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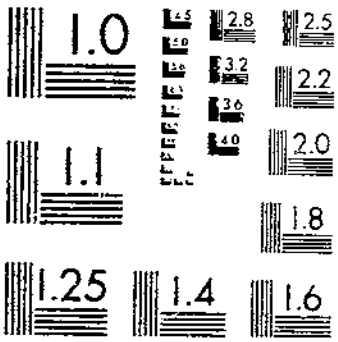

MICROCOPY RESOLUTION TEST CHART
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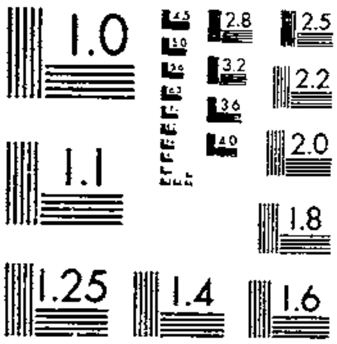

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Strains of the European Corn Borer in the United States[1]

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CONTENTS

SUMMARY

In the period 1937 to 1940, inclusive, experiments were conducted at New Haven, Conn., to determine whether inherently different strains of the European corn borer (*Pyrausta nubilalis* (Hbn.)) occur in the United States and to study the physiological relationships of strains.

These experiments were conducted under controlled laboratory conditions utilizing techniques that have been found suitable by other workers for handling this insect.

Larvae were collected from fields near New Haven for test insects from the Eastern States, and from Toledo, Ohio, Mount Clemens, Mich., and Rochester, N. Y., for test insects from the Lake States.

The material from New Haven was found to represent a homozygous multiple-generation strain, and no evidence was obtained to indicate the occurrence of a single-generation strain in that locality. The Toledo material was heterozygous, a complex of multiple- and single-generation strains occurring together. A homozygous single-generation strain was isolated from the Toledo material, but attempts to isolate a homozygous multiple-generation strain were not successful. Some larvae went into a disapause before pupation in every one of

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[2] Assistance in this investigation from the following men in the Bureau of Entomology and Plant Quarantine is gratefully acknowledged: W. A. Baker, in charge of European corn borer research, under whose leadership the study was conducted; A. M. Vance, who had general supervision of the study at its inception; R. T. Everly, who assisted in 1937, and C. H. Batchelder, in charge of the substation at New Haven, Conn., where the work was conducted. For much helpful advice in the conduct of the experiments the writer is indebted to D. F. Jones, geneticist, L. C. Curtiss, assistant geneticist, and Roger B. Friend, entomologist, of the Connecticut Agricultural Experiment Station, New Haven, Conn., and to D. F. Poulson, geneticist, Osborn Biological Laboratory, Yale University.

four successive generations of every pedigreed line that was selected in the attempt to produce a purely multiple-generation strain from this stock. Hence the genetic factors responsible for the single-generation habit evidently persisted as recessive characters.

Larvae of the single-generation strain from Toledo grew more slowly than those of the multiple-generation strain from New Haven. The multiple-segregates (heterozygous) from Toledo material entered the pupal stage more slowly than the multiple-generation strain from New Haven.

Moths from the New Haven and Toledo field stocks, respectively, showed a preference for mating among individuals from their own locality rather than crossing between the stocks. Mating of New Haven females with Toledo males was accomplished in only a few cases, apparently because of a racial inhibition to mating, the nature of which was not discovered.

In crosses between the two regional stocks, heterosis was evident in the F_1 generation, and larval mortalities were very high in the F_2 generation. The Toledo female × New Haven male cross produced progeny which resembled the paternal parent. The reciprocal cross produced (1) diapause and nondiapause phenotypes and a preponderance of males in the nondiapause part of the F_1 generation, and (2) a retarded development of larvae in the F_2 generation, both results being evidence of the paternal disapause characters.

The author feels that the existence of distinct biological strains of the European corn borer has been demonstrated by these experiments.

INTRODUCTION

An investigation was conducted during the period April 1, 1937, to June 1, 1940, at New Haven, Conn., to determine some of the inherent differences between strains of the European corn borer (*Pyrausta nubilalis* (Hbn.)) now occurring in the United States and to study their physiological relationships. An understanding of these relationships is important in the formulation of quarantine regulations intended to prevent the introduction of the insect into new regions. It is also of value in research on methods for controlling the pest, and to control methods in practice, since physiologically different strains of the species may require modified or even different means for their control. Furthermore, biological control programs become more effective when the influence of recognized physiological strains of the borer on its parasites is known. This investigation was designed primarily to determine whether the single-generation borer, predominant in the Lake States, is biologically distinct from the multiple-generation insect, prevalent in the Eastern States.

The occurrence in the field of a partial second generation in the Lake States, at about the time this study was initiated, as reported by Vance (*19*)[3] and Ficht (*10*), was probably responsible for the heterogeneity in corn borer material from the vicinity of Toledo, Ohio, used in the experiments. Prior to 1936 the corn borer near Toledo had principally only one generation a year, although a few pupae were observed each summer, as Bottger and Kent (*5*) reported summer pupation of the first generation at Monroe, Mich., before 1931.

Caffrey and Worthley (*7*) reported the results of experiments con-

[3] Italic numbers in parentheses refer to Literature Cited, p. 19.

ducted from 1921 through 1924 in which individuals of the European corn borer were transferred between western New York (one-generation area) and eastern Massachusetts (two-generation area) and allowed to develop in cages exposed to the climatic conditions of the other region. Each group of transferred material adhered to its original seasonal rhythm of development. In addition to this finding these authors state: "The results have shown that individuals taken from both areas, as well as individuals from various foreign localities, cross-breed and are capable of producing fertile eggs."

A study to determine the reasons why different numbers of generations occur in apparently similar climatic complexes, such as are found in western New York and eastern Massachusetts, has been made by the writer. His conclusion is that in the United States where the strain best suited to survive in an environment spreads into a region after a strain less well adapted to that environment has been present for several years, the strain best suited to the environment will become more abundant.

Prebble (*15, p. 452*) has reported a similar complex of strains in *Diprion hercyniae* (Htg.) (=*Gilpinia polytoma* (Htg.)), of which he says:

> * * * there are different "lines" within the species, with respect to the inherent tendency towards diapause or continued development; * * *. The character of the population in different areas seems to have been fashioned out of the various elements within the species, by the interaction of climatic and genetic factors with respect to diapause and survival

TECHNIQUE AND EQUIPMENT

The following technique was employed in the conduct of the investigation: Hibernating corn borer larvae were stored within a tight metal cabinet, fitted inside a household refrigerator operating at 33° to 36° F., that provided a humid environment beneficial to the larvae. Pupation and emergence were obtained in a room-sized incubator operating at 80° and at a relative humidity of 90 to 100 percent. The mating tests were conducted in this incubator room or in a laboratory workroom where the temperature could be varied between 65° and 80°, as desired. The workroom conditions were used for all tests in which the moths were observed for mating and the incubator room for tests in which egg fertility was the index of mating. All the rearing, including the incubation of eggs and also the feeding of larvae for a study of developmental rate, was done in the incubator room. All other feeding, involving removal of the larvae from their experimental environment for short intervals, was done in the laboratory workroom at a temperature of 70° to 80°.

In the initial tests of mating, moths were placed in oviposition cages of the type described by Bottger (*4*), which consisted of a pint-size, cardboard food container with a copper-screen bottom. The later mating tests required a cage that facilitated observation. In the first two of these later tests each moth combination was confined in a wooden frame cage, the top, bottom, and ends of which were of solid wood, 5 by 7 inches, and the front and back were removable 7- by 7-inch wooden frames covered with ⅛-inch-mesh bobbinet. This type of cage was used for all mating work in 1939. In the last test, in which mating of moths of both sexes from the two localities was observed, the moths were confined in a wooden frame cage 15 by 12 by 9 inches covered with bobbinet. A wad of absorbent cotton saturated with water was placed on the floor of the cage, and the inside of the cage was sprayed at approximately 2-hour intervals to provide drinking water for the moths.

Moths were confined for oviposition in round, pint-size cardboard food cartons having 18-mesh copper-screen bottoms and removable tops. The inner surfaces of the top and side walls were lined with white typewriter paper on which moths deposited their eggs. A fine mist was sprayed into the cages twice daily to provide drinking water for the moths. The paper linings with attached eggs were removed from the cages daily, or at more frequent intervals, and cut into small sections, each bearing one egg mass. The paper sections were then pinned onto linoleum disks and retained in pint-size food cartons during the embryonic development of the eggs. Egg fertility was determined by a count of the eggs showing embryonic development 3 days after deposition. (The dark pigmented head capsule of an embryonic larva is visible through the translucent chorion of the egg.)

A few hours before they were expected to hatch, eggs were placed on food in rearing cages. Insofar as practicable, 100 fertile eggs were allotted to each cage. In many cases less than this number of eggs from a particular source were ready to hatch on a given day, and consequently smaller numbers were placed in some cages. When the number of larvae in isochronous cages became less than 50 because of mortality or pupation, the survivors were combined in fewer cages, within the limit of 100 per cage.

Rearing cages 9 inches long and 15 inches wide with wooden sides, 80-mesh copper-screen bottoms, and glass tops, and essentially the same as those described by Mathes (*12*) were used throughout the 3 years of study, except that in 1939 a cage made from a 1-pound, metal, coffee can, with an 80-mesh copper-screen bottom and glass top, was utilized for larvae during their first 5 days (first feeding period) of life. The feeding method used by Mathes was modified to fit the needs of these experiments. The food consisted of cut green beans for the first 5 days. For the two subsequent feedings at 5-day intervals, and daily thereafter, green peas in the pod with the ends of the pods cut off were used. The number of eggs hatching was determined by a count of the unhatched embryos remaining after an interval of 5 days. Larval survival, size (instar), and pupation were determined by counting the numbers alive on the tenth and fifteenth day after the eggs were placed in the rearing cage, and daily thereafter until the fortieth day. Pupae formed in the rearing cages were removed daily. After their sex was determined the pupae were isolated in shell vials large enough to allow wing expansion of the emerging moths.

Hibernating corn borer larvae were collected in the early spring of each year of study from the vicinity of Toledo, Ohio, for test insects from the Lake States, and from New Haven, Conn., for test insects from the Eastern States. In 1938, material was procured from Mount Clemens, Mich., which appeared to be of the same developmental type as that from Toledo and is so considered throughout this discussion. Mature, active larvae were collected in August 1938, from Rochester, N. Y., and retained in cold storage until the following spring. From the last material one pair of moths mated, the progeny were single-generation phenotypes, and they are included in the records of the single-generation strain utilized to show differences in the rate of development.

In 1937, crosses were made between moths from New Haven and Toledo, and moths from each region were also bred separately. The results of these matings, the resultant egg production and fertility, the larval development, and the pupation are discussed hereafter, with the results from the work of other years, in the sections dealing with the various morphological stages of the insect. In 1938 emphasis was placed on the study of mating and mating technique and the isolation of homozygous strains to facilitate studies of strain characters and in 1939 the studies of strain characters themselves were made.

TERMINOLOGY

The term "field stock" is used to designate material from field collections in which no selections had been made, "strain" is used to designate the homozygous "diapause" and "nondiapause" material from selections, and "multiple segregate" is used to designate the material from the heterozygous Lake State field stock selected for the nondiapause character but which produced in each generation phenotypes that entered the diapause.

RESULTS OF THE INVESTIGATIONS

Field Stock

During the 3 years of the investigation, 3,298 corn borers, progeny of 2 stocks, were reared to maturity, i. e., to pupae or full-fed larvae which spun hibernacula. The general data on these rearings appear in table 1. Of this total, 1,402 individuals were from New Haven stock, and all these pupated within 35 days from the time of hatching. No evidence of a single-generation strain was found among progeny from field stock collected from New Haven. This result is indicative of the existence in this region of a homozygous multiple-generation strain of the European corn borer having the inherent ability to reproduce through successive generations without a larval diapause. This strain is apparently adaptable to a wide climatic range.

TABLE 1.—*Progeny rearings of the European corn borer from 2 areas, New Haven, Conn., and Toledo, Ohio, on the basis of selections for the multiple-generation phenotypes in successive generations*

Source of original field stock	Year	Progeny reared							
		First generation		Second generation		Third generation		Total	
		Larvae[1]	Pupae	Larvae[1]	Pupae	Larvae[1]	Pupae	Larvae[1]	Pupae
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
New Haven, Conn.	1937	0	150	0	240			0	390
	1938	0	383	0	117	0	17	0	517
	1939	0	204	0	225	0	66	0	495
Total (3 years)		0	737	0	582	0	83	0	1,402
Toledo, Ohio	1937	40	129	96	28			136	157
	1938	140	331	49	1			189	332
	1939	621	99	137	198	10	17	768	314
Total (3 years)		801	559	282	227	10	17	1,093	803

[1] Mature—40 days old.

The 1,896 progeny from the Toledo stock included both multiple- and single-generation strains. The second generation in 1937 and 1938 and the second and third generations in 1939 were progeny from multiple-generation phenotypes. Among progeny reared in 1938 and 1939 from individual pairs of moths from the Toledo stock, pupation ranged from 0 to 100 percent. A homozygous single-generation strain was segregated in one generation from the Toledo stock by selecting single-generation phenotypes distinguished by inherent diapause factors and incapable of reproducing through successive generations until the diapause requirements of full-fed larvae have been satisfied. The diapause requirements of this strain have been reported and discussed by Babcock (*1, p. 49*), who stated

> * * * the fact that larvae of the normal one-generation stock are not at all successfully forced into pupation when bred from the egg under incubator conditions show[s] that the specific requirement for emergence from hibernation in the corn borer includes something more than the mere accumulation of temperatures above a threshold of development; * * *.

Baker and Mathes (*2*) state that in an average season these borers should not be collected from the field before October 1 (for parasite data) and that these larvae should not be removed from their cool environment before April 24, a period of 6 months. In the present study about 6 months in cold storage was used to satisfy the diapause requirements of laboratory-reared larvae of this strain. When the multiple-generation phenotypes which all pupated in the first generation were used, however, as parents of a second generation, some single-generation phenotypes were produced. In fact, 4 successive generations of these selections, discussed in the section on larval development, did not eliminate the single-generation phenotypes. It is thus apparent that although the multiple-generation (nondiapause) character is dominant in this stock the single generation (diapause) factor persists in recessive form. These data show that the Toledo stock is a mixed population consisting of multiple- and single-generation strains intermingled and apparently interbreeding in the field, as well as possibly breeding among themselves.

Mating of Moths

In 1937, single pairs of corn borer moths that were placed in mating and oviposition cages of the type described by Bottger (*4*) did not produce fertile eggs. When five moths of each sex were confined together, however, mating occurred with each of the four combinations utilized, as determined by the resulting fertile eggs from each combination. The combinations were as follows: (1) Toledo females with Toledo males, (2) New Haven females with New Haven males, and (3 and 4) reciprocal sex combinations from the two regions. In the New Haven female × Toledo male group a preponderance of infertile eggs (76.23 percent) was obtained in subsequent oviposition studies, a fact suggesting that mating was perhaps not so successful in this as in the other groups in which infertile eggs averaged from 3.03 to 18.23 percent. On the other hand, unmated females sometimes deposited their infertile eggs, and the low egg fertility in the one group could have resulted from this type of oviposition by unmated females together with a deposition of fertilized eggs by only a few mated females in the cage.

Matings with groups of moths were tested again in 1938. In these tests apparently no mating was obtained in the New Haven female × Toledo male groups, as no fertile eggs were laid by the females. Fertile eggs were laid in these tests by the other three combinations, however, again denoting successful mating.

The foregoing results suggested that a study of mating might yield information relative to physiological differences between stocks and possibly between strains. Also, in order to investigate differences between strains in other stages or activities of the insect, it was desirable to perfect a mating technique to provide eggs from which further rearings could be made. Mating experiments were therefore conducted in which the moths were observed for prenuptial activity and copulation, which are described by Caffrey and Worthley (7), and mated females were isolated for egg production. The eggs were observed for fertility and used in progeny-rearing experiments.

The tests of mating were conducted with each of four combinations of moths. The data on moths used in the experiment and the frequency of mating are given in table 2.

TABLE 2.—*Mating of European corn borer moths in two tests in 1938, when each sex was confined with the opposite sex from each of the 2 field stocks, Toledo, Ohio, and New Haven, Conn.*

Test	Stock of moths		Moths available for mating		Pairs mated
	Female	Male	Female	Male	
			Number	*Number*	*Number*
1	Toledo	Toledo	10	10	2
2	do	do	15	5	0
Both	do	do	25	15	2
1	New Haven	New Haven	10	10	2
2	do	do	15	15	11
Both	do	do	25	25	13
1	Toledo	do	10	10	2
2	do	do	15	15	3
Both	do	do	25	25	5
1	New Haven	Toledo	10	10	0
2	do	do	15	25	0
Both	do	do	25	35	0

Test 1 was begun August 31, 1938, and the moths were observed continuously for 87½ hours; test 2 was begun October 10, 1938, and the moths were observed continuously for 164 hours. Test 1, with 10 moths of each sex available for mating in each category, resulted in 2 matings of Toledo females and males, 2 of New Haven females and males, and 2 of Toledo females with New Haven males. No mating occurred in the New Haven female and Toledo male combination, although typical prenuptial activity of males was observed and the females were not observed to avoid copulation. In test 2 a preponderance of males (25 to 15) was included in the New Haven female and Toledo male group as an additional stimulus to mating. This change necessitated a corresponding reduction in the number of Toledo males confined with Toledo females. Fifteen moths of each sex were used in the other 2 lots. No mating of Toledo females and males was obtained and again, although the same activity of the moths was noted as in test 1, there was no mating between New Haven females and Toledo males.

In a third test, moths were marked for identification by spraying them with two colors of waterproof ink. All moths of both sexes from the two localities were then confined in one cage. Many of these moths died, probably owing to an excess of marking material, and these were removed from the cage during the first 5 hours. Observations were continuous for 77 hours, beginning at 8:00 a. m. November 9, and ending at 1:00 p. m., November 12, 1938. The data from this experiment, presented in table 3, show the number of females from each source that mated or lived throughout the experiment, the number of males from both localities, and the pairs obtained from each of the four possible combinations.

TABLE 3.—*Mating of European corn borer moths when both sexes from the 2 field stocks, New Haven, Conn., and Toledo, Ohio, were confined in the same cage for 77 hours*

Females		Males mated from each field stock [1]		
Field stock	Available for mating	Toledo (53)	New Haven (18)	Both stocks (71)
	Number	*Number*	*Number*	*Number*
Toledo	39	7	4	11
New Haven	38	1	14	15
Both stocks	77	8	18	26

[1] Figures in parentheses are numbers available for mating.

One hundred and forty-eight moths, 77 females and 71 males, either mated and were removed from the cage or lived throughout the experiment. All the 18 New Haven males mated, the last one after 52 hours. Only 8 of the 53 Toledo males mated. The last mating observed for a Toledo pair was at the end of 53 hours. The prevalence of mating between moths from the same source contrasts sharply with its limited occurrence between those from different sources, and it is noteworthy that only 1 pair of the New Haven female × Toledo male cross was obtained. Here, as in the first 2 tests, mating was more frequent among New Haven individuals than among those from Toledo.

The number of living moths of each sex from each source which were available for mating during the test are illustrated in figure 1. The number of each at given times during the experiment, reduced as mating removed individuals from the cage, is represented in the illustration by a designated line. The first pair which mated, represented by the first coincident reduction in their respective lines, consisted of a New Haven female and a Toledo male. No other Toledo male mated until the forty-ninth hour. This and all later matings of Toledo males occurred only with Toledo females. Two periods of activity were evident, the first of much less magnitude than the second. The first of these periods was from 30 to 33 hours and the second from 43 to 53 hours after the beginning of the test.

Figure 2 shows the proportion of moths coupled in the various combinations by lines connecting circular areas representing the

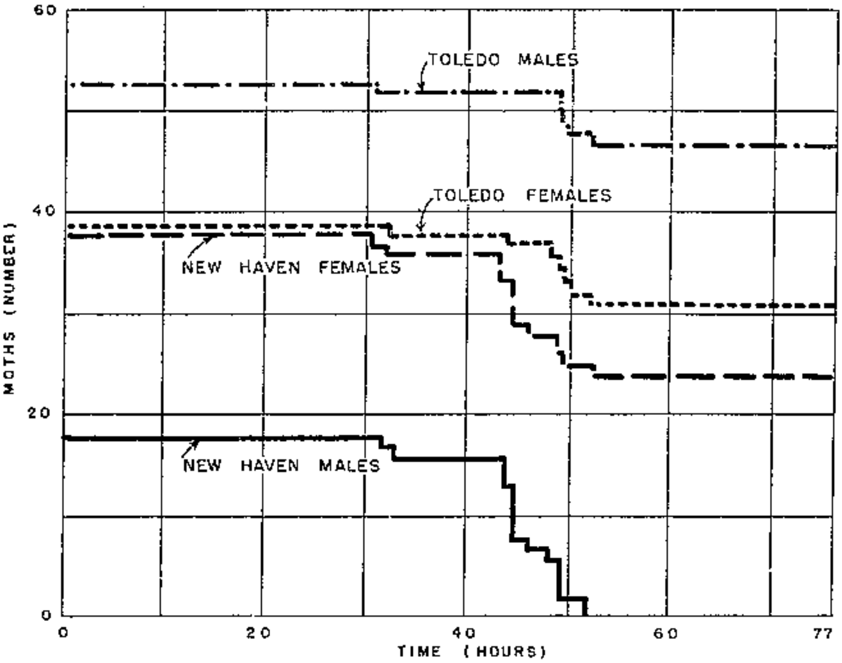

FIGURE 1.—Occurrence of mating of moths of both sexes of the European corn borer from Toledo, Ohio, and New Haven, Conn.

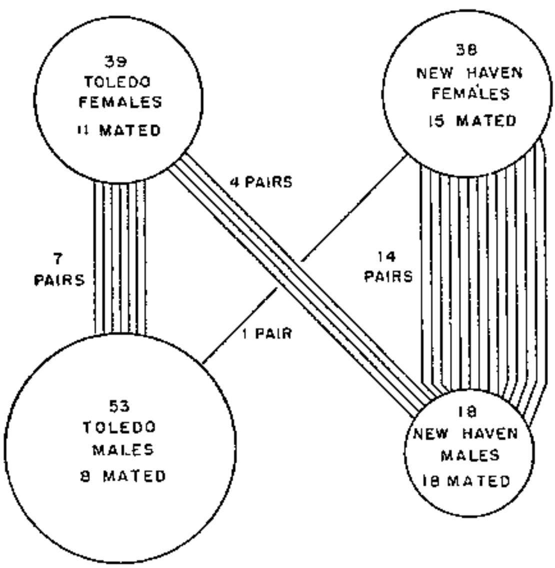

FIGURE 2.—The number of moths of the European corn borer of each sex, from Toledo, Ohio, and New Haven, Conn., confined in one cage and the number of pairs that mated.

moths available in each category. Each line connecting two circles represents one mating pair of moths.

In this test the female moths that mated were isolated, and the number and fertility of the eggs laid by each were observed. Of 14 New Haven females mated with New Haven males, 13 produced eggs some of which were fertile in every case. Five of the 7 Toledo females mated with Toledo males produced fertile eggs, but 2 females in this category died without ovipositing. All pairs that were cross matings produced fertile eggs. Following the period during which the moths were observed for mating in the test, all unmated females were isolated in oviposition cages for observation. A few of these produced infertile eggs, but none deposited fertile ones. This substantiates the belief of the observers that no unobserved mating occurred.

The data indicate a preference of moths for mating with others from the same strain over those from different strains. There appeared to be some hindrance of a physical nature to the mating of New Haven females and Toledo males since, in three tests, normal prenuptial activity did not lead to mating, except in the case of one pair, whereas mating in the other three combinations did occur. This is indicative of the existence of physical differences, between stocks of the species, that have not yet been determined. In an extensive discussion of isolating mechanisms between strains Dobzhansky (*9, p. 268*) states: "The experimental evidence in favor of mechanical isolation is scanty and is confined mostly to a single order, namely, *Lepidoptera.* * * *" Physically, these stocks of *Pyrausta nubilalis* exhibit intraracial isolation very similar to that between strains of *Drosophila pseudoobscura* and *D. miranda* found by Dobzhansky in some of his experiments.

The differences in time of pupation under natural field conditions of overwintered larvae of the single and multiple-generation strains, reported by Vance (*19*), suggest that there may be a partial temporal isolation of the two phases of the insect coexistent in the Toledo area. Thompson and Parker (*18*) were unable to show that occurrence on different host plants was responsible for inherent differences between strains of the corn borer.

Egg Production and Fertility

It has been shown that the Toledo stock of the corn borer was a mixture of strains and that the stock from New Haven was homozygous for the nondiapause character. Although the composition of the Toledo stock precluded analysis of the inherent behavior of strains, some information was obtained from crosses made in 1937 between groups of moths from the two stocks.

The eggs produced by groups of moths, five females and five males in each cage, were used in this experiment. These moths were from the two stocks, Toledo and New Haven. Matings were accomplished within each stock and reciprocal crosses were also made between these stocks. Moths of the multiple-strain phenotype, discussed later, were reared from each and inbred within each of the four combinations.

Egg production was calculated per moth-day by dividing the total number of eggs laid by the total days of life of all moths in the group. This was used as the basis for comparing moth productivity. These data, presented in table 4, with the data on egg fertility, show a uniformity of production for the two generations of the New Haven strain.

TABLE 4.—*Egg production of European corn borer moths reared from field-collected larvae and the first generation reared from their multiple-generation phenotypes; from New Haven, Conn., and Toledo, Ohio, field stocks, and their reciprocal crosses*

Crosses of moths		Stock or generation	Moth-days	Egg production		Fertility of eggs
Female	Male			Total	Per moth-day	
			Number	*Number*	*Number*	*Percent*
New Haven	New Haven	Field stock	36	2,322	64.5	85.8
Do	do	First generation	32	2,211	69.1	80.6
Toledo	Toldeo	Field stock	38	2,737	72.0	97.0
Do	do	First generation	78	2,981	38.2	71.0
Do	New Haven	Field stock	39	1,843	47.3	81.2
Do	do	First generation	55	4,329	78.7	92.1
New Haven	Toledo	Field stock	65	2,541	40.2	23.8
Do	do	First generation	28	1,646	58.8	96.9

The cross between New Haven females and Toledo males from field stock showed the lowest fertility (23.8 percent), which was probably due to nonmating of some of the moths as already discussed in the section on mating of moths, while fertility in the F_1 generation of this cross was 96.9 percent, comparable with that in the inbred field material from Toledo. When the F_1 moths from each cross were inbred, fertility was nearly as high as that of the original Toledo strain when inbred.

The uniformity of egg production and fertility of the New Haven strain is considered indicative of the homogeneity of this material found in studies of other morphological stages. Performance of the Toledo stock is thought to be evidence of its heterogeneity.

LARVAL DEVELOPMENT

To study differences between stocks of the European corn borer, it was found necessary to separate and define the characters of the strains to be utilized as an index to racial performance. To accomplish this objective, the segregation of three strains was undertaken.

The first of these was the multiple-generation strain from the vicinity of New Haven characterized by a complete lack of larval diapause under the experimental conditions. In the 3 years of study and through four successive generations in the laboratory in 1939, this strain produced only nondiapausing larvae.

The second was the single-generation strain from Toledo characterized by obligatory larval diapause before pupation. Test insects of this strain segregated in 1937 rearings did not hibernate successfully and only one moth was produced from this material. In 1938, however, material of this segregate was reared successfully by retaining mature larvae in cold storage for 6 months for the production of F_1 moths in 1939. Seven pairs of these moths were mated, and all progeny of 6 pairs entered a diapause. The progeny of the other pair comprised both diapausing and nondiapausing individuals.

The third desired strain was to be of pure multiple-generation type from Toledo stock. Through four successive generations reared in 1939 both diapausing and nondiapausing progeny were produced from multiple-generation phenotypes in each preceding generation.

The isolation of this strain was not accomplished, and the nondiapausing progeny from this material will be referred to in this paper as "multiple segregates."

This study showed that, under the experimental conditions, the multiple-generation strain reproduces continuously with no evidence of a larval diapause, and also that the single-generation strain is distinct from the multiple-generation strain and is characterized by a larval diapause which prohibits continuous development of successive generations without a larval resting period preceding pupation. Bradley and Arbuthnot (*6*), in discussing the effect of the physiology of the European corn borer on one of its parasites, *Chelonus annulipes* Wesm., show that this parasite is affected by the host physiology so that it conforms to the seasonal cycle of the host, producing one generation annually when reared on a host strain which enters a diapause and more than one when reared on a multiple-generation strain of the host.

During the rearings in 1937 and 1938 and of the first generation of the multiple-generation strain and multiple segregates in 1939 it was evident that there was a difference in the rate at which the strains were developing under the controlled experimental conditions. As a measure of this difference, the number of specimens in each morphological stage was recorded on each observation date.

The multiple-generation strain used in the study consisted of F_2 progeny from three and F_3 progeny from two pairs of moths of the New Haven stock, all of which pupated before 35 days of larval life, as shown in table 5.

Table 5.—*Number of progeny and percent pupation of the European corn borer in different generations of the single- and multiple-generation strains and in the multiple segregates*

Strain	Locality source of parents	Generation	Progeny surviving after—					Pupation within 35 days
			10 days	15 days	20 days	25 days	35 days	
			Number	*Number*	*Number*	*Number*	*Number*	*Percent*
Single generation	Rochester, N. Y.	F_1	58	36	19	19	14	0
	Mount Clemens, Mich.	F_2	54	47	27	24	20	0
	Toledo, Ohio	F_2	316	234	165	135	125	0
	Total		428	317	211	178	159	0
Multiple generation	New Haven, Conn.	F_2	298	238	180	161	156	100.0
	do	F_3	111	94	78	69	64	100.0
	Total		409	332	258	230	220	100.0
Multiple segregates.	Toledo, Ohio	F_2	1,061	695	442	384	341	29.4
	do	F_3	67	57	50	38	30	40.0
	do	F_4	274	192	138	127	110	20.0
	Total		1,402	944	630	549	481	30.0

The single-generation strain consisted of F_1 progeny from one pair of moths from Rochester, N. Y., and F_2 progeny from one pair of moths of Mount Clemens, Mich., stock and from five pairs of moths of Toledo stock. All these became full-fed fifth instars and entered a diapause, none pupating within 40 days after hatching from the egg.

The multiple segregates consisted of F_2 progeny from seven pairs and F_3 and F_4 progeny each from one pair of moths from Toledo.

These were the successively pupating parts of each generation, in each of which some larvae (35.7 to 92 percent) entered the diapause. The diapausing and nondiapausing larvae were indistinguishable except by observation of their pupation or nonpupation. Attempts

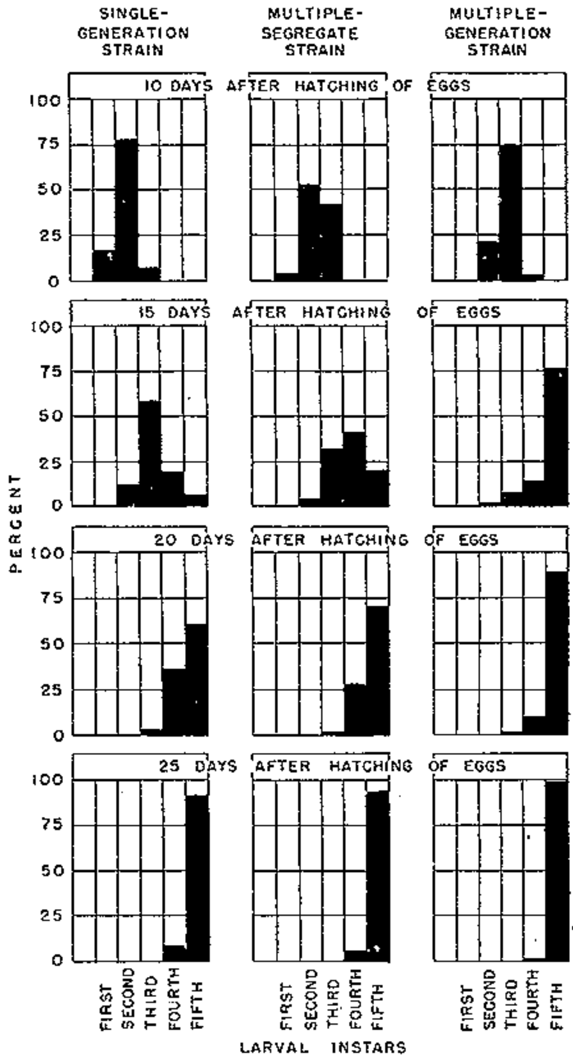

FIGURE 3.—Percent of progeny of the European corn borer in each larval instar in the single- and multiple-generation and multiple-segregate strains, 10, 15, 20, and 25 days after hatching from the egg.

to separate this population into proportionate parts of single- and multiple-generation strains, based on the observed pupation of each family [4] and on the differences in growth rates of the two strains, hereinafter discussed, were not successful.

In figure 3 are illustrated the percentages of the progeny in each of

[4] The descendants from one pair of moths inbred through successive generations are designated as a "family."

the three strains which were in each stage of development 10, 15, 20, and 25 days after hatching of the eggs. In table 6, data are presented to show the differences in the development of the three strains. These data show considerable difference in the attained developmental stages of the strains. The multiple-generation strain developed most rapidly and the single-generation strain most slowly, whereas the multiple segregates were intermediate in development between the other two.

TABLE 6.—*Differences in development of progeny of the single-generation, multiple-segregate, and multiple-generation strains of the European corn borer observed 10, 15, 20, and 25 days after hatching.*

Period after hatching (days)	Strain	Individuals	Larvae that had developed beyond given instar			
			First	Second	Third	Fourth
		Number	*Percent*	*Percent*	*Percent*	*Percent*
10	Single-generation	426	83.4	5.8	0	0
	Multiple-segregate	1,402	95.7	42.9	1.4	0
	Multiple-generation	409	100.0	77.3	2.0	0
15	Single-generation	317	97.8	86.4	26.8	6.0
	Multiple-segregate	914	100.0	95.7	62.9	21.2
	Multiple-generation	332	100.0	99.4	90.7	75.6
20	Single-generation	211	100.0	100.0	97.2	60.7
	Multiple-segregate	630	100.0	100.0	98.7	69.7
	Multiple-generation	258	100.0	100.0	99.2	88.0
25	Single-generation	178	100.0	100.0	100.0	91.5
	Multiple-segregate	549	100.0	100.0	100.0	94.2
	Multiple-generation	230	100.0	100.0	100.0	99.6

The multiple segregates at 10, 15, and 20 days also differed significantly from the single-generation strain, although less markedly; they were intermediate between single- and multiple-generation strains. They may have been a mixture of the other strains, or a strain, or strains, different from either of the others.

To determine the possibility of these three strains having been drawn from a homogeneous population, the data were subjected to the chi-square test. Since the computed chi-square values were far beyond the limit of the table ($P=0.01$), a highly significant departure from the requirements of a homogeneous population for the single- and multiple-generation strains was shown.

LARVAL SURVIVAL

Rearings from the eggs, discussed in the section on egg production and fertility, provided data on larval survival among the stocks and strain crosses. Data on the number of cages or replicates, number of larvae hatched, and number and percent that survived after 15 days are presented in table 7.

The F_1 generation larvae from inbred field material from the New Haven and Toledo stock had lower survivals than the reciprocal crosses between these two stocks. The higher survival from the crosses was probably due to heterosis.

Moths from nondispausing larvae from these four combinations were inbred. In the second, or F_2, generation of larvae reared, survivals were lower than in the F_1 generation and much lower in the crosses than in the inbred stock, possibly owing to the combination of recessive lethal characters from the two stocks.

TABLE 7.—*Survival of European corn borer larvae at the end of 15 days after hatching in rearings of 2 generations of field stocks from New Haven, Conn., and Toledo, Ohio, and their reciprocal crosses*

Parent stock	Source of parent moths		Replicates	Larvae in rearing test			
	Female	Male		Generation	Hatched	Survived	
			Number		*Number*	*Number*	*Percent*
Field material	New Haven	New Haven	4	F_1	404	263	65.1
F_1 generation [1]	do	do	8	F_2	778	400	51.4
Field material	Toledo	Toledo	4	F_1	410	205	50.0
F_1 generation [1]	do	do	4	F_2	389	172	44.2
Field material	do	New Haven	4	F_1	403	304	75.4
F_1 generation [1]	do	do	8	F_2	687	169	24.6
Field material	New Haven	Toledo	3	F_1	295	229	77.6
F_1 generation [1]	do	do	6	F_2	475	118	24.8

[1] Nondiapausing.

Each group of progeny was tested for homogeneity of survival between replicate cages by calculating the χ^2 of the individual cage survival from the group mean. The between-cage variation of the first generation of the New Haven and Toledo stocks was greater than would be expected in homozygous material, but in all other groups a uniformity between replicates within each group of progeny was indicated. The apparent absence of uniformity found between replicates in the first-generation larvae of the New Haven strain indicates that characters affecting survival are possibly not so homogeneous in this field material as those for the nondiapause characters. Lack of uniformity in the Toledo material is not unexpected, since this material is heterozygous. The uniformity of performance of the crosses is indicative of heterosis.

PUPATION

Among the progeny that pupated without a larval diapause, discussed in the section on larval development, there was a difference in the rate of pupation between the multiple-generation strain and the multiple segregates. The former began pupating at 16 days of larval life, and all progeny had either died or pupated by 31 days. There were 220 pupae in the F_2 and F_3 generations. Of the 481 multiple-segregate individuals surviving 35 days (table 5), 173, or 36 percent, pupated. The first pupa was found on the seventeenth day and the last was formed by the thirty-fourth day after hatching. Those larvae that survived longer than 34 days behaved like the single-generation strain, i. e., they diapaused and did not pupate, although they remained in the same environment for at least 5 additional days.

Pupation progressed more rapidly in the multiple-generation strain than in the multiple segregates.

Table 8 contains the data on the number and percentage of larvae that pupated and shows the differences in the rate of pupation of these two lots. The rate of pupation of the multiple-generation strain was significantly more rapid then that of the multiple segregates. It may be that the heterozygous diapause characters, in the multiple segregates, discussed in the section on larval development, caused retarded development in this material. Further evidence on this point is presented in the discussion of pupation of crosses between the New Haven and Toledo stocks.

TABLE 8.—*Difference in the rate of pupation between the multiple segregates and the multiple-generation strain of the European corn borer*

Time after hatching (days)	Pupae		Accumulated pupation	
	Multiple-segregates	Multiple-generation strain	Multiple segregates	Multiple-generation strain
	Number	*Number*	*Percent*	*Percent*
Through 16	0	1	0.0	0.5
17 through 19	11	31	6.4	14.5
20 through 22	73	108	48.5	63.0
23 through 25	46	45	75.1	84.1
26 through 28	23	23	88.4	94.5
29 through 31	12	12	95.4	100.0
32 through 34	8	0	100.0	100.0
Mean days after hatching	23.6	22.2		

From the pupation of the larvae discussed in the section on larval survival, data on pupation were also obtained on the Toledo and New Haven stocks and their reciprocal crosses. The rate at which pupation occurred among the multiple-generation phenotypes is illustrated in figure 4. The two generations of the New Haven stock pupated at about the same rate, this uniformity confirming the evidence of homogeneity found in data on other morphological stages of material from this locality. In the first generation the rate of pupation was slower in the Toledo stock than in the New Haven stock. In the second generation, part of the Toledo group pupated at about the same rate as the first generation, but there was also a well defined second peak of lesser magnitude similar to that which had been observed in the original multiple segregates.

In the crosses between the two field stocks, the first generation pupated at about the same rate as the New Haven stock. The F_2 generation of the Toledo female and New Haven male cross pupated at a slightly slower rate than the New Haven stock, and the F_2 generation of the reciprocal cross had a well defined peak at the same time. A number of individuals developed very slowly, however, resembling the single-generation strain in rate of development, but when they became full fed the larvae did not enter a diapause. The last pupae were formed on the fiftieth day after they had hatched.

The New Haven strain and the Toledo female × New Haven male cross all pupated in both generations, the last pupa having been formed by the thirty-fifth day of larval life. Of the 168 first-generation progeny of the Toledo stock, 39, or 23.2 percent, pupated, and 96, or 77.4 percent, of 124 of the second generation pupated. Females and males occurred in about equal numbers in all three groups. The New Haven female × Toledo male cross behaved quite differently. There were 203 mature F_1 larvae, of which 131, or 64.5 percent, pupated. Of these 131 pupae, 17 were females and 114 were males, or a sex ratio of approximately 1 female to 7 males. All the second generation from the multiple-generation phenotypes pupated as noted in the discussion on rate of pupation. The characters which cause diapause thus seem to be present in the hybrid group and in the Toledo stock, as evidenced by the second peak of pupation. These data also indicate that the characters for diapause are associated with the sex chromosomes, which, probably, as in other Lepidoptera, are heterogametic in the female sex as pointed out by Cockayne (*8*), who says of Lepidoptera, ' The female sex is heterogametic, and has an X- and a Y-chromsosome. * * *''

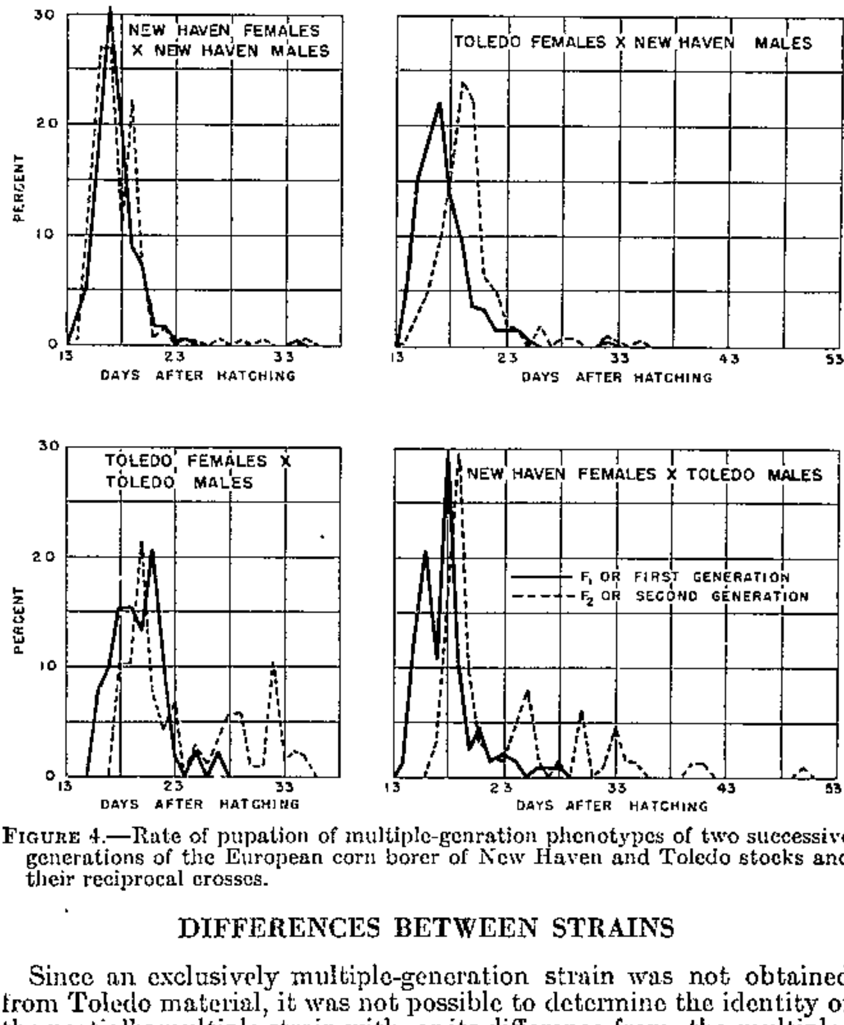

FIGURE 4.—Rate of pupation of multiple-genration phenotypes of two successive generations of the European corn borer of New Haven and Toledo stocks and their reciprocal crosses.

DIFFERENCES BETWEEN STRAINS

Since an exclusively multiple-generation strain was not obtained from Toledo material, it was not possible to determine the identity of the partially multiple strain with, or its difference from, the multiple-generation strain from New Haven. The differences, as summarized in table 9, in (1) the mating activity of the moths, (2) their egg production, (3) the rate of larval growth, (4) the number of days to pupation, and (5) the persistence of diapausing phenotypes in selections for the nondiapause characters, all may have been due to the presence and physiological effect of recessive characters for diapause in the persistently heterozygous Toledo stock.

TABLE 9.—*A summary of differences found between the European corn borer from New Haven, Conn., and Toledo, Ohio*

Compared material	New Haven	Toledo
Field stock	A homozygous multiple-generation (nondiapause) strain occurs in this region with no evidence of a single-generation (diapause) strain.	A heterozygous multiple- and single-generation strain complex prevails in this region. The single-generation (diapause) strain characters are possibly recessive to those of the multiple-generation strain. A single-generation strain was isolated from this stock, but a homozygous multiple-generation strain was not isolated. The phenotypes, selected for the nondiapause character, were heterozygous after four successive generations.
Strains	Larvae: The multiple-generation strain larvae from New Haven grew more rapidly than did those of the single-generation strain, isolated from the Toledo stock.	Larvae: The single-generation strain larvae isolated from Toledo stock grew more slowly than those of the multiple-generation strain from New Haven, and larvae of the multiple-segregates from the Toledo stock grew at a rate intermediate between those of the pure strains.
	Pupae: The multiple-generation strain larvae pupated in advance of those of the multiple-segregates from Toledo.	Pupae: The multiple-segregate larvae pupated at a slower rate than those of the multiple-generation strain. The single-generation strain larvae did not pupate until their diapause requirement had been satisfied.
Crosses between field stocks	Moths showed a preference for mating within rather than crossing between the 2 stocks. New Haven females seldom mated with Toledo males. Reciprocal crosses between the 2 stocks, New Haven and Toledo, gave the following results: 1. The Toledo female × New Haven male cross showed, in both the F_1 and the F_2 generation, development much like that of the paternal parent (nondiapause) but showed heterosis in the F_1 generation and a decline in vigor, evident as low egg fertility and high larval mortality, in the F_2 generation. Sexes occurred in a 1-to-1 ratio in each generation. 2. The F_1 generation of the reciprocal cross differed in that it had (a) low egg fertility, possibly due to failure of many to mate, (b) 22.6 percent diapause of the larvae, and (c) in the multiple-generation phenotypes, a ratio of 1 female to 7 males. 3. Also, in the latter cross, the F_2 generation declined in vigor, evident as low egg fertility and high larval mortality, and pupation occurred much more slowly although no progeny diapaused. This retardation of pupation was due, possibly, to the effect of the characters for diapause, when they are heterozygous.	

This instance of the existence of characters which produce intermediates in crosses of Lepidoptera is not unique. This subject has been discussed by Ford (*11*) with reference to other species. He found that on numerous occasions, crosses involving contrasting characters lead to the production of intermediates. It is evident from the present study that crossing between different strains of the European corn borer has occurred and probably is continuing to occur in the field in the Toledo, Ohio, area.

Plotnikov (*14*) has reported the results of crossing *Locusta migratoria* and *L. danica*. Eggs of the latter hatch in 16 days but those of the former hibernate. In a cross of *L. danica* male and *L. migratoria* female some of the eggs hibernated and some did not. When the same male was mated with a *danica*-like female of *migratoria*, all eggs hibernated. This author regards *L. danica* as a variety in the act of separating from the parent species. This may be what is happening in the Toledo region, i. e., a multiple-generation strain is separating from the single-generation strain. If this is the case, it would be unlikely that the resulting multiple-generation strain would be identical with the New Haven multiple-generation strain. On the other hand, the mixed strains in the Toledo region may be the result of crosses between a multiple-generation strain of the type isolated from New

Haven material and a single-generation strain of the type isolated from the Toledo material. Little information on the origin and relationship of the different strains was obtained, however, from the present study.

The multiple- and single-generation strains are distinguishable by different rates of larval development. There may be other differences which were not observed because of the fact that these experiments were conducted in a uniform environment of temperature, moisture, food, and light, and diapause may be expressed differently under other environmental conditions. The effect of environmental factors on diapause in *Loxostege sticticalis* L. have been reported by Pepper (*13*), who found that temperature gradually lowered to below freezing for several days broke the diapause, and by Steinberg and Kamensky (*17*), who found that temperature and food affected the incidence of diapause for the same insect. Sabrosky et al. (*16*) also found that light played an important role in breaking the diapause in *Acrydium arenosum augustum* Hancock. The physiology of diapause in grasshopper eggs was discussed by Bodine (*3*) and he showed the effect of temperature on rates of oxygen consumption by diapause eggs as well as by eggs of no diapause. In the European corn borer, difference in growth rate are indicative of different rates of metabolism.

LITERATURE CITED

(1) Babcock, K. W.
1927. THE EUROPEAN CORN BORER PYRAUSTA NUBILALIS HUBN.: I. A DISCUSSION OF ITS DORMANT PERIOD. Ecology 8: 45–59.

(2) Baker, W. A., and Mathes, R.
1931. PYRAUSTA NUBILALIS HUBN. HANDLING OF SINGLE-GENERATION LARVAE TO SUPPLY PARASITE DATA. Jour. Econ. Ent. 24: 404–413, illus.

(3) Bodine, J. H.
1932. HIBERNATION AND DIAPAUSE IN CERTAIN ORTHOPTERA; II. RESPONSE TO TEMPERATURE DURING HIBERNATION AND DIAPAUSE. Physiol. Zool. 5: 538–548, illus.

(4) Bottger, G. T.
1940. PRELIMINARY STUDIES OF THE NUTRITIVE REQUIREMENTS OF THE EUROPEAN CORN BORER. Jour. Agr. Res. 60: 249–257.

(5) ——— and Kent, V. F.
1931. SEASONAL-HISTORY STUDIES ON THE EUROPEAN CORN BORER IN MICHIGAN. Jour. Econ. Ent. 24: 372–379, illus.

(6) Bradley, W. G., and Arbuthnot, K. D.
1938. THE RELATION OF HOST PHYSIOLOGY TO DEVELOPMENT OF THE BRACONID PARASITE, CHELONUS ANNULIPES WESMAEL. Ent. Soc. Amer. Ann. 31: 359–365, illus.

(7) Caffrey, D. J., and Worthley, L. H.
1927. A PROGRESS REPORT ON THE INVESTIGATIONS OF THE EUROPEAN CORN BORER. U. S. Dept. Agr. Bul. 1476, 155 pp., illus.

(8) Cockayne, E. A.
1938. THE GENETICS OF SEX IN LEPIDOPTERA. Cambridge Phil. Soc. Biol. Rev. 13: 107–132.

(9) Dobzhansky, T. G.
1941. GENETICS AND THE ORIGIN OF SPECIES. Ed. 2, rev., 446 pp., illus. New York.

(10) Ficht, G. A.
1936. THE EUROPEAN CORN BORER IN INDIANA. Ind. Agr. Expt. Sta. Bul. 406, 24 pp., illus.

(11) Ford, E. B.
1937. PROBLEMS OF HEREDITY IN THE LEPIDOPTERA. Cambridge Phil. Soc. Biol. Rev. 12: 461–503.

(12) MATHES, R.
1936. THREE FACTORS AFFECTING LABORATORY REARING OF EUROPEAN CORN BORER LARVAE. Jour. Econ. Ent. 29: 691–697, illus.

(13) PEPPER, J. H.
1937. BREAKING THE DIAPAUSE IN THE SUGAR-BEET WEBWORM, L. STICTICALIS L., BY MEANS OF CHEMICALS. Jour. Econ. Ent. 30: 380.

(14) PLOTNIKOV, [V.]
1915. REPORTS ON THE WORK OF THE TURKESTAN ENTOMOLOGICAL STATION IN 1912, 1913, 1914, AND PART OF 1915. 60 pp. Tashkent. (Abstract in Rev. Appl. Ent. 4: 209–211. 1916.)

(15) PREBBLE, M. L.
1941. THE DIAPAUSE AND RELATED PHENOMENA IN GILPINIA POLYTOMA (HARTIG). V. DIAPAUSE IN RELATION TO EPIDEMIOLOGY. Canad. Jour. Res. Sec. D. 19: 437–454.

(16) SABROSKY, C. W., LARSON, I., and NABOURS, R. K.
1933. EXPERIMENTS WITH LIGHT UPON REPRODUCTION, GROWTH AND DIAPAUSE IN GROUSE LOCUSTS (ACRIDIDAE, TETRIGINAE). Kans. Acad. Sci. Trans. 36: 298–300.

(17) STEINBERG, D. M., and KAMENSKY, S. A.
1936. LES PRÉMISSES OECOLOGIQUES DE LA DIAPAUSE DE LOXOSTEGE STICTICALIS L. (LEPIDOPTERA, PYRALIDAE). Bul. Biol. de la France et Belg. 70: 145–183, illus.

(18) THOMPSON, W. R., and PARKER, H. L.
1928. HOST SELECTION IN PYRAUSTA NUBILALIS HUBN. Bul. Ent. Res. 18: 359–364.

(19) VANCE, A. M.
1939. OCCURRENCE AND RESPONSES OF A PARTIAL SECOND GENERATION OF THE EUROPEAN CORN BORER IN THE LAKE STATES. Jour. Econ. Ent. 32: 83–90, illus.

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