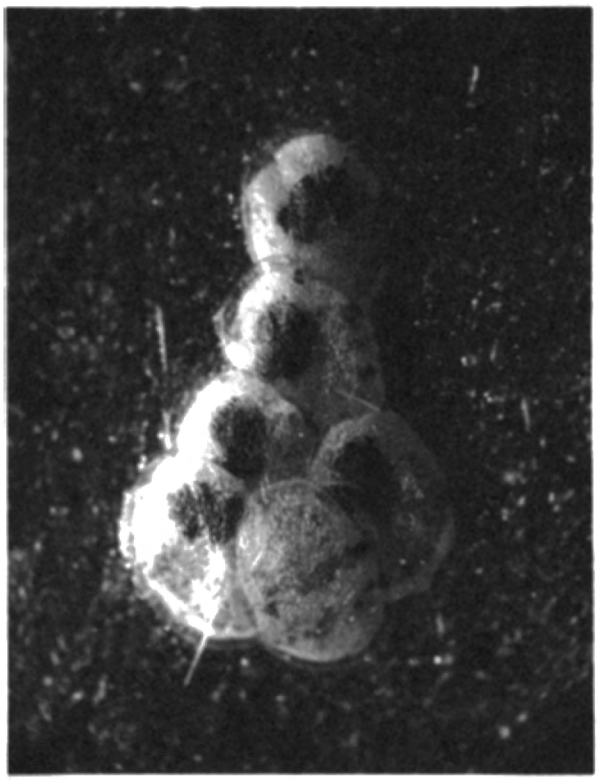
A

B

C

D

Figure 2. Eggs of Zeadiatraea grandiosella (Dyar) at post-oviposition intervals of (E) five days, (F) six days, (G) six days (photographed with transmitted light), (H) three days (infertile mass). Magnification: E, F and H 20X; G, 40X.



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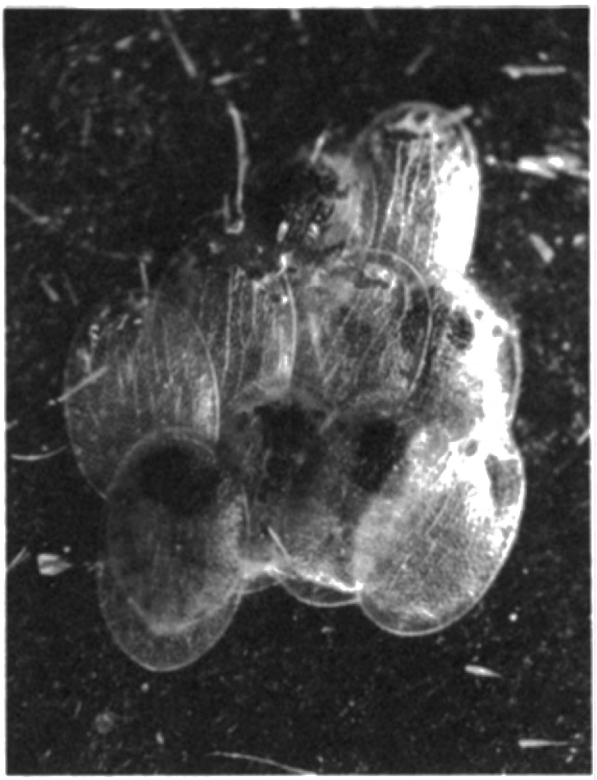

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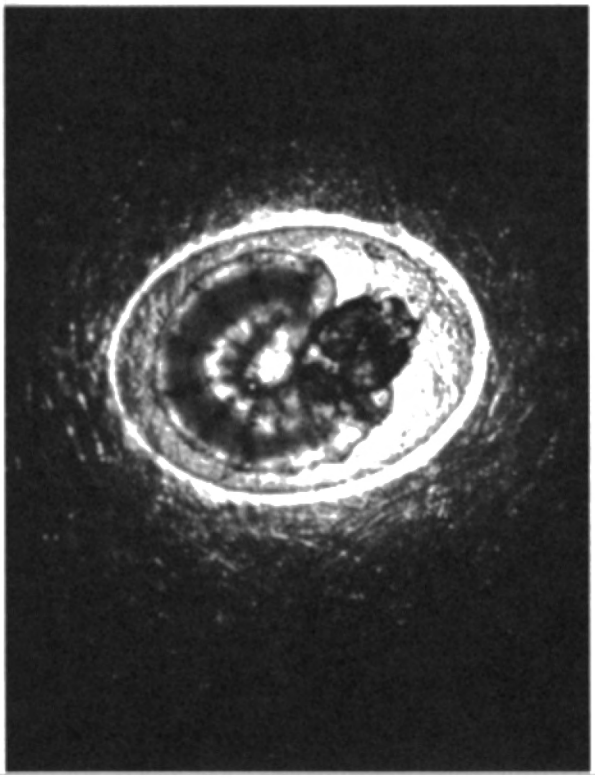
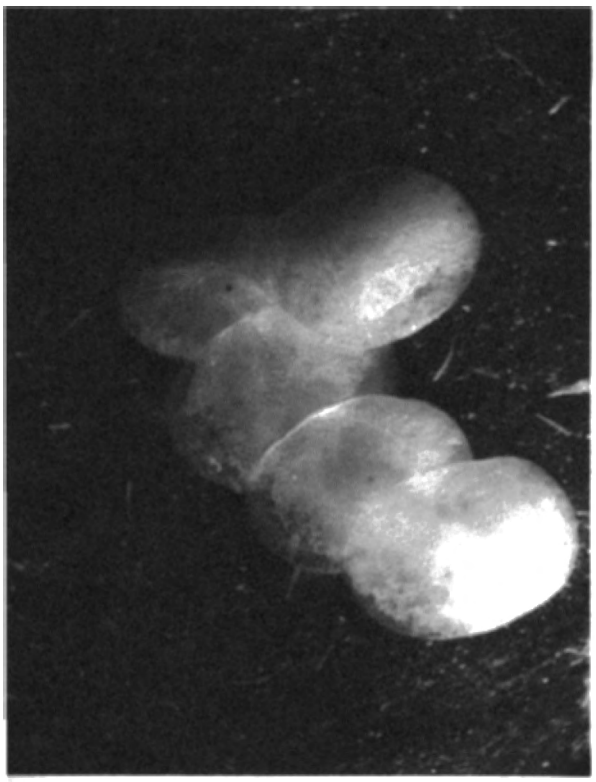


G

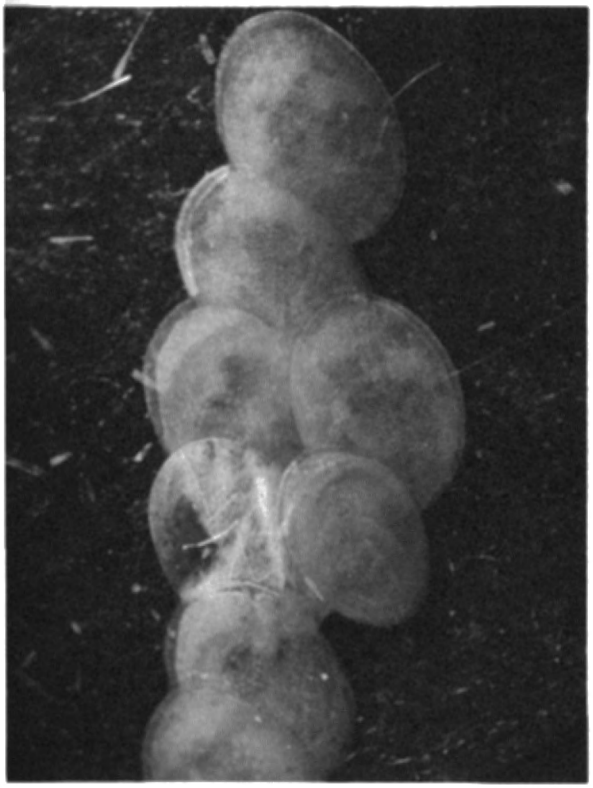
H

Figure 3. Eggs of Diatraea crambidoides (Grote) at post-oviposition intervals of (A) one day, (B) two days, (C) three days, (D) four days. Magnification 20X.

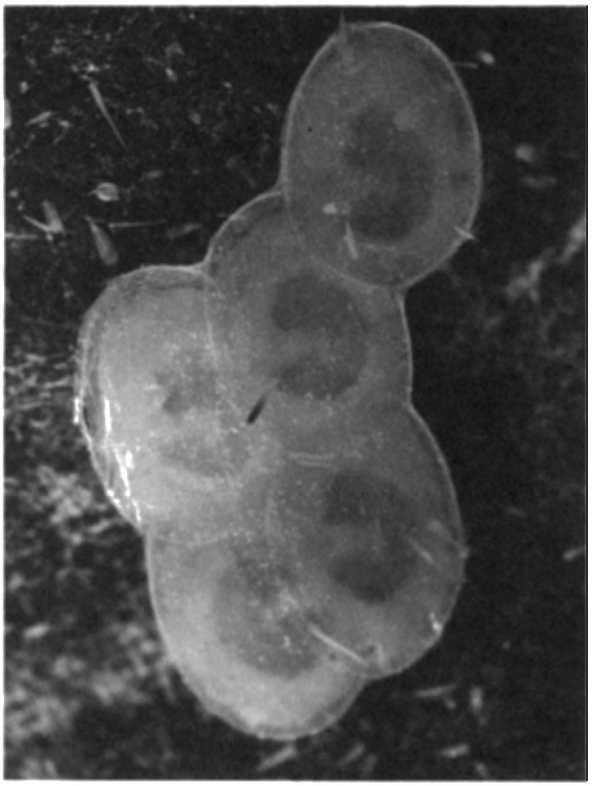


A

B



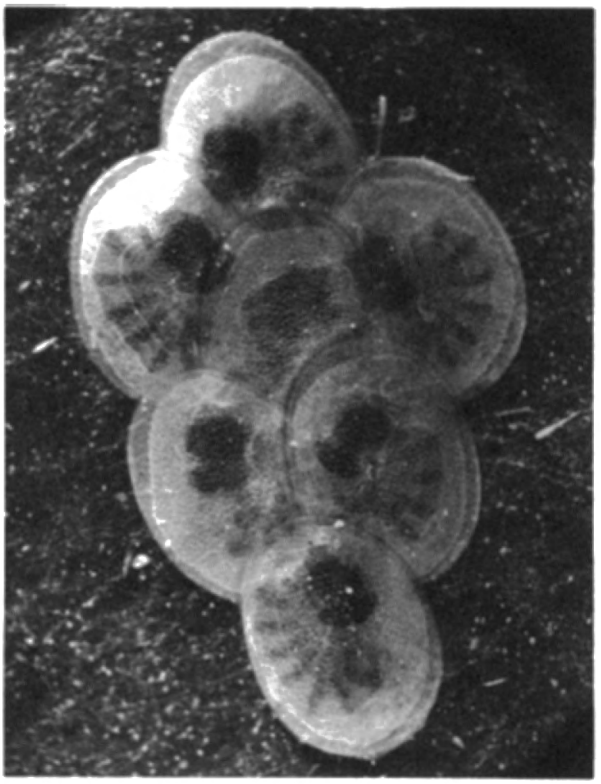
C



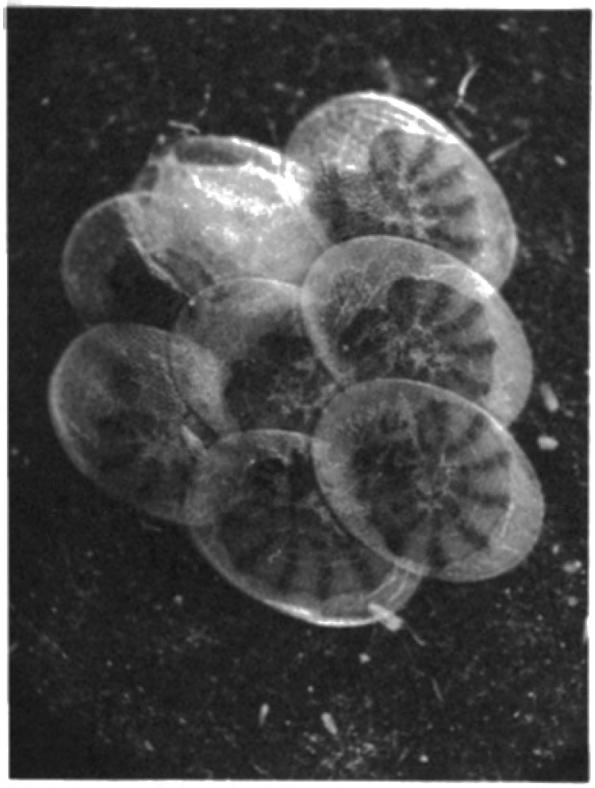
D

Figure 4. Eggs of Diatraea crambidoides (Grote) at post-oviposition intervals of (E) five days, (F) six days, (G) seven days, (H) seven days (hatched mass). Magnification: 20X.

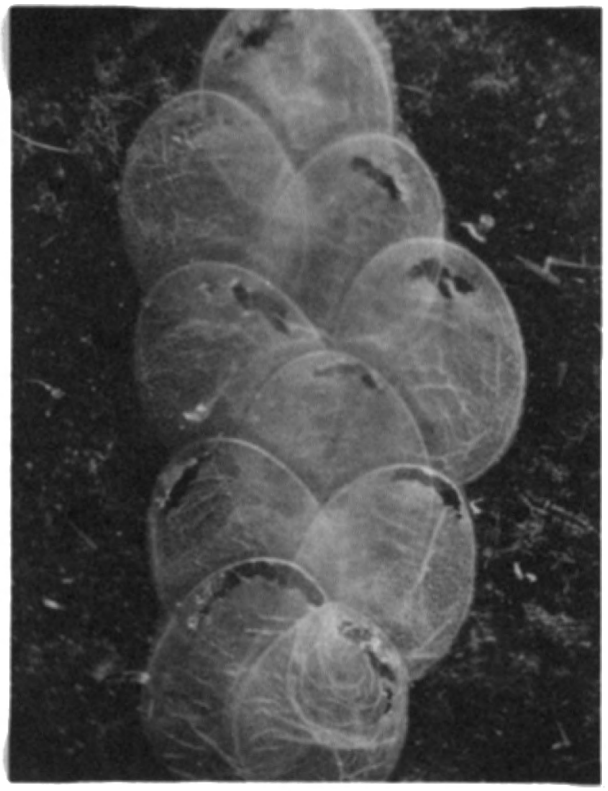
E



F



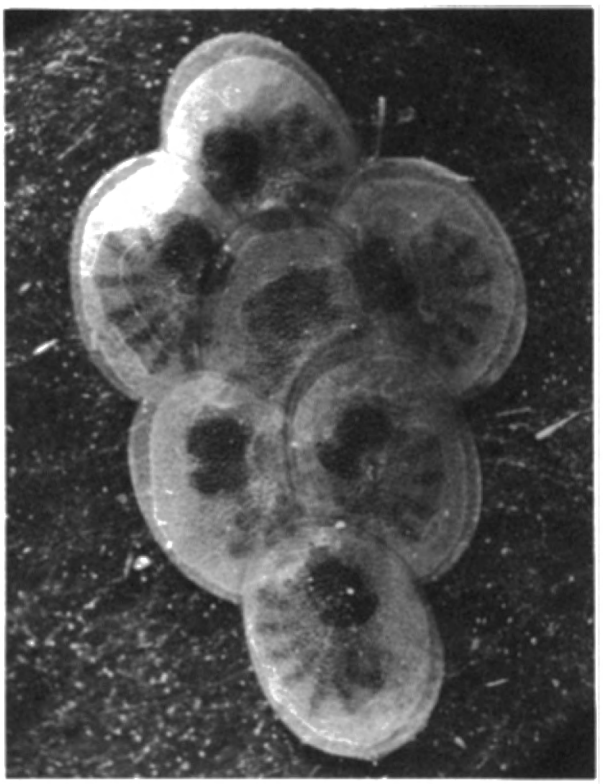
G



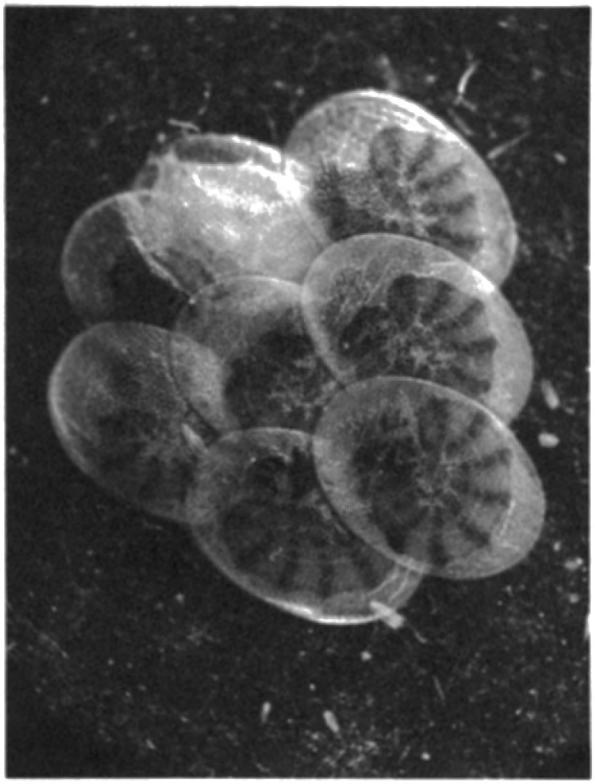
H

Figure 5. Eggs of Diatraea saccharalis (Fabr.) at post-oviposition intervals of (A) one day, (B) two days, (C) three days, (D) four days. Magnification: 20X.

E



F



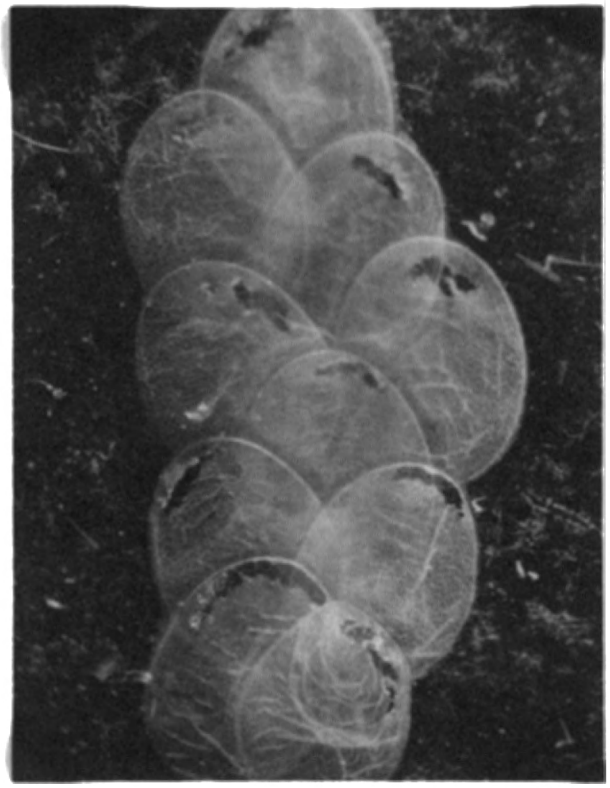
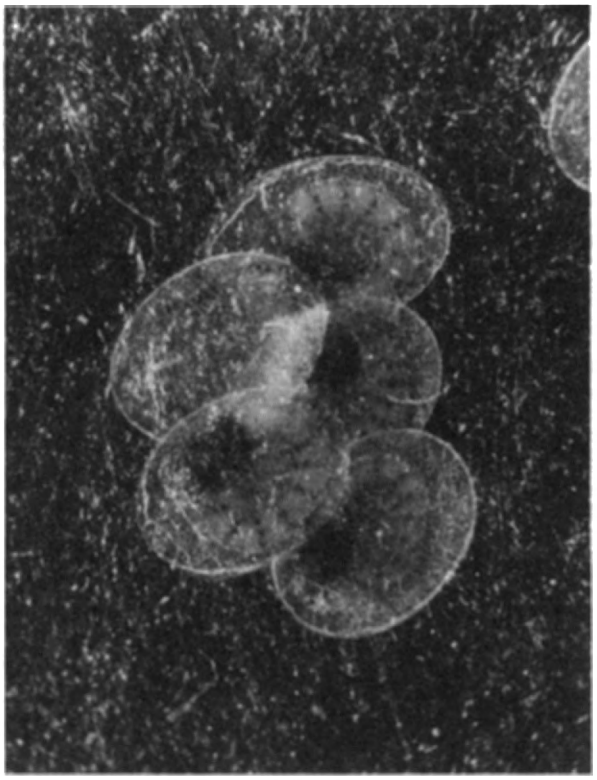


Figure 6. Eggs of Diatraea saccharalis (Fabr.) at post-oviposition intervals of (E) five days, (F) six days. Magnification: 20X.

E



F

incubation, the white cytompasm of the yolk appears to be more opaque than in other periods of incubation since it completely masks the developing embryo (C, D). The head capsule of the embryo is visible in most eggs that are five days old (E). Partially disintegrated bars can still be seen in one egg in this mass. It was probably deposited a few hours later than the others. A partially eclosed mass is shown in F. Some of the larvae emerged from eggs while this mass was being photographed. Davis et al. (1933) found the incubation period of eggs of this species in Arizona was five days in the field and five to six days in the laboratory.

About six hours before eclosure, the embryo becomes active within the egg, moving its head from side to side. When observed with the aid of a microscope, a peristaltic movement is noticed in the alimentary canal and the embryo can be seen ingesting the yolk content of the egg including the diffused particles and strands of red material that appeared as bars in three to four-day-old eggs. This material is visible as a dark line in the alimentary canal of the embryo shown in G, which was photographed with the aid of transmitted light. Rolston (1955 b) observed that first instar larvae of this species possessed a reddish cast. This color probably results from ingestion of the yolk by embryos prior to eclosure. Embryos of D. crambidoides and D. saccharalis were also observed ingesting yolks prior to eclosure. An infertile, three-day-old mass is shown in H. When first deposited, an infertile mass can not be distinguished from one that is fertile, but after two to three days time has elapsed, it appears collapsed and shrunken. Cleavage planes

and dark areas also appear in the white cytoplasm. In the field, infertile masses seldom remain on plants as long as fertile ones.

Eggs of D. crambidoides (Figures 3 and 4, A-H) differ from those of Z. grandiosella in color. Leiby (1920) in his publication on the biology of D. crambidoides states "When first laid, the egg is milky white in color but as the embryo develops, the egg assumes a pale yellow tint, the fully developed embryo within the egg giving it finally an orange-yellow color." His observations are essentially correct, however the eggs of this species are also characterized by the appearance of three transverse bars during the medial period of incubation. These bars appear similar to those of Z. grandiosella except that they are pale orange. Similar pale orange bars were also observed in eggs of D. saccharalis. In both species, these bars were so faintly colored that they are not visible in any of the photographs.

There is little visible change in the color and internal organization of eggs of D. crambidoides that are one and two days old, (A and B). The shaded areas in the centers of eggs three days old (C) are probably the young embryos. A collapsed infertile egg is situated at the lower left center of this mass. The embryo, which is dark orange, is visible in four-day—old eggs (D) and the abdominal segments of the embryo are visible in five-day—old eggs (E). It appears to be fully formed in six-day—old eggs (F). Eclosure occurred on the seventh day (G). The incubation period of eggs deposited in the field is reported as eight days in North Carolina (Leiby 1920) and five and one half days in South Carolina (Cartwright 1934). The appearance of the hatched egg in the upper left corner of G is misleading. Reflection of light from

the shell during photography gives it the appearance of an infertile egg. A hatched mass is shown in H. A small irregular hole is visible near the periphery of each egg shell. These holes were made by larvae as they chewed through the shells during emergence. The translucent appearance of the egg shells indicate that embryos prior to emergence, ingested the extraneous yolk matter. The shells of hatched masses appear similar in all three species.

The eggs of D. saccharalis (Figures 5 and 6, A-F) are quite similar in color to those of D. crambidoides during the entire period of incubation. Holloway et al. (1928) state "They are at first white but later an orange hue develops." This orange appearance does not result from a uniform orange color. In the medial period of incubation, the embryo is dark orange and pale orange bars are present, however the remainder of the yolk surrounding the embryo is white. Eggs that are one day old (A) are opaque white and appear similar in color to those of the other two species at that period of incubation. The embryo which is pale orange in two-day-old eggs appears as a shaded, curled mass in the eggs in B. It is much darker in three-day-old eggs (C) and in four-day-old eggs (D). The abdominal segments are visible in five-day-old eggs (E), and the fully formed embryo can be seen in six-day-old eggs (F). Females of this species apparently deposit more uniformly shaped masses than those of the other two species. Holloway et al. (1928) indicate that in Louisiana, eggs of this species require five days incubation.

Comparison of the larval life cycles of the three species. Under laboratory rearing conditions at a temperature of 27 degrees C., larvae

of the three species fed corn, did not undergo a consistently uniform number of instars prior to pupation. Of 72 larvae of each species isolated for rearing, 49 D. crambidoides, 54 Z. grandiosella and 60 D. saccharalis larvae were reared from eggs to pupae. The incidence of pupation of larvae after completion of various instars is presented graphically for each species in Figure 7. Of the 60 D. saccharalis larvae reared through pupation, 19, 34 and 7 completed five, six and seven instars, respectively, prior to pupating. Of 54 Z. grandiosella larvae, 23, 28, and 3 completed six, seven and eight instars respectively, before pupating. Twenty-seven, 17 and 5 D. crambidoides larvae, respectively, pupated after instars seven, eight and nine. These data indicate a difference among species in range of number of instars prior to pupation. A range in number of instars of three to ten is given by Holloway et al. (1928) for D. saccharalis. Davis et al. (1933) state that Z. grandiosella larvae undergo from five to nine instars and Leiby (1920) did not determine the number of instars for D. crambidoides because of rearing difficulties.

The duration of instars occurring in each species is presented in Table 4. With the exception of the first instar, the mean duration of instars in all three species progressively lengthened as the number of instars increased. Z. grandiosella larvae required an average of 34.6 days to develop from hatch to pupation as compared to 48.7 and 25.2 days, respectively, for D. crambidoides and D. saccharalis. Ranges in length of larval life cycles presented by other authors are : Z. grandiosella,

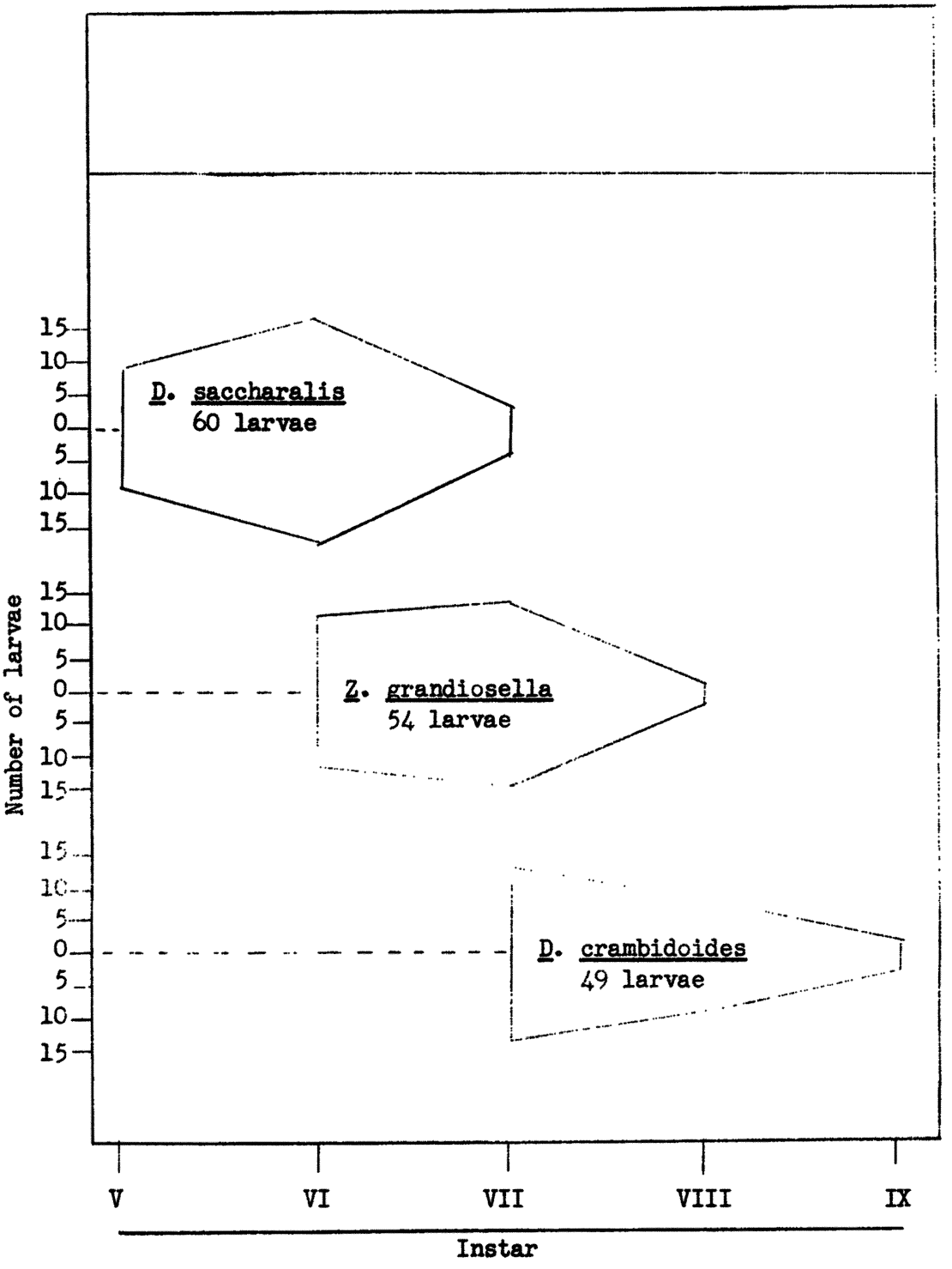


Figure 7. Instars completed prior to pupation by larvae of Diatraea saccharalis (Fabr.), Zeadiatraea grandiosella (Dyar) and Diatraea crambidoides (Grote) reared on corn at a temperature of 27 degrees C., Stillwater, Oklahoma, 1956.

Table 4. Duration of larval instars of three species of the Diatraea complex reared on corn at a temperature of 27 degrees C. Stillwater, Oklahoma, 1956.

	Instar									All instars
	I	II	III	IV	V	VI	VII	VIII	IX	
Zeadiatraea grandiosella (Dyar)										
No. of larvae	54	54	54	54	54	54	31	3	—	54
No. of days:										
Minimum	2	2	3	3	5	6	7	10	—	23
Maximum	4	4	5	6	8	13	12	11	—	43
Mean	3.2	2.7	3.5	4.4	6.1	8.8	9.7	10.3	—	34.6
Diatraea crambidoides (Grote)										
No. of larvae	49	49	49	49	49	49	49	22	5	49
No. of days:										
Minimum	2	2	3	4	5	5	8	11	11	39
Maximum	4	4	5	6	8	11	15	16	15	75
Mean	3.3	3.1	4.2	4.5	5.9	8.1	12.0	13.0	14.0	48.7
Diatraea saccharalis (Fabr.)										
No. of larvae	60	60	60	60	60	41	7	—	—	60
No. of days:										
Minimum	2	2	2	3	4	5	8	—	—	23
Maximum	4	5	5	6	8	11	13	—	—	39
Mean	3.0	2.8	3.3	3.9	6.0	7.4	9.0	—	—	25.2

Table 5. Width in millimeters of head capsules of larval instars of three species of the Diatraea complex reared on corn at a temperature of 27 degrees C. Stillwater, Oklahoma 1956.

	Instar								
	I	II	III	IV	V	VI	VII	VIII	IX
Z. grandiosella									
Number	54	54	54	54	54	54	31	3	—
Width:									
Minimum	.33	.48	.73	1.07	1.52	1.86	2.32	2.59	—
Maximum	.37	.57	.93	1.43	2.14	2.63	3.10	3.03	—
Mean	.36	.52	.86	1.28	1.80	2.27	2.68	2.87	—
D. crambidoides									
Number	49	48	49	49	49	49	49	22	5
Width:									
Minimum	.35	.50	.70	.96	1.24	1.65	2.01	2.17	2.53
Maximum	.37	.63	.92	1.34	1.76	2.12	2.55	2.80	2.76
Mean	.36	.54	.81	1.12	1.54	1.92	2.27	2.48	2.66
D. saccharalis									
Number	60	60	60	60	59	41	7	—	—
Width:									
Minimum	.32	.47	.67	.92	1.21	1.73	2.33	—	—
Maximum	.35	.56	.90	1.30	2.01	2.53	2.87	—	—
Mean	.34	.52	.82	1.17	1.82	2.16	2.67	—	—

25-35 days (Davis et al. 1933); D. saccharalis, 25-35 days (Holloway et al. 1928); D. crambidoides, 24-38 days (Leiby 1920).

The head capsule widths for each laboratory-reared, larval instar are presented in Table 5. As the number of instars increased, the width of head capsules became more variable with wider ranges in this measurement. Overlapping in range occurred between instars five (max. 2.14 mm.) and six (min. 1.86 mm.) of larvae of Z. grandiosella. In D. crambidoides, overlapping occurred between instars four (max. 1.34 mm.) and five (min. 1.24 mm.) and in these same instars, four (max. 1.30 mm.) and five (max. 1.21 mm.) in larvae of D. saccharalis. Overlapping in ranges of width also occurred between comparable instars of the three species. Rolston (1955 b) used the mean and range of width of head capsules that occurred in each instar of approximately 50 reared Z. grandiosella larvae to assign field collected larvae to their respective instars. His data show ranges in width of field collected larvae overlap in all instars except one and two but that instars of most larvae could be determined by comparison with the measurements of the reared series.

Very few differences in color or morphological characters were observed in larvae of the three species during rearing. First instar larvae of Z. grandiosella could be distinguished from those of the other two species by a definite reddish color. This color appeared to be restricted to the alimentary canal and gradually disappeared as larvae fed and developed. Immediately after hatching, first instar larvae of D. crambidoides appeared to be slightly longer than those of the other

two species, however the difference was not measurable with preserved specimens. The head capsules and prothoracic shields of larvae of all three species were dark brown during the first instar and became increasingly lighter during later instars. In mature larvae, just prior to pupation, the color of these structures ranged from yellow to light brown in all three species.

The larvae of D. saccharalis could be distinguished from those of the other two species by a difference in color. The dark pigmented pinacula (spots), located dorsally on the abdominal segments of D. saccharalis larvae, are surrounded by a light brown pattern of coloration that is not present in larvae of Z. grandiosella and D. crambidoides. This distinctive coloration is present in all instars and in the over-wintering form of D. saccharalis. It is responsible for the dirty white appearance of larvae of this species as compared to the more clearly contrasting black and white appearance of larvae of Z. grandiosella and D. crambidoides.

The author was unable to separate larvae of the three species by utilizing existing keys. The key presented by Holloway (1916) for separating D. crambidoides larvae from those of D. saccharalis emphasizes size, shape and color of the pinacula, color of the head and spiracles and the angle formed by the alpha and beta setae (Fracker 1915) of abdominal segments two, three or four as valuable diagnostic characters. The setal angle was emphasized as especially valuable because it was believed to be the only character that could be utilized to separate the over-wintering forms of the two species. In examining

19 specimens of D. saccharalis and 7 of D. crambidoides, Holloway found that when lines were projected through setae alpha and beta, on any of abdominal segments two, three or four, they met on the meson at angles ranging from 18 to 41.5 degrees (mean 30.2 degrees) for D. saccharalis and 41 to 69.5 degrees (mean 53.8 degrees) for D. crambidoides. In examining 124 D. saccharalis larvae collected near Houma, Louisiana and 91 D. crambidoides larvae collected near Florence, South Carolina, the author was unable to find any consistent difference between species in the setal angles measured by Holloway. It was found that this character was influenced more by differences in size and instar of larvae and the solutions larvae were preserved in, than by interspecific differences. The only diagnostic character, presented by Holloway, of value in separating larvae of these two species during this study was the color of the pinacula, dark brown to black in D. crambidoides and light brown in D. saccharalis.

Peterson (1948) also presents a key for separation of larvae of D. saccharalis, D. crambidoides and Z. grandiosella, that is based on the chaetotaxy of the head, shape and number of mandibular dentes and the angle formed by alpha and beta setae of abdominal segments. None of these characters were adequate for separation of all specimens examined during this study.

Since the base color of D. saccharalis appears distinct in all instars and in both winter-form and summer-form larvae from that of the other two species, considerable time was spent attempting to find some character that could be used to separate the larvae of Z. grandio-

sella from those of D. crambidoides. The chaetotaxy of the head, pattern and color of the pinacula, various setal patterns and the number and arrangement of crochets were studied critically. No single character or combination of characters was found that was sufficiently consistent in all instars and in both winter-form and summer-form larvae to be of value in separating these species.

Although of little value taxonomically, a consistent difference in the thickness of the cranial wall as well as the arms of the tentorium was noticed in the cast head capsules of comparable instars of Z. grandiosella and D. crambidoides. Both the cranial wall and tentorial arms of Z. grandiosella appear thicker than those of D. crambidoides.

The pupal stage of these three species was not studied in detail but one reliable taxonomic character was noticed. The two tubercles on the vertex of the pupae are smooth and pointed in D. saccharalis and rough and blunt in the other two species. These tubercles are described by Holloway et al. (1928) and Davis et al. (1933) as "upward horn-like projections of the frons".

Diapause in larvae of the Diatraea complex. During July of 1954, a small number of inactive, mature Z. grandiosella larvae, that appeared morphologically similar to the over-wintering form of this species, were collected from drought killed corn plants at Stillwater, Oklahoma. These larvae lacked the dark spotted pigmentation of summer-form larvae and were located below ground level in cells in the crowns of plants. When isolated in vials in the laboratory, they refused to feed on pieces of cornstalk provided as food and constructed cells in which they remained inactive.

In contrast to the few larvae found in the crowns of drought-killed plants in July, large populations of spotted summer-form larvae were present in green stalks in adjacent irrigated fields.

The appearance and behavior of the larvae collected from drought-killed plants was similar in every respect to that of the over-wintering form of this species described by Davis et al. (1933) as being in a state of hibernation that is believed to be caused by the onset of cooler fall temperatures. Although morphologically similar to the over-wintering form described by Davis et al., the larvae found in July could not have been in hibernation. It was more likely that they had entered diapause as a result of the poor quality or lack of food available in drought-killed plants. It also became apparent that larvae of this species do not cease feeding in the fall and over-winter as a result of the onset of cooler temperatures but because such temperatures have caused their host plant (corn) to become unsuitable as food.

The moisture content of cornstalks appears to have had little effect on diapause of Z. grandiosella since the diapause form was found in dry plants in July and in plants with a high moisture content in the fall. Photoperiod apparently was not a contributing factor since the diapause form was present in July in drought-killed plants in unirrigated fields, while living plants in nearby irrigated fields contained only non-diapause spotted summer-form larvae. There appeared to be no association of a specific generation with diapause since the larvae collected in July were probably of the second generation while those entering diapause in the fall were third or fourth generation forms. Of the possible explana-

tions of the inception of diapause in Z. grandiosella, poor quality or lack of food appears to be the most logical.

In Trinidad, mature larvae of Z. lineolata, a species closely related to Z. grandiosella enter diapause at the beginning of the dry season and only resume development, followed shortly by pupation, when another rainy season begins several months later. Kevan (1944) demonstrated experimentally, that larvae of this species in diapause would pupate after several weeks, but prior to the beginning of the rainy season, when adequately wetted with free water. Since Z. grandiosella is remarkably similar in appearance and biology to Z. lineolata, a similar study was initiated here.

Attempts were made in September to collect diapause-form larvae from corn stubble killed by drought in July. In examining several hundred drought-killed plants, only 41 were found with crowns that contained cells of Z. grandiosella. Twenty pupal cases, five pupae, fourteen dead larvae and two living winter-form larvae were recovered. The larvae pupated shortly after being isolated in vials in the laboratory. From June 15 to August 1, only .15 inch of precipitation was recorded as compared to 2.27 inches during the period August 1-September 15. It is apparent that the few larvae that were present in the crowns of drought-killed plants in July entered diapause and then pupated in the fall after the moisture contents of stalks became higher, since only pupal cases, pupae and larvae approaching pupation were found in similar plants examined in September.

When diapause-form larvae could not be collected in adequate numbers

from drought-killed plants, they were obtained in October from fields that had previously been irrigated. The plants in such fields were senescent and contained large numbers of larvae in diapause in cells in the crowns and a few spotted larvae in the lower nodes. When brought into the laboratory and provided with green succulent corn, many of the spotted larvae fed readily and soon pupated, however a few fed for a few days and then molted to the winter-form and constructed cells, in which they remained inactive without feeding. None of the diapause-form larvae collected from cells in crowns fed in the laboratory.

On October 16, 100 diapause-form larvae were isolated individually in vials containing cotton and kept in a cabinet at a temperature of 27 degrees C. Fifty of these larvae received application of free water at three day intervals and fifty received no water. The pupation and mortality that occurred in the two groups of larvae from October 16, 1954 to April 14, 1955 are shown in Figure 8, A and B. Eighty-two per cent of the larvae exposed to water (B) pupated and the remaining 18 per cent succumbed. In comparison only 6 per cent of the larvae without water (A) pupated and 22 per cent died. Seventy per cent of the larvae that received water pupated during the period December 30 to February 28, or from 75 to 135 days after the experiment was initiated. None of the larvae without water pupated after the first 90 days of the experiment.

Since a significant increase in rate of pupation did not occur during the first 60 days of the preceding experiment, regardless of treatment, an attempt was made in 1955 and 1956 to determine if a period of time existed after larvae entered diapause in which exposing them to

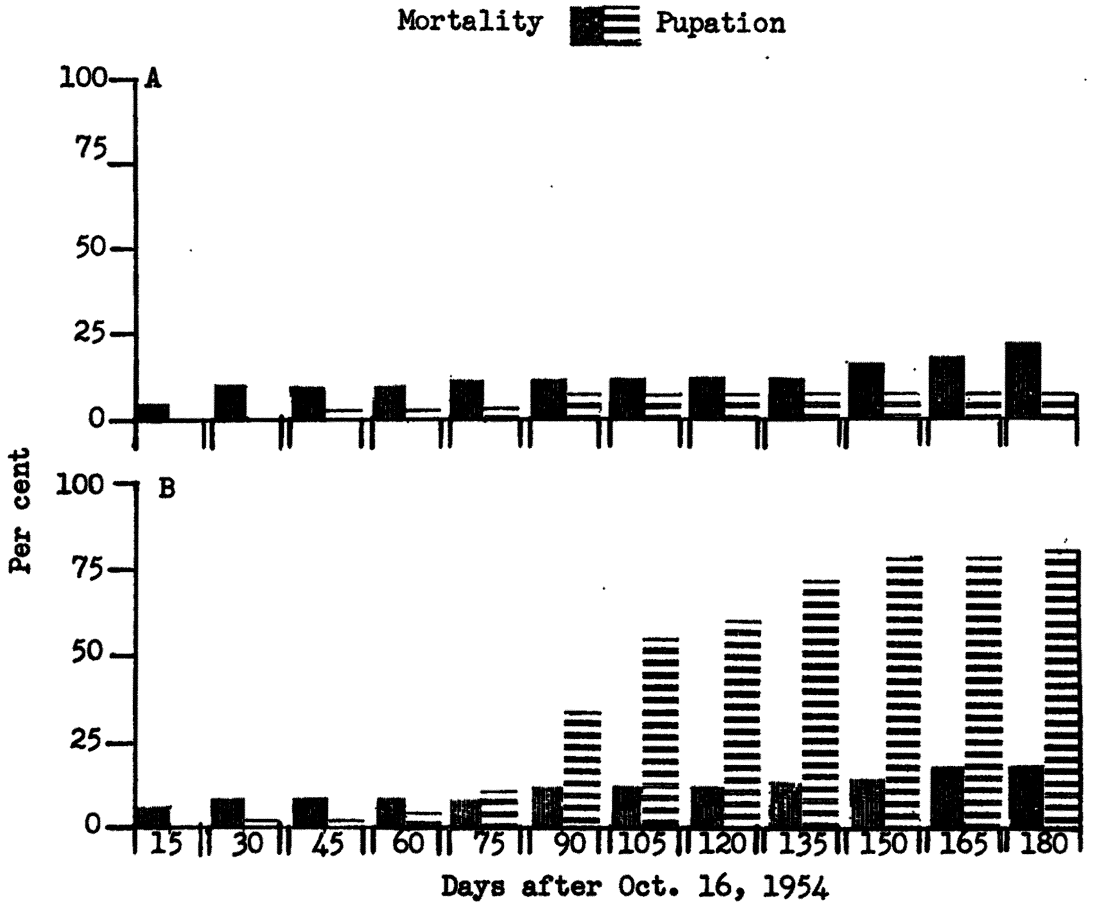


Figure 8. Accumulated mortality and pupation of Zeadiatraea grandiosella (Dyar) larvae kept (A) without and (B) with access to free water during the period October 16, 1954 to April 14, 1955. Stillwater, Oklahoma.

water was not effective in inducing pupation. Approximately 70 larvae in diapause were collected from the crowns of dead corn stubble on November 1, 1955 and isolated in vials containing cotton at a temperature of 27 degrees. On November 7, two groups, each composed of 20

larvae were randomly selected from the collection. One group was exposed to free water at three days interval after that date while the other group was kept without water during the entire experiment. Beginning on December 19, a third group of 20 larvae were exposed to free water at three day intervals. The experiment was not terminated until all larvae in each treatment had pupated or died.

The pupation and mortality that occurred among the different groups of larvae are presented in Figure 9, A-C. A comparison of larvae that were exposed to free water beginning November 7 (B) with those exposed to free water beginning December 19 (C) indicates little difference in rate of pupation or mortality. All the larvae in these groups had pupated or died by January 30 and most of the pupation occurred during the period December 19-January 30. Although the rate of mortality of larvae, held without access to free water (A), gradually increased as the experiment continued, no appreciable increase in rate of pupation occurred until after April 23. At the termination of the experiment May 21, 60 per cent of the larvae had pupated and 40 per cent had died. Approximately 80 per cent of the total pupation in this group occurred from April 23 to May 21. The ten pupae produced during this period were abnormal in the sense that a few were deformed, all were extremely small and only 50 per cent moth emergence occurred. This pupation was coincident with that of the over-wintering brood in the field which occurred after a period of rainfall.

It was not determined in preceding experiments that larvae exposed to free water pupated as a result of contact with water or because the

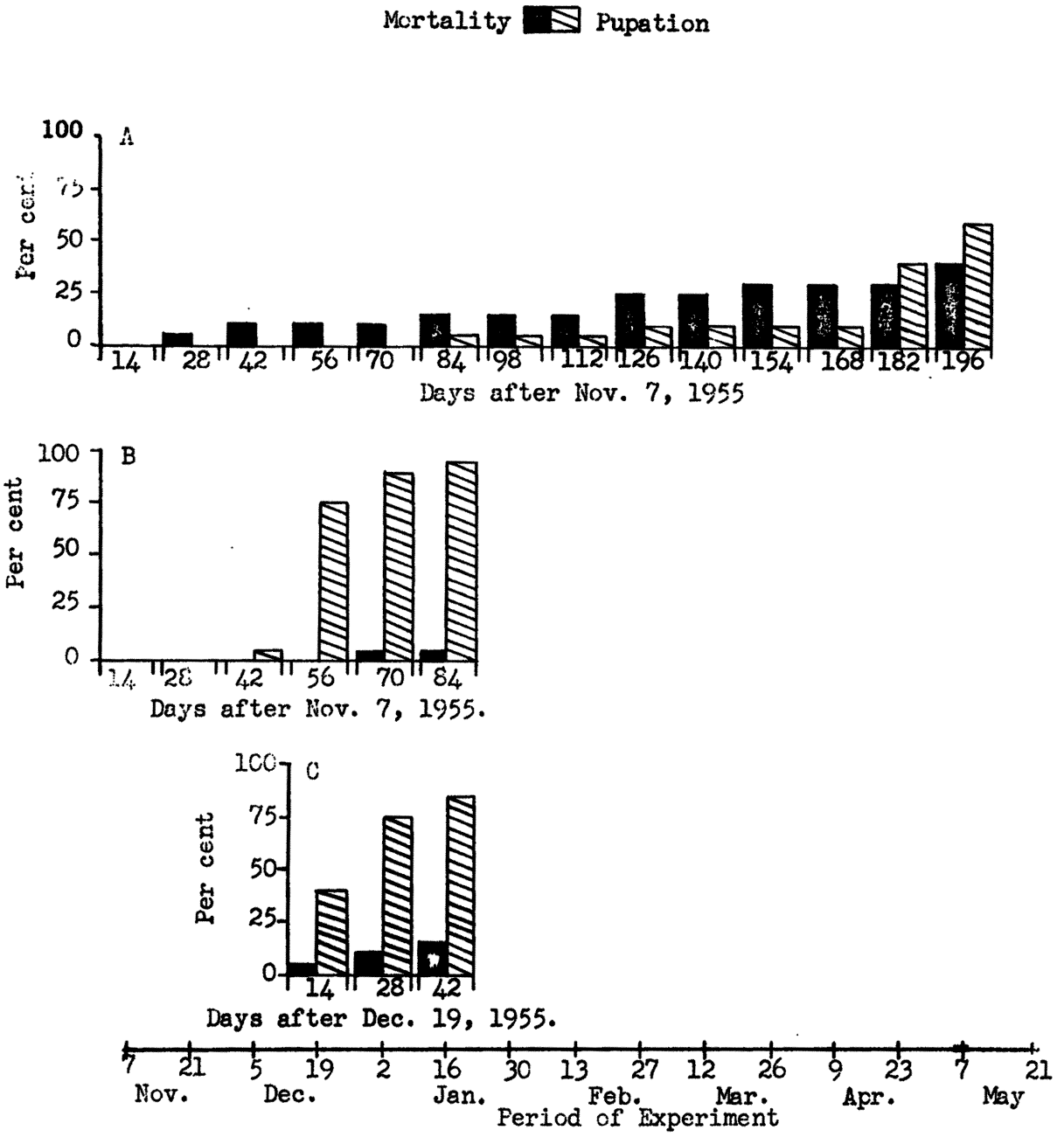


Figure 9. Accumulated mortality and pupation of Zeadiatraea grandiosella (Dyar) larvae kept (A) without access to free water from November 7 to May 21, (B) with access to free water from November 7 to January 30, (C) with access to free water from December 19 to January 30, Stillwater, Oklahoma, 1955-1956.

water, especially that retained in cotton in vials, created adequate humidity conditions. Therefore, experiments were conducted in 1956 and 1957, in which groups, each composed of 20 larvae in diapause were kept in humidities ranging from approximately 30 to 90 per cent at temperatures of 12, 22, and 32 degrees C. The humidities were established and maintained by placing saturated solutions of the chemicals shown in Table 1 in sealed gallon jars. Two groups, each composed of 40 larvae on cotton in vials, were also kept at each temperature. One of these groups was exposed to free water at three day intervals after November 7, 1956 while the other was kept without access to water. The pupation and mortality that occurred in these experiments from November 10, 1956 to May 7, 1957 are presented in Figures 10, 11, and 12.

No pupation occurred in groups of larvae that were kept at a temperature of 12 degrees C. and in constant humidities ranging from 37 to 96 per cent (Figure 10). Apparently this temperature is below the threshold of normal pupation since only five per cent occurred regardless of humidity or moisture conditions. Two of 40 larvae given access to free water at three days interval and kept at humidities of 30-60 per cent pupated approximately 150 days after the experiment was started, however they were deformed and died. At this temperature, mortality of larvae was very erratic. Forty, 35 and 30 per cent mortalities were recorded at constant humidities of 37, 96, and 78 per cent, while less than 20 per cent occurred at constant humidities of 47, 59, and 73 per cent. In the two groups of larvae held at humidities that varied from 30-60 per cent, 7.5 per cent mortality

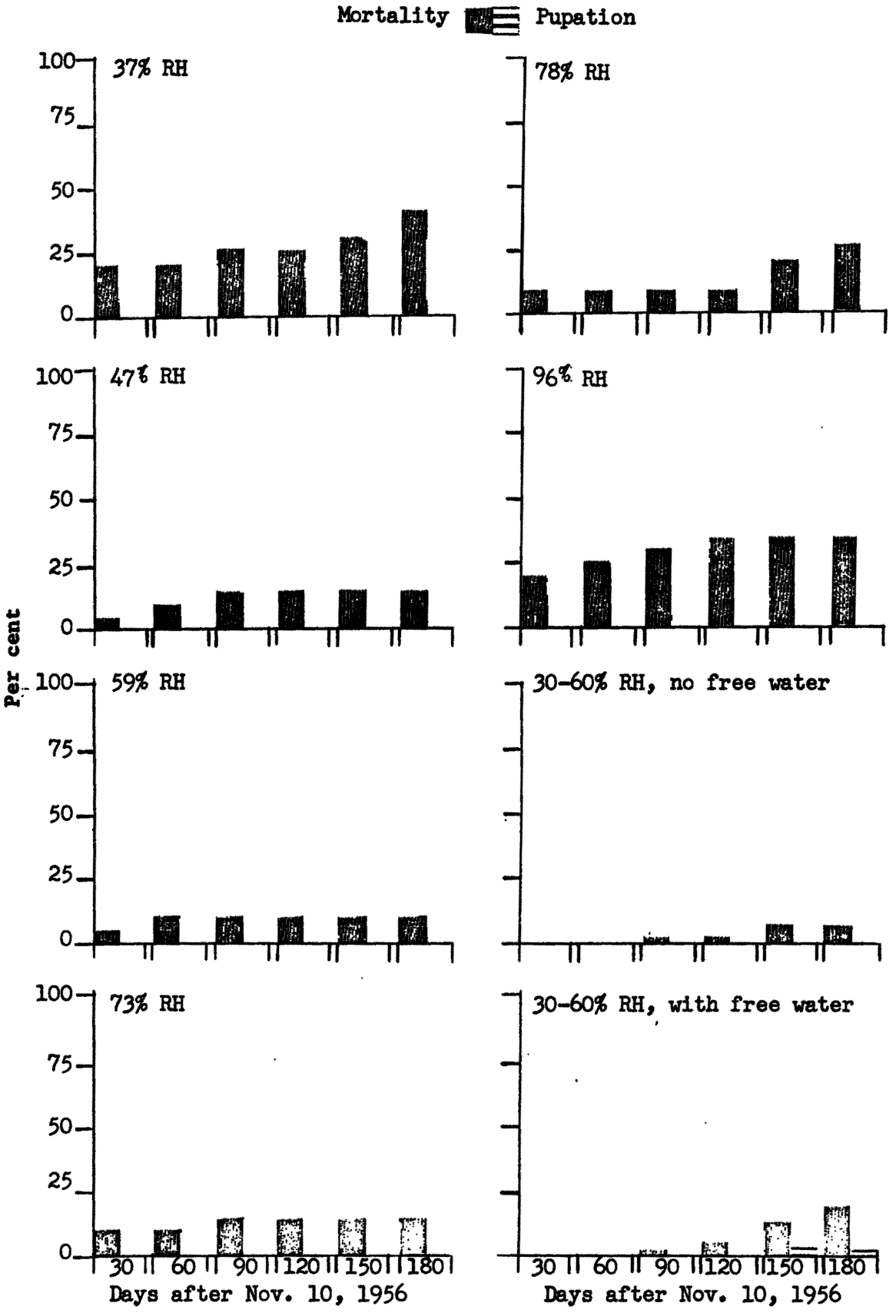


Figure 10. Accumulated mortality and pupation of Zeadiatraea grandiosella (Dyar) larvae at 12 degrees C. and various relative humidities. Stillwater, Oklahoma, 1956-57.

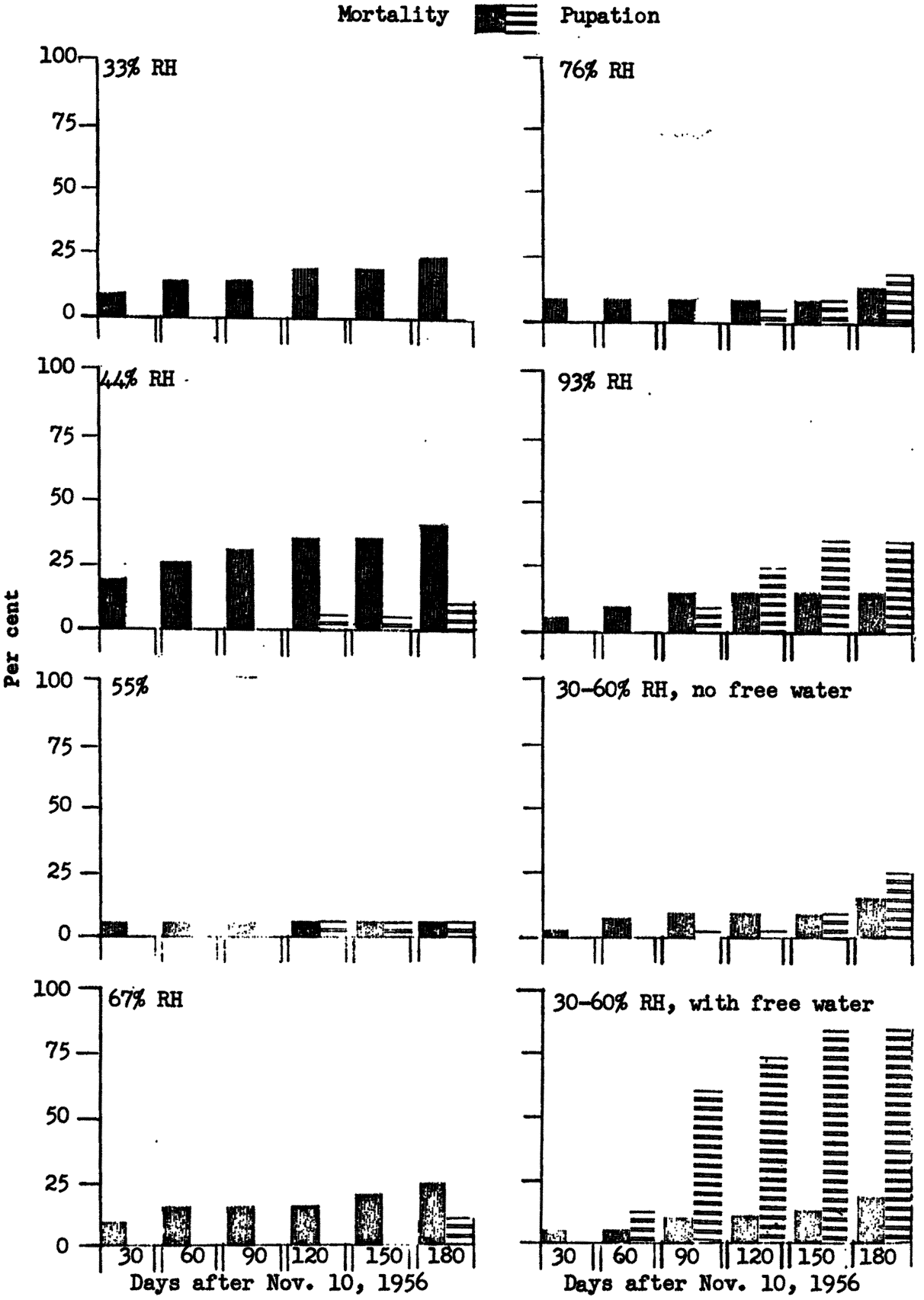


Figure 11. Accumulated mortality and pupation of Zeadiatraea grandiosella (Dyar) larvae at 22 degrees C. and various relative

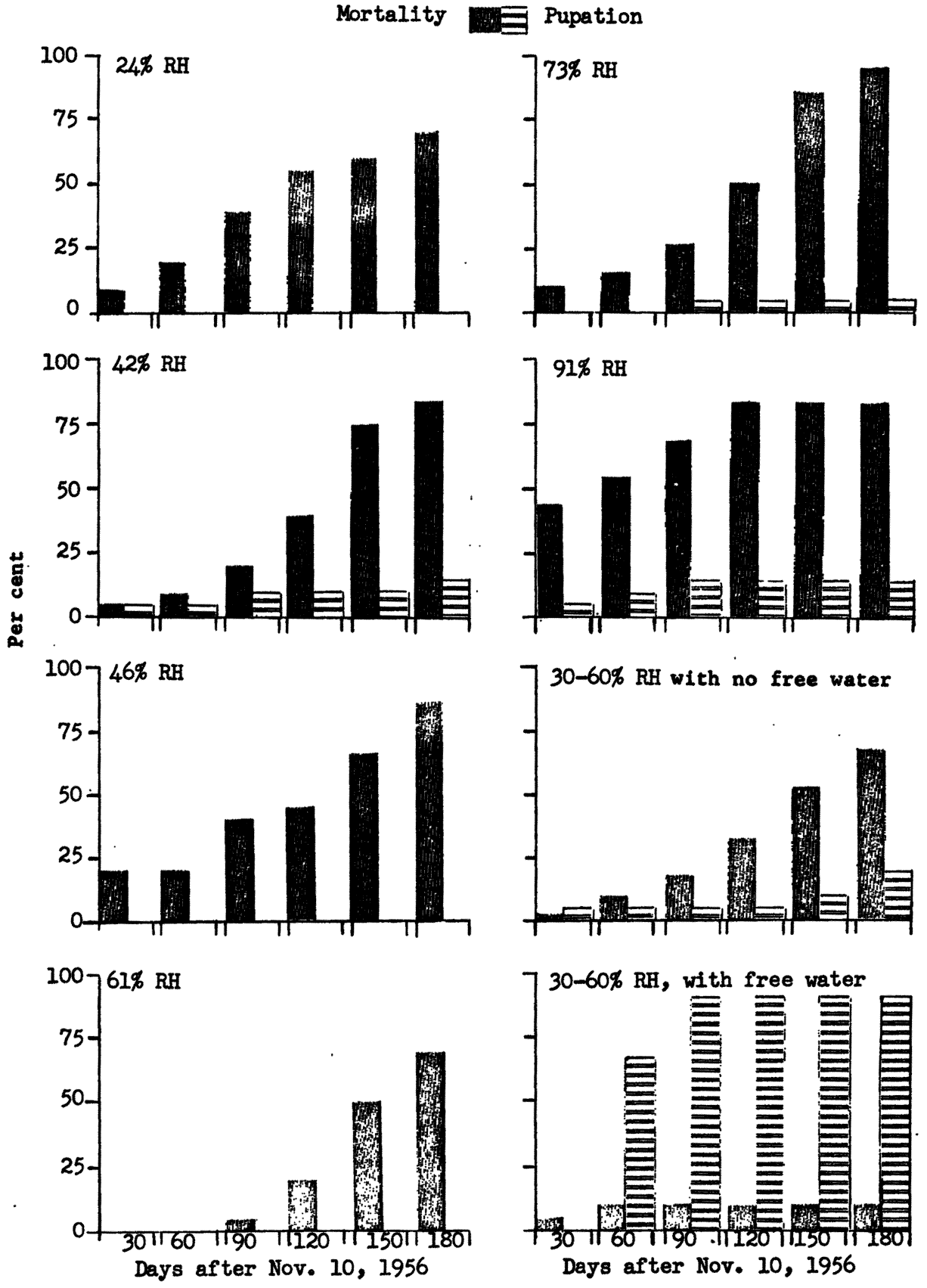


Figure 12. Accumulated mortality and pupation of Zeadiatraea grandiosella (Dyar) larvae at 32 degrees C. and various relative

occurred among the group not given access to free water and 25 per cent among those given access to free water.

At a temperature of 22 degrees C. (Figure 11), no pupation occurred among larvae kept in a constant humidity of 33 per cent. Ten, 5, 10, 20, and 35 per cent pupation occurred respectively at constant humidities of 44, 55, 67, 76, and 93 per cent. In the two groups of larvae held at humidities that varied from 30 to 60 per cent, twenty-five per cent pupation occurred among the group not exposed to free water and 82.5 per cent among those exposed to free water. At this temperature the percentages of mortality was also extremely erratic at the various humidities.

With the exception of the group of larvae given access to free water and held at humidities ranging from 30-60 per cent, mortality was extremely high at a temperature of 32 degrees C. (Figure 12). At least 70 per cent mortality occurred in all other groups of larvae. In the group given free water, mortality was probably low because 90 per cent pupation occurred during the first 90 days of the experiment.

In January of 1956, earlier than normal pupation in the laboratory was induced by exposing winter-form larvae of D. crambidoides and D. saccharalis as well as those of Z. grandiosella to free water at three days interval. The moths which emerged from these pupae were utilized to establish breeding colonies of each species in the laboratory in order to obtain eggs with which to initiate the comparative rearing work reported elsewhere in this study.

Eighty-one D. saccharalis larvae, including two distinct physiological types that were collected near Houma, Louisiana were received

December 1. Sixty-six of these larvae were apparently winter-form specimens since they refused to feed on sections of succulent corn stalks provided as food. These larvae constructed cells in the vials they were isolated in and remained inactive from December 1 to January 2. The remaining 15 larvae appeared to be immature. They fed readily on corn, completed from one to three additional instars in development and then pupated.

Sixty-three D. crambidoides larvae that were collected near Clemson, South Carolina were received December 19. These larvae were winter-form and so similar to that form of Z. grandiosella that one species could not be morphologically differentiated from the other. In order to compare the two imported species with that present locally, 60 Z. grandiosella larvae were collected and kept without food from December 3 to January 2.

The larvae of all three species were kept from the date received until January 2 in a cabinet at 27 degrees C. No appreciable mortality occurred among larvae of Z. grandiosella and D. saccharalis, but 25 of the 63 D. crambidoides larvae succumbed during the period December 19-January 2. Apparently these larvae were injured in transit or were kept in unsuitable conditions after arrival.

Beginning January 2, the colonies of larvae were exposed to free water at three days interval until all larvae had pupated or died. When the experiment was terminated March 14, 6 of 38 D. crambidoides, 9 of 60 D. saccharalis and 14 of 58 Z. grandiosella larvae had succumbed. The percentages of pupation occurring weekly in each species are presented graphically in Figure 13.

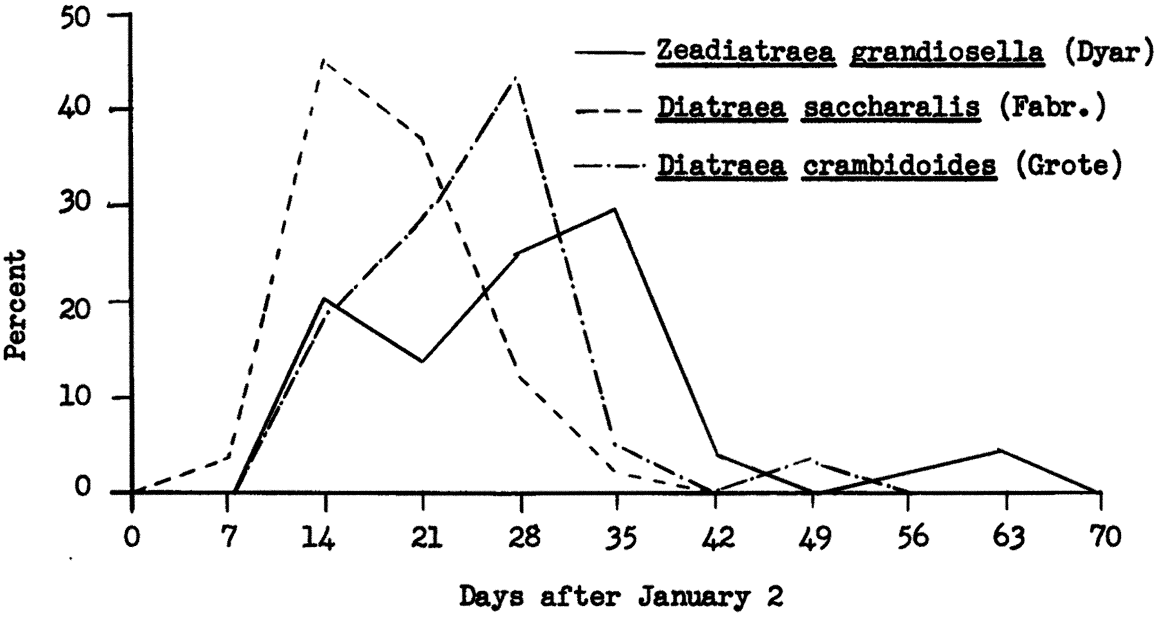


Figure 13. Pupation of over-wintering larvae of three species of the Diatraea complex at 27 degree C. Stillwater, Oklahoma, 1956.

More than 90 per cent of the larvae of each species that pupated did so within 42 days after the beginning of the experiment and prior to February 13. An interpretation of the difference in rate of pupation among species is not attempted since each developed in a different ecological habitat prior to the beginning of the experiment.

Attempts to induce larvae to enter diapause. Several attempts were made in the laboratory to induce larvae of Z. grandiosella to enter diapause by feeding them sections of cornstalk obtained from senescing corn plants. At various times of the year, groups of first instar larvae were isolated in vials and provided with food that

consisted of progressively older sections of mature cornstalk made available to the larvae at three-day intervals. Mortality was extremely high and few larvae survived through the third instar. None were induced to enter diapause, however an attempt to induce diapause in larvae developing on corn in a greenhouse was successful.

On May 28, 1956, 25 mature corn plants that had been growing in a greenhouse since the previous January were each infested with 20 first instar larvae. These larvae were obtained from first generation eggs deposited in the field by moths of the over-wintering brood. When infested, the cornstalks had already tasseled and possessed ears that contained kernels in the hard-dough stage of maturity, however the stalks and leaves were still succulent. When dissected July 25, the plants were senescent with brown dead leaves and dry stalks lacking in chlorophyll. Few larvae had survived and only twenty-one forms (4.2 per cent) were recovered during dissection. These forms included 13 mature larvae in diapause and eight pupal cases. A single diapause-form larva was located below ground level in the crown of each of 13 plants. Eight of the plants had been girdled a few inches above ground level. This type of plant injury is typical of diapause-form larvae in the fall. When isolated in vials and exposed to free water at three days interval, six of the larvae died and seven pupated during the period October 3 to November 17.

PART V

DISCUSSION

Differences among immature forms of the three species. The most important differences in the eggs of the three species are the strikingly different color of Z. grandiosella eggs and the larger size of those of D. crambidoides. During the medial stages of incubation the eggs of Z. grandiosella possess three transverse red bars (Figure 1-C) whereas similar structures in D. crambidoides and D. saccharalis are pale orange. This character is adequate for differentiation of eggs of Z. grandiosella from those of the other species. Although the eggs of D. crambidoides (Figures 3 and 4) and those of D. saccharalis (Figures 5 and 6) are quite similar in color, those of D. crambidoides are much larger. They averaged 1.54 and .90 mm. respectively in length and width as compared to 1.20 and .79 mm. for the same measurements in eggs of D. saccharalis. This difference in size is probably not adequate for differentiating eggs of the two species in the field since an overlap occurs in both length and width (Table 3).

Holloway et al. (1928) and Leiby (1920) imply, respectively, that the eggs of D. saccharalis and D. crambidoides are uniformly orange in color. When causually viewed, they appear orange but when examined microscopically, it is apparent that the yolk cytoplasm is white. The

dark orange embryo and the presence of three pale orange transverse bars during the medial stages of incubation probably cause the orange appearance of eggs of both species. Holloway et al. also describe two small black spots in the eggs of D. saccharalis as the "eyes of the embryo". These spots were not seen in any of the eggs examined during this study.

Morphologically, the larvae and pupae of Z. grandiosella and D. crambidoides are remarkably similar. No single character or combination of characters was observed that would allow visual separation of the species. The cranial wall and tentorial processes of Z. grandiosella larvae are consistently thicker than those of D. crambidoides, however this difference is masked in living or preserved larvae by the dark pigment present in the head capsules of both species, It can only be detected by removing and clearing head capsules of both species or by collecting those cast by larvae during molts.

There is a pronounced difference in the base color of larvae of D. saccharalis and those of the other two species. The paper by Holloway (1916) which contains a complicated key, emphasizing differences in setal angles, has led to a belief that larvae of D. saccharalis and D. crambidoides are quite similar in appearance. This key was not adequate for separating all larvae of the two species during this study. The difference in base color, especially that surrounding the dorsal pinacula, was more reliable. This color is light brown in D. saccharalis and white in D. crambidoides and Z. grandiosella. During this study and two years of field work with D. saccharalis, this character was sufficiently

pronounced to allow separation of this species from the other two, regardless of instar or seasonal form of larvae.

The larvae of D. evanescens possess a ground coloration similar to D. saccharalis, but are smaller and possess pinacula that appear darker colored. This species is found on Paspalum spp. in the areas where sugar cane is grown in Louisiana. There is some possibility that it and D. saccharalis are the same species and that the differences in size and color are associated with a difference in host plants. In Oklahoma, when Z. grandiosella larvae develop on Sorghum halepense (Johnson grass), they are consistently smaller and more darkly pigmented than larvae that develop on corn.

Few differences in the larval life cycles of the three species were noted during rearing. Wide ranges in duration of instars (Table 4) and size of head capsules (Table 5) occurred in all species and these ranges became more variable as development of larvae progressed. A constant number of instars prior to pupation did not occur in any of the species (Figure 7). The range in number of instars completed by each species was: D. saccharalis 5-7, Z. grandiosella 6-8 and D. crambidoides 7-9. The average duration of the larval life cycle in days (Table 4) was D. saccharalis 25.2, Z. grandiosella 34.6 and D. crambidoides 48.7. Although Holloway et al. (1928) reported pupation of D. saccharalis larvae after completion of three instars, none of the larvae reared here successfully pupated before completion of five instars. The few larvae that attempted pupation after four instars developed into partially formed pupae and died. It is believed that all three species

require at least five instars prior to pupation and that they may undergo as many as ten instars depending on the quantity and quality of food available and climatic conditions.

Molting during the non-feeding period in winter-form larvae was not considered as indicating additional instars. When specimens of all three species were kept in the laboratory during the winter, they molted periodically. As many as eight molts occurred in Z. grandiosella larvae during the period November-May. Measurement of the cast head capsules collected after each molt indicated a decrease in head capsule size as molting continued.

Since the range of Z. grandiosella is reported to partially overlap that of D. crambidoides and also to include areas that are within 50 miles of the range of D. saccharalis, the following means of identifying immature forms in the field are presented. The base color of both winter-form and summer-form larvae of D. saccharalis is light brown while that of Z. grandiosella and D. crambidoides is white. The pupae of D. saccharalis possess two tubercles located on the vertex that are decidedly pointed whereas similar structures in the other two species are rounded. Although no characters were found that would separate either the larvae or pupae of Z. grandiosella and D. crambidoides, the eggs of these species are distinct. During the medial stages of incubation, the embryo and bars of eggs of Z. grandiosella are red whereas those in D. crambidoides are orange.

Diapause in larvae of the three species. The possession of a resting stage is a common and often necessary occurrence among organisms that

inhabit inconstant environments. In these forms the dormant state is usually accompanied by a temporary halt in development, by reduced metabolism and often by increased resistance to cold or drought which allows survival during periods of food scarcity and when climatic or other environmental factors are not conducive to continuous development. It is significant that in insects, a group in which normal growth is usually discontinuous, examples of "resting stages" are most numerous and varied.

Too often entomologists have directly associated dormancy occurring in species of insects with hibernation or estivation. More critical examination of these phenomena often revealed that such climatic factors were secondary and that scarcity or unsuitability of food resulted in arrested growth. It is evident that dormancy in larvae of Z. grandiosella should not be termed hibernation simply because the majority of dormant forms are found in the winter; such similar dormant forms can be found in drought-killed plants in July. It is also evident that climate plays an important secondary role in dormancy in this species since it is the most important factor in causing scarcity or unsuitability of food. Perhaps it is more appropriate to define dormancy in Z. grandiosella as diapause since this term implies arrested development in an organism and is not specifically associated with, but may encompass such conditions as hibernation or estivation.

The term diapause was introduced by Wheeler (1893) in defining a stage in embryogenesis of the grasshopper as the phase or halt between anatrepsis and catatrepsis. Henneguy (1904) lifted the term from its

rather rigid definitive setting and applied it, not to a stage of embryogenesis, but to the conditions of arrested growth occurring in insects regardless of stage of development. The meaning of the term for many years implied all forms of arrested growth, even simple inhibition of insect activity during short periods of extremely cool or warm temperatures.

Shelford (1929) suggested that the use of the term should be limited to cases in which development or activity is arrested spontaneously and is not immediately resumed when the environment is favorable for development. He also suggested that the term quiescence be used for cases in which development is temporarily inhibited by unfavorable environment and is immediately resumed when the environment becomes favorable for development. The terms "diapause vrai" and "pseudo diapause" (Roubaud 1930) are based on similar distinctions. Perhaps Shelford's definitions are not entirely appropriate since it is difficult to assign many cases of arrested development to one or the other category, however the distinction of diapause from other more simple forms is of undoubted utility in ecology.

Andrewartha (1952) stated that in considering diapause it is helpful to think of development in terms of its morphological and physiological aspects. Diapause may then be considered, at least for ecological purposes, as a physiological stage that must be completed as a prerequisite for resumption of morphogenesis. He defined the term <u>diapause stage</u> as the state of arrested development in which there is little or no apparent morphological change and defines

diapause development as the physiological development or physiogenesis that occurs during the diapause stage in preparation for resumption of morphogenesis. He also states that in nature, clear-cut morphologically recognizable differences between diapause and non-diapause stages of a species are unusual.

Perhaps Z. grandiosella affords entomologists a rare opportunity to study diapause since there is a distinct morphologically recognizable difference between the diapause and nondiapause larval forms. The conspicious butter yellow color and absence of dark pigment in the pinacula of the diapause-form serves as a "marker" in differentiating this form from the dark pigmented (spotted) nondiapause-form. With adequate investigation this change in form might be utilized to chronologically determine the onset of diapause. At present it is known that fully fed, spotted larvae molt to the diapause form immediately prior to or during construction of cells in the crowns of corn plants in which they later become dormant. Larvae in diapause also pupate without resumption of feeding activity shortly after the termination of diapause when environmental conditions are appropriate for morphogenesis. This change in metamorphosis could also serve as a marker for determining the termination of diapause.

In classifying examples of diapause, the terminology introduced by Steinberg and Kamensky (1936) is of considerable utility. In many insects diapause does not occur in every generation and is said to be facultative. The onset of diapause in these species seems always to be influenced by the environment and can either be induced or averted by appropriate

external conditions. Some other species possess an obligatory diapause in the sense that when they are reared under variable conditions, every individual enters diapause in each generation regardless of environmental conditions. Diapause in Z. grandiosella is obviously facultative, since only a portion of the larvae of any one generation enter diapause and also because its inception may occur in any generation throughout the year. The study here parallels the work by Kevan (1944) with a closely related species in Trinidad. The neotropical corn borer Z. lineolata may go through several successive generations without interruption, but in certain conditions especially when the food is less succulent, the fully fed larvae enter diapause which may endure for a few weeks or several months. In nature, development is resumed, followed shortly by pupation when another wet season begins. Kevan demonstrated experimentally that larvae in diapause obtained from the field would pupate after several weeks if they had been adequately wetted with water. On the other hand, larvae feeding in senescing corn plants entered diapause during the rainy season despite the presence of free water. This apparent ready response of resting stage larvae to applications of water led Simmonds (1948) to suggest that this phenomenon is not a true diapause. However Andrewartha (1952) considered it a true example since the resting stage occurred in senescing, less succulent, corn plants despite the presence of free water. Lees (1955) also interprets this as diapause and also associated its inception with the poor value of the food. Both of these authors are undoubtedly correct in their interpretation since in a closely related species, Z. grandiosella, the diapause-form

was collected during July, 1954 in Oklahoma from drought-killed fields of corn in which there was an absence of free water in plants and also in the fall (September-November) from fields that had been irrigated and contained plants in which there was an abundance of free water.

Photoperiod apparently is not associated with inception of diapause in Z. grandiosella since in July nondiapause (spotted) form larvae were found in irrigated corn fields and the diapause-form in adjacent unirrigated drought-killed fields. Further support for this conclusion is also furnished by the difference in day length in July when the diapause-form was collected from drought-killed plants and in the fall when it was collected from senescent plants. Since moisture content in plants differed during these periods it is also evident that scarcity or poor quality of food is the most logical explanation of the cause of diapause in Z. grandiosella.

At this point it should be appropriate to mention that the work in Louisiana by Kevan and the work here may possibly have been done with the same species of Zeadiatraea. Although the author has not had opportunity to study the characters of adult genitalia used by Box (1956) to separate the two species, there are certain striking similarities in biology, immature stages and distribution that should be pointed out. The description of immature stages and methods of injury to corn of Z. lineolata (Kevan 1943, 1944) are remarkably similar to that published by Davis et al. (1933) for Z. grandiosella. Both species are reported to possess eggs with red bars during the medial stage of incubation.

The larvae of both species are reported to be spotted during

active feeding stages and butter-yellow and without spots during the resting stage. The diapause stage of both species occurs in the crowns of corn stubble. During the period 1911-1927, Z. grandiosella was referred to as Z. lineolata in southwestern United States. Painter (1955) states that Z. lineolata is closely related taxonomically to Z. grandiosella. Both species are found in Mexico with Z. lineolata reported as occurring no further north than central Mexico and Z. grandiosella restricted to northern Mexico (Box 1931). Both species are recognized as major economic pests of corn.

The role of free water in causing pupation of diapause-form Z. grandiosella larvae is interpreted, not as a stimulus that evokes diapause development and ultimately results in completion of the diapause stage, but only as an aid in creating conditions that are favorable for development after diapause is terminated. The data shown in Figure 9 (B and C) indicate that relatively uniform pupation occurred simultaneously in January in two groups of larvae, one of which was exposed to free water after November 7 and the other after December 19. These data indicate that during the first 42 days of the experiment, application of free water was not effective in inducing pupation. Further proof that a period exists in which free water is not effective in inducing pupation is presented in Figures 8, 11 and 12. Rates of pupation in the groups of pupae exposed to free water did not increase appreciably until 60-90 days after the start of each experiment. It is believed that this inherent periodism represents the true diapause stage and that after its termination, the application

of free water was effective in inducing pupation because it created adequate moisture conditions coincident with adequate temperature requirements of the species for development.

In Oklahoma, larvae of Z. grandiosella normally enter diapause in the fall and pupate during the spring, six to seven months later. It is probable that during the first two or three months of dormancy the larvae are in diapause and complete diapause development or physiogenesis. After that period, they apparently resume morphogenesis but do not pupate until spring because environmental conditions including temperature and moisture are not appropriate. Food does not appear to be required by larvae for resumption of morphogenesis, because larvae in appropriate moisture and temperature conditions in the laboratory pupated without feeding shortly after completion of diapause and resumption of activity.

The data presented in this study do not allow a clear-cut interpretation of the role of free water in post-diapause development of Z. grandiosella larvae. When a group of 2[illegible] diapause larvae were kept at a temperature of 27 degrees C. without access to free water from November 1[illegible] to May [illegible] (Figure 4 A), 60 per cent pupated and 40 per cent died. Most of the pupation in this group occurred in April and May. In contrast, approximately 90 per cent pupation occurred in January in similar groups of larvae kept at 27 degrees C., one of which had access to free water after November 7 (B), the other after December 19 (C). Perhaps an explanation of the activities of these three groups of larvae will aid in understanding the need for free water in post-diapause development.

Shortly after isolation in vials in the laboratory in November, the larvae in all three groups constructed cells in which they became dormant. This dormancy continued until mid-December regardless of whether the larvae were receiving applications of free water (B) or were kept without access to free water (A and C). In mid-December, the larvae in all three groups became active in the sense that they emerged from their cells and crawled aimlessly around the interior of the vials. Many constructed tunnels in the cotton that extended from the bottoms to the screen caps at the tops of vials. In the two groups of larvae that were given access to free water, one after November 7 (B) and the other after December 19 (C), pupation started in early January and was completed by January 30. The pupae in these groups were of normal size from which normal moths emerged. However in the group that did not receive water (A), very little pupation occurred in this group. These larvae continued to crawl aimlessly around vials and occasionally molted. After each molt the larvae appeared smaller. Most larvae of these vials died between January and May. They also appeared abnormally wrinkled and dessicated. When pupation eventually occurred all of the pupae were abnormally small. From a total of 16 larvae that pupated in this group in April and May, only five small moths emerged.

Although the possibility that applying free water to the larvae and cotton in the vials merely created appropriate humidity conditions is not entirely ruled out, these data are interpreted as indicating that larvae of Z. grandiosella require free moisture for healthy post-diapause development. Less (1967) indicates that free moisture is required for

post-diapause development in larvae of Chilo simplex Butl., and that most of it enters through the mouth and only a small amount is admitted through the cuticle.

The eventual abnormal pupation of larvae deprived of free water is not readily explained. Most of it occurred in April and May and was coincident with pupation of diapause larvae in the field. Since atmospheric humidity is often high in the spring in Oklahoma, it is possible that water from the atmosphere condensed in the rearing vials and became available to larvae, however this condition was not observed. Regardless of the cause of pupation, the small size of pupae and moths and high mortality indicate abnormal development.

Although recognized as a common technique, the use of saturated salts was not satisfactory for maintaining constant humidities in which larvae were kept at various temperatures (Figures 10, 11 and 12). When the jars were removed from the cabinet and opened for examination, condensation often occurred in vials containing larvae, especially those kept in high humidities and temperatures. Leaks developed in jar lids on several occasions and the solutions had to be resaturated. The possibility of adverse effect of some of the salt solutions on larvae cannot be overlooked since mortality was extremely erratic and apparently followed no trend that could be associated with range of humidity.

Regardless of the humidity they were kept in, none of the larvae maintained at a temperature of 12 degrees C. successfully pupated (Figure 10). Two larvae of the group provided with free water and kept in humidities ranging from 30-60 per cent formed pupae that were

deformed and which later died. This temperature is probably very near the threshold required for pupation of Z. grandiosella. A comparison of the larval mortality that occurred at 12 degrees C. (Figure 10) with that which occurred at 32 degrees C. (Figure 12) indicates much higher mortality of larvae at the latter temperature.

At a temperature of 22 degrees C. (Figure 11), most of the group of larvae kept in 30-60 per cent humidities and provided with free water pupated within 90-150 days, while 20 per cent of those held at similar humidities without water pupated from 150-180 days after the experiment started. Many of these pupae, which were abnormally small, were distorted and died. Thirty-five per cent pupation occurred at a relative humidity of 93 per cent. In contrast to the small distorted pupae that resulted when larvae were held without water, those that pupated at 93 per cent humidity were normal in size. Since the pupation coincided with that occurring at the same temperature in larvae provided with free water and, also, because the pupae were normal in size, it is believed that condensation of moisture in vials provided the larvae with free water. No interpretation is made of the small per cent of pupation that occurred at other humidities. It has been observed that when larvae of this species are kept in the laboratory in the winter at temperatures above 20 degrees C. that occasionally one will pupate regardless of moisture conditions.

With the exception of those provided with free water, most of the larvae died at temperatures of 32 degrees C. (Figure 12). In the group provided with water, mortality would have probably been higher except

that most of the larvae pupated from 60 to 90 days after the beginning of the experiment. The pupation that occurred in this experiment is explained along lines similar to that given for Figure 11.

A comparison of larvae provided with water and kept at 22 degrees C. (Figure 11) and those given similar treatment and kept at 32 degrees C., (Figure 12) shows that pupation occurred more rapidly and the pupation period was shorter at the higher temperature. Lees (1955) indicates that the rate of diapause development is increased at higher temperatures in some species and decreased in others.

The only data indicating that D. crambidoides and D. saccharalis also possess a diapause stage is presented in Figure 13. This experiment was not designed to study diapause, since its primary purpose was to obtain moth colonies for egg production and subsequent rearing of larvae. However winter-form larvae of all three species responded to applications of free water in mid-winter and pupated several weeks prior to their normal pupation periods in the field. Although the pupation curves, which are based on small numbers of larvae, are erratic, the data indicate little differences among species in rate or time of pupation.

The publications of Leiby (1920), Phillips et al. (1921) and Cartwright (1934) present descriptions of the biology of D. crambidoides which are almost identical to that presented for Z. grandiosella by Davis et al. (1933). Both species over-winter in a dormant state in cells in the crown roots of corn. The over-wintering larvae of D. crambidoides are so similar to those of Z. grandiosella that during

this study they could not be morphologically differentiated. It is probable that D. crambidoides possesses a diapause stage and as in the case with Z. grandiosella, its inception is caused by the poor quality or lack of food available in senescent corn plants in the fall rather than by the onset of cooler fall temperature as reported by Leiby.

Unfortunately the recognition of a diapause stage in D. saccharalis is more difficult. Holloway et al. (1928) state that all instars of this species hibernate but that "hibernation is not what may be called complete since the larvae become active on warm days and continue to feed to some extent". They also indicate that smaller larvae increase slowly in size during the winter and by spring all hibernating larvae are of the same size.

During two years field work in Louisiana from 1957-1959, actively feeding populations of D. saccharalis larvae were not observed in the winter after the sugar cane crop had been harvested and the tops of stalks left in the field had rotted. During 1957, no pupae were collected after November and no moths or egg clusters were observed in December. In contrast butter yellow mature larvae were observed as early as September in cells in internodes of mature sugar cane stalks. The incidence of this form increased as the crop became more mature. It is believed that larvae of this type are in diapause and that they are the only larvae capable of over-wintering from one crop season to the next. The larvae present in fields at the end of the harvest season that are not mature enough to enter diapause probably die from lack of

food. This interpretation may partially explain the poor correlation that has existed in the past between winter survey data, which has been based on all forms recovered, and the following year's infestation.

PART VI

SUMMARY

The extent of differences which occur among immature stages of three species of the Diatraea complex was studied in the laboratory and field from 1954 to 1957. The morphologies and life cycles of larvae and the eggs of D. saccharalis (Fabr.), D. crambidoides (Grote) and Zeadiatraea grandiosella (Dyar) were compared under uniform laboratory rearing conditions. The relationship of water and food to diapause in larvae of Z. grandiosella was investigated in the laboratory and field.

No morphological characters were recognized that would allow taxonomic separation of the larvae of D. crambidoides and Z. grandiosella. The base color of D. saccharalis differs sufficiently to allow separation of all larval instars of that species from those of the other two species. This color is light brown in D. saccharalis larvae and opaque white in larvae of D. crambidoides and Z. grandiosella. Existing keys relating to the larvae of the three species were determined to be inadequate for separating all specimens.

The eggs of D. saccharalis and D. crambidoides appear similar in color, but those of D. crambidoides are larger and are deposited in smaller masses than those of D. saccharalis. The eggs of Z. grandiosella are intermediate in size to those of the other two species, but differ significantly in color.

The pupae of D. saccharalis possess two tubercles on the vertex that are pointed, whereas analogous structures in the other two species are rounded. The pupae of Z. grandiosella and D. crambidoides appear morphologically similar.

When kept in the laboratory at a temperature of 27 degrees C. and fed corn, the mean duration in days of the life cycles of larvae of each species was : D. saccharalis 25.2, Z. grandiosella 34.6 and D. crambidoides, 48.7. The range in number of larval instars prior to pupation was : D. saccharalis 5-7, Z. grandiosella 6-8, D. crambidoides, 7-9. The head capsules of reared larvae of the three species did not differ sufficiently in size, shape or color to be useful for taxonomic purposes.

The ecological data presented indicate that dormant winter-form larvae of Z. grandiosella are in diapause and not in hibernation, and that the inception of diapause is associated with poor quality or lack of food rather than lower temperature. Earlier than normal pupation occurred when applications of free water were made to larvae in diapause in the winter. Experiments in which larvae in diapause were subjected to various conditions of humidity and temperature indicate that humidity has no causal relation with termination of diapause.

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