EFFECTS ...

BIOLOG...

REPRODUCTI...

SURVIVAL AND BEHAV...

CEREAL LEAF BEETLE,

Oulema Melanopus (Linnaeus),

UNDER LABORATORY CONDITIONS

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

YOUNG MOK YUN

1967

YOUNG MOK YUN

Ph. D.

EFFECTS OF SOME PHYSICAL AND BIOLOGICAL FACTORS ON THE REPRODUCTION, DEVELOPMENT, SURVIVAL, AND BEHAVIOR OF THE CEREAL LEAF BEETLE, Oulema Melanopus (Linnaeus), UNDER LABORATORY CONDITIONS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
YOUNG MOK YUN
1967

This is to certify that the

thesis entitled

EFFECTS OF SOME PHYSICAL AND BIOLOGICAL FACTORS ON THE REPRODUCTION, DEVELOPMENT, SURVIVAL, AND BEHAVIOR OF THE CEREAL LEAF BEETLE, Oulema melanopus (Linnaeus), UNDER LABORATORY CONDITIONS.

presented by

Young Mok Yun

has been accepted towards fulfillment
of the requirements for

PhD degree in Entomology

Richard V. Connin

Prof. Richard V. Connin
Major professor

Date November 16, 1967

O-169

ABSTRACT

EFFECTS OF SOME PHYSICAL AND BIOLOGICAL FACTORS ON THE REPRODUCTION, DEVELOPMENT, SURVIVAL, AND BEHAVIOR OF THE CEREAL LEAF BEETLE, Oulema melanopus (Linnaeus), UNDER LABORATORY CONDITIONS

by Young Mok Yun

From June 1964 to December 1966, a series of laboratory studies were conducted to investigate the effects of various physical and biological factors on the reproduction, development, survival, and behavior of the cereal leaf beetle, Oulema melanopus (Linnaeus).

Diapause development in the summer adults seems to be completed after 90 days of storage at 38° F. in the laboratory. The free flight by the overwintered adults occurred at 68° F. and a minimum of 55° F. seems to be required for an effective initial mating and egg deposition. More consistent egg laying was observed when the temperature reached 70° F. or more.

After 110 days of storage at 38° F., one laboratory-reared female laid 1,251 eggs over a period of 153 days in a lamp chimney cage. A field-collected active spring female laid 256 eggs during a 28-day period under the same laboratory conditions. An isolated virgin female laid 129 eggs but none of them hatched.

Optimum environmental conditions for the active spring adults seem to be 80° F., 95 to 100 percent relative humidity, and an 18-hour light period. Over 93 percent of the eggs were deposited during a

16-hour light period and only 7 percent during an 8-hour dark period under laboratory conditions.

The cereal leaf beetle was able to complete its development within a temperature range of 58° F. to 90° F. A more complete range of temperature for the development is estimated to be between 54° F. and 92.5° F. Thresholds of development were calculated as 52° F. for the egg, 46.6° F. for the larva, 54° F. for the pupa, and 51.6° F. for the entire immature stage. A complete development from an egg to adult requires an average of 92 days at 58° F., 52 days at 67° F., 28 days at 80° F., and 23 days at 90° F. Davidson's (1944) logistic and linear regression equations were used to express the relationship between temperature and the rate of development in the eggs, larvae, pupae, and entire immature stages.

The overwintering adults seemed to be the most resistant of all stages against the cold followed by eggs, pupae, larvae, active summer adults, and lastly by active spring adults. The inactive summer adults were the most resistant stage to the high temperatures of 110° F. and 120° F. followed by larvae, eggs, pupae and active summer adults, and by spring adults.

Treatments of the summer adults with various combinations of temperature and light period have failed to break diapause in this beetle. Some of the early eggs laid by the summer adults were sampled and raised to adults for three consecutive generations, but no apparent non-diapausing strain of the beetle was detected.

Age-specific life and fertility tables were constructed for the laboratory and field populations of the cereal leaf beetle. Out of

1,000 eggs, 202 adults that are comparable to the spring adults survived after 90 days of storage at 38° F. Based on the 90-day storage period at 38° F., the net reproduction rate for the laboratory population was calculated as 3.94. The net reproduction rate of the field population was estimated at 0.94 when the field mortality data of Dr. R. F. Ruppel were applied.

EFFECTS OF SOME PHYSICAL AND BIOLOGICAL FACTORS ON THE REPRODUCTION, DEVELOPMENT, SURVIVAL, AND BEHAVIOR OF THE CEREAL LEAF BEETLE, *Oulema melanopus* (Linnaeus), UNDER LABORATORY CONDITIONS

By

Young Mok Yun

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Entomology

1967

ACKNOWLEDGEMENT

I am grateful to Dr. Gordon E. Guyer, Chairman of the Department of Entomology, for his constant encouragement and support throughout the entire period of this study.

My sincere appreciation is extended to Dr. Robert F. Ruppel under whose guidance this study was initiated and to Professor Richard V. Connin under whose constant supervision this investigation was successfully undertaken and concluded.

Special thanks are expressed to Drs. James W. Butcher and Dean L. Haynes of Entomology Department and Dr. Fred C. Elliott of Crop Science Department for their valuable suggestions and criticism.

I am also indebted to Messrs. David L. Cobb and George Lawson of Entomology Research Division, U.S.D.A., and to Messrs. John C. Arnsman and Melvin S. Gomulinski of Michigan State University for their aid in accomplishing this study.

My appreciation is further extended to Dr. Reynold G. Dahms of Entomology Research Division, U.S.D.A., for providing necessary funds and facilities for conducting this investigation, and also to Mr. Maurice E. Turner of Plant Pest Control Div., U.S.D.A. and Messrs. Dean F. Lovitt, John C. Dreves, and Howard Martin of Michigan Department of Agriculture for furnishing valuable information regarding infestation and distribution of the beetle.

Finally my thanks are due to Mrs. Arthur L. Wells for typing this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The cereal leaf beetle, Oulema melanopus (Linnaeus), is one of the oldest pests of small grains in the Old World. In the United States, the pest was first reported in 1962 from Berrien County, Michigan. Numerous individual and cooperative research projects have been set up and carried out by investigators in Michigan, Indiana, Ohio, and U. S. Department of Agriculture. Major areas of studies were on biology, ecology, host plant resistance, and chemical and biological control of the pest. During the five-year period from 1962 to 1967 these investigators have produced a large amount of information regarding various aspects of the beetle.

In spite of the large-scale chemical control effort, the population has increased and continued to spread. The pest was first found in Ohio in 1963 (USDA, 1963), in Illinois in 1965 (USDA, 1965), and in Pennsylvania in 1967 (USDA, 1967). In 1965 the pest was also found in Ontario, Canada (personal communication with Mr. Maurice E. Turner of Plant Pest Control Division, USDA).

European literature on the cereal leaf beetle is rather limited and observations and results are often incomplete and inconsistent. Apparently no systematic studies on this pest have ever been carried out in Europe. Most European papers are simple records of field and laboratory observations. The natural history of the cereal leaf beetle has been described and a brief outline of the life cycle of the pest is given in some European literature.

Systematic studies on the natural history of the cereal leaf beetle in the United States were initiated by Guyer (1962), Ruppel (1964), Castro (1964), and Castro, et al. (1965). The information obtained from these studies will be extremely useful in later studies on population dynamics, host plant resistance, and optimistically the eventual control of the pest. Their research, however, consisted largely of field studies and many specific questions regarding biology and ecology of the cereal leaf beetle are still left unanswered.

The author conducted a series of studies on the reproduction, growth, and survival of the cereal leaf beetle under various laboratory conditions during the two and a half year period from 1964 to 1966. Laboratory results were compared with field observations wherever it was feasible. It is hoped that the information obtained from this study will be useful in future studies on biology, ecology, and control of the pest.

DISTRIBUTION AND ECONOMIC IMPORTANCE

The cereal leaf beetle has been known as a pest of small grains in Europe since at least 1737 (Kadocsa, undated). According to the available literature from Europe, the beetle is distributed throughout the humid and subhumid regions of the western Paleoarctic zone. This area roughly covers the whole continent of Europe, parts of northern Africa, Iran, Turkey, and central Siberia eastward (Hodson, 1929; Balachowsky and Mesnil, 1935; Urquijo, 1940; Venturi, 1942; Sengupta and Behura, 1957; USDA, 1958; and Balachowsky, 1963).

The pest is generally considered as scarce and sporadic throughout most of the range. However, more frequent and severe damage to small grains has been recorded in the region of the Balkans, the Ukraine and the Transcaucasia area of the Soviet Union. This region has a continental climate and the abundance of the beetle is apparently favored by an early warm spring followed by a relatively dry summer (Kadocsa, 1916; Balachowsky and Mesnil, 1935 and Urquijo, 1940).

The cereal leaf beetle was first identified in the United States in July of 1962 from specimens collected in Berrien County, Michigan. How and when the pest reached the southwestern corner of Michigan is still not known. A few farmers in Galien, Michigan reported that they sprayed their oat fields as early as 1959 to control the then unidentified pest. The infestation of the pest and damage to the small grains, especially spring-planted crops, became heavier and heavier during the subsequent years (unpublished survey report by Michigan Department of Agriculture, 1962).

A survey conducted in summer of 1962 indicated that Berrien and Cass Counties in Michigan and adjacent St. Joseph and LaPorte Counties in Indiana were infested with the cereal leaf beetle (USDA, 1962). The beetle was first found in Ohio in 1963 (USDA, 1963) and the infested area has continued to expand mainly to the north, east, and south following the directions of prevailing winds. The pest has moved westward into Illinois in 1965 (USDA, 1965). In the same year the beetle was discovered for the first time in Harrow and Essex Counties in Ontario, Canada (personal communication with Mr. Maurice E. Turner of Plant Pest Control Div., ARS, USDA, Lansing, Michigan). The pest has moved further eastward into Pennsylvania in 1967 (USDA, 1967). The number of counties infested with the beetle in five states is given in Table 1 and the general distribution of the pest in the United States from 1962 through 1967 is shown in Figure 1. The population density of the cereal leaf beetle is still highest around the original sites of infestation in 1962, namely southwestern Michigan and adjacent counties in northern Indiana.

The actual yield reductions caused by spring adults and larvae of the pest have been estimated at 12 to 28 percent in winter wheat and 15 to 35 percent in spring oats and barley. It is also estimated that in Michigan, a minimum annual loss of 1.93 million dollars from the beetle damage can be expected in the areas where the beetle population is high. An annual loss of as much as 11.85 million dollars can be expected if the pest becomes established in the entire state (from unpublished data by Dr. R. F. Ruppel in 1965).

TABLE 1.--Total number of counties infested with the cereal leaf beetle by state and year (from USDA 1966b and 1967)

	Total Number of Counties Infested					
State	1962	1963	1964	1965	1966	1967
Michigan	2	15	34	43	53	60
Indiana	2	25	32	38	54	65
Ohio	0	1	18	49	63	83
Illinois	0	0	0	3	3	6
Pennsylvania	0	0	0	0	0	4
Total	4	41	84	133	173	230

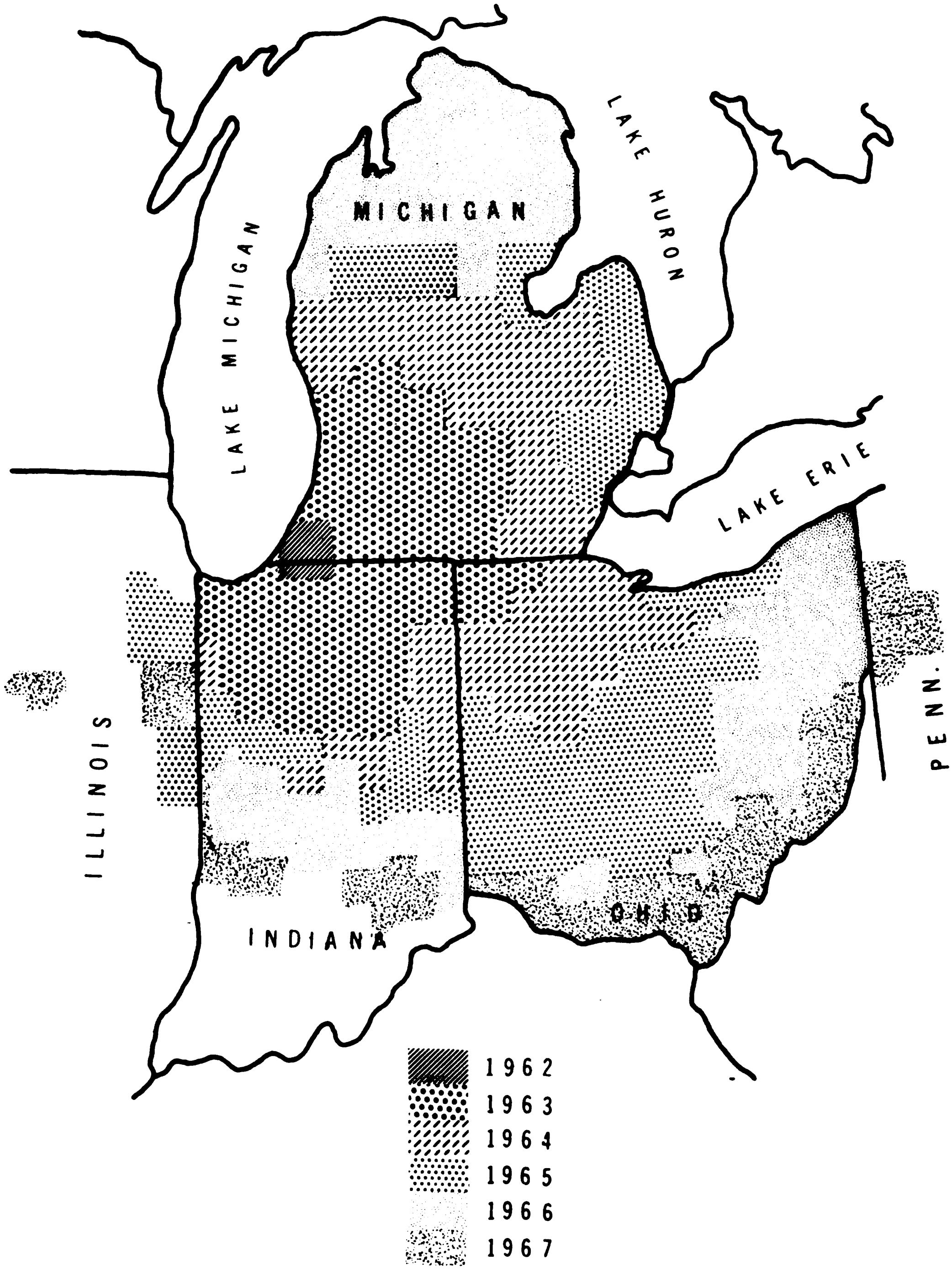

Fig. 1 – Spread of the cereal leaf beetle in the United States from 1962 through 1967.

LITERATURE REVIEW

The general life cycle of the cereal leaf beetle, *Oulema melanopus* (Linnaeus), has been described to various extents by workers in Russia (Megalov, 1925; and Vassiliev, 1913), Rumania (Manolache, 1932; and Knechtel and Manolache, 1936), Hungary (Kadocsa, 1916), Italy (Venturi, 1942), France (Mesnil, 1931; Balachowsky and Mesnil, 1935), Sweden (Borg, 1959), Spain (Urquijo, 1940), Great Britain (Hodson, 1929), and more recently in the United States (Guyer, 1962; Castro, 1964; and Castro, *et al.*, 1965). Some of the results of these studies on the biology of the cereal leaf beetle are summarized in Table 2. Other articles that have been published in the United States and describe at least a part of the biology of the cereal leaf beetle include Favinger (1962), Castro and Guyer (1963), Ruppel (1964), Ruppel and Castro (1964), Shade and Wilson (1964), Wilson and Ruppel (1964), Wilson and Shade (1964 a and b; 1966), Janes and Ruppel (1965), Connin, *et al.* (1966a), and Connin, *et al.* (in press).

Overwintering

The available literature from Europe and the United States notes that the cereal leaf beetle overwinters as an adult in forest litters, grasses, tree bark, in any small crevice, or in a variety of tight places that are protected from excessive heat and cold (Hodson, 1929; Balachowsky and Mesnil, 1935; Knechtel and Manolache, 1936, and

TABLE 2.--Summarization of published life cycle statistics

Country	No. Eggs /Female Per Day	No. Eggs /Female Per Season	Duration of Stages in Days				Investigator
			Egg	Larva	Pupa	Total	
Russia			13-14	12-13	14-15	39-42	Megalov, 1925 & 1927
Britain	1-3	50	11-13	13-16	17-26	43-50	Hodson, 1929
France			7-8	15	25		Balachowsky and Mesnil, 1935
Rumania	2-18	50-150	10-12 4(86°F.) 8(69.8°F.) 23(59°F.)	14-15 12(75.2°F.) 21(68°F.)	16-18 5(86°F.) 6(78.8°F.)	40-45	Knechtel and Manolache, 1936
Spain					25		Urquijo, 1940
Italy	3-12	100-150	15(Apr.) 7-8(May)	20(Apr.) 12-14(May)	20-25	40-62	Venturi, 1942
Sweden	5-6						Borg, 1959
Europe		100-150	7-15	12-20	20-25		USDA, 1958
Michigan		150~400(80°F.)	4~7(80°F.) 13~21(60°F.)	9-12(80°F.)	12-15(80°F.)		Castro _et al_. 1965

Castro, _et al_., 1965). An overwintering experiment by Castro (1964) shows that the lowest winter mortality was observed among experimental adult beetles placed at ground level.

Laboratory studies indicated that the diapausing adults could be held at 43° F. for a considerable period of time. The mortality of the adults at 43° F. ranged from 1 to 5 percent after 70 days, 30 percent after 90 days, 39 percent after 120 days, 50 percent after 160 days, and 65 percent at the end of 190 days. The beetle survived much better when they were provided with water (Castro, _et al_., 1965).

Further laboratory studies by Castro (1964) showed that the diapausing adults, after a 20-day holding period at 43° F., suffered 3 percent mortality after 10 days and from 9 to 10 percent mortality after 25 days at 25° F. At 15° F., after 20 days of storage period at 43° F., there was 17 percent mortality after 10 days, 32 percent after 15 days, and 100 percent mortality at the end of 20 days. At 0° F., there was 22 percent kill of the adults after 3 days, 91 percent after 7 days, and 100 percent mortality after 10 days at 0° F.

Spring Adults

The overwintering adults become active in April or May when the mean daily temperature reaches 50° F. (Knechtel and Manolache, 1936). In Michigan, the adults emerge from their hibernation sites during the first warm days of late winter or early spring. The first active adults were found as early as the 4th of March in 1964 by Mr. N. Remington of the Michigan Department of Agriculture. The adults seem to be active when temperature reaches 55° F. or more (Castro, _et al_., 1965).

The flight of the adult beetles is said to occur freely at 62° F. by Hodson (1929) or at 62.6° F. by Balachowsky (1963). The greatest amount of flight was observed in the field in late April when temperatures were 72° F. and up to 88° F. at the plant level (Castro, et al., 1965).

Several workers have indicated that the cereal leaf beetle shows marked preferences for host plants. Gallun and Ruppel (1963) found more eggs on oats and barley than on wheat during their host resistance studies near Galien, Michigan. Wilson and Shade (1964a) reported that oats were preferred to barley or wheat for oviposition. Castro, et al. (1965) mentioned that oats and barley were more severely damaged by all stages of the beetle than were wheat and grasses. Most reports from Europe state that succulent spring grains are more attractive to the spring adults than winter crops.

The overwintered adults mate in 4 to 5 days and the first eggs are laid about a week after the mating (Hodson, 1929; and Knechtel and Manolache, 1936). The mating takes place without any special courtship behavior and it may last for several hours. Multiple mating seems to be the rule in this species (Castro, et al., 1965; and Merino M., 1966). Merino M. (1966) also stated that definite homosexual behavior exists in the males of this species.

Oviposition

The eggs of the cereal leaf beetle are usually laid one or two at a time on the upper surface of the leaves of host plants (Balachowsky and Mesnil, 1935; and Castro, 1964). Castro (1964) reported that 98.5 percent of the eggs were found on the upper surface, 59.8 percent in the basal third region of the leaf, and 83.9 percent of the eggs occurred either singly or in pairs. Castro, _et al._ (1965) noted that the greater number of eggs were laid on plants 4 to 4-3/4 inches tall.

One female beetle in spring lays 1 to 3 eggs a day in England (Hodson, 1929), an average of 3 eggs and up to 12 eggs per day in Italy (Venturi, 1942), 5 to 6 eggs per day in Sweden (Borg, 1959), and 2 to 18 eggs per day in Rumania (Knechtel and Manolache, 1936). The oviposition activity continues for 45 to 60 days and during this period one female lays up to about 50 eggs in England (Hodson, 1929), 100 to 150 eggs in Italy (Venturi, 1942), 50 to 150 eggs in Rumania (Knechtel and Manolache, 1936) and an average of 96 eggs in Michigan (unpublished data by Dr. R. F. Ruppel of Michigan State University). In the laboratory, however, the total egg production was estimated to be 150 to 400 eggs per female (Castro, _et al._, 1965). Hodson (1929) stated that a single mating is sufficient for a female to lay fertilized eggs for several weeks. The eggs are observed in the field from April to as late as 19th of July in Europe. Some of the spring adults can survive for two years and may deposit some eggs during the second season (Megalov, 1927; and Hodson, 1929).

Egg

As Table 2 indicates, the incubation period for the egg ranges from 7 to 15 days in the field. Knechtel and Manolache (1936) reported that the threshold for development of the egg is 53.6° F. The eggs would fully develop in 4 days at 86° F., 8 days at 69.8° F., and 23 days at 59° F. Castro (1964) stated that the eggs hatched in 4 to 7 days at 75° to 80° F., 13 to 21 days at 60° F., and there was no egg hatching at a constant 50° F. with 16 hours of light and 60 percent relative humidity. Total egg hatching at 80±3° F. ranged from 62 to over 80 percent.

In the field, the egg mortality was about 50 percent in winter wheat and 10 percent in spring oats. The greater part of the egg mortality was attributed to low temperatures but a considerable portion of the kill was also due to a coccinellid predator, *Coleomegilla maculata lengi* Timberlake (Castro, *et al.*, 1965). The importance of this lady beetle as a predator of the cereal leaf beetle eggs was also mentioned by Yun (1964) and Yun and Ruppel (1964).

Larva

Young larvae upon eclosion start feeding on the upper surface of the larvae, leaving the lower cuticle largely intact. It was estimated in the laboratory that one larva consumed approximately 119.35 mg. of leaf tissue to complete its development while an adult consumed 25.9 mg. of leaf tissue per day and the total amount of 1,040 mg. during its 40-day active period (Castro, *et al.*, 1965).

As in the egg, the larval development also starts at 53.6° F. according to Knechtel and Manolache (1936).

Pupa

Threshold for the development of the pupa is given as 48.2° F. by Knechtel and Manolache (1936). It takes from 12 to 15 days for a pupa to complete its development at 80° F. and only 40 to 60 percent of the larvae that entered the soil for pupation emerged as adults (Castro, *et al.*, 1965). Castro (1964) believes that the high pupal mortality was largely due to an improper soil moisture content. The pupal mortality was even higher in the field. The newly emerged adults accounted for only 10 to 30 percent of the larvae that were present in the field (Castro, et al. 1965).

Summer Adult

Most workers in Europe and the United States seem to agree that new adults emerge from the soil in early to mid-summer and feed on young succulent grasses for about two weeks. During this period the summer adults are extremely active and some of them were caught at 1,000 feet in airplane traps (Wilson and Ruppel, 1964). Wilson and Ruppel (1964) and Shade and Wilson (1964) assumed that wind-borne dispersion could be of great significance in this species.

Mesnil (1931) stated that some new adults emerge in summer while others stay in the soil until the following spring. Vassiliev (1913) and Vereshtchagin (1914), on the other hand, reported that all of the new adults stay within the pupal cases in the soil until the following spring.

The total developmental periods from egg to adult are given as 39 to 42 days in Russia, 40 to 45 days in Rumania, 40 to 62 days in Italy, and 43 to 50 days in Britain (Table 2).

Although the males occasionally attempted to mate, no successful mating of the summer adults was observed (Castro, et al., 1965). Kumararaj (1964) and Hoopingarner, et al. (1965) reported that the gonads of the female do not normally develop until they have been subjected to a low temperature for a certain period of time.

Most European literature indicates the presence of only one generation per year in this species except Averin (1914) who claims the existence of the second generation in Russia. Hodson (1929) and Borg (1959) also mentioned the possibility of the second generation in Great Britain and Central Europe. A group of field-collected summer adults produced a second generation in the laboratory (Castro, et al., 1965) and oviposition by summer adults in field cages was noted in Indiana by Wilson and Toba (1963). In Michigan, however, the cereal leaf beetle seems to have a univoltine life cycle with an obligatory diapause in adult stage under field conditions (Castro, et al., 1965).

Diapause

Extrinsic and intrinsic factors that affect the onset and duration of diapause are: 1) photoperiod, 2) temperature, 3) moisture, 4) diet, and 5) maternal physiology (Wigglesworth, 1953; Andrewartha and Birch, 1954; Lees, 1955; de Wilde, 1962; and Harvey, 1962).

In the cereal leaf beetle, photoperiod studies in the laboratory showed that some newly emerged adults mated and oviposited under a 16-hour photoperiod in 15 days after their emergence from the soil. Similarly treated adults, however, failed to reproduce under a 12-hour light period. Exposure of immature stages to a 12-hour or 16-hour photoperiod did not affect the reproductive behavior in the adult stage. Diapausing beetles under a 12-hour light period started to lay eggs when they were transferred to a 16-hour photoperiod. The cereal leaf beetle seems to respond more readily to a long-day photoperiod of 16 hours than to a short-day 12-hour light period (Castro, 1964; Castro, et al., 1965).

The adults of Colorado potato beetle, Leptinotarsa decemlineata, go into diapause when they are exposed to a short photoperiod of 8 to

12 hours but they may be reactivated by a long photoperiod (de Wilde, et al., 1959). In certain bivoltine races of Bombyx mori, nearly all the females developed from eggs that had been exposed to a long photoperiod laid diapausing eggs; while the females developing from eggs that were exposed to short days laid only non-diapause eggs (Kogure, 1933). In some cases diapause has been shown to be quite independent from the photoperiod. Examples are the greenbottle fly, Lucilia sericata, and vegetable weevil, Listroderes obliquus (Dickson, 1949).

Attempts to break diapause by subjecting the diapausing adults to various cold temperatures for a short period of time failed to yield satisfactory results. The diapausing adults were induced to reproduce by subjecting them to 0° F., 15° F., 25° F., and 43° F. for 15 to 25 days but egg production was highly inconsistent. More consistent egg production was obtained when the adult beetles were placed in a lamp chimney cage without any hiding places. Castro (1964) doubted the effectiveness of such short-period cold treatments in breaking diapause. The diapause seemed to be completely broken in the beetles that were subjected to 43° F. for at least 100 days (Castro, et al., 1965).

In general, low temperatures favor the onset of diapause and arrest of growth while high temperatures tend to avert diapause (Lees, 1955). In long-day insects, high temperatures are effective in reactivating the diapausing insects (Way and Hopkins, 1950; and Beck and Hanec, 1960). In the corn earworm, Heliothis zeae, diapause is induced by low temperatures during larval stage (Ditman, et al., 1940).

One of the surest ways to bring the diapause-stage to an end is to expose the diapausing insects to adequate low temperature. With many species, diapause-development proceeds most rapidly at some temperature above 0^o C. (Andrewartha and Birch, 1954).

The effect of moisture on diapause is apparently complicated. Squire (1940) showed that diapause in the pink bollworm Platyedra gossypiella is induced by lack of moisture in the food. Andrewartha (1952), however, stated "the evidence indicates that the absorption of water during aestivation or hibernation is rarely if ever the primary stimulus which initiates diapause-development".

Both quantity and quality of food substances apparently influence the onset of diapause in some insects. Diapause in the cornstalk borer, Diatraea lineolata (Pyralidae) is sometimes induced by starvation (Kevan, 1944). The larvae of Euproctis phaeorrhoea (Lymantriidae) which normally go into diapause in the fall will complete development without diapause if fed with tender apple foliage (Grison, 1947).

The physiological constitution of the females of Bombyx mori affect the diapause pattern of the following generation (Kogure, 1933). The sensitive and responsive stage, therefore, may be far apart. In addition to above mentioned factors, wounding or artificial shock treatments such as electric shock, pricking with a needle, and dipping in or exposure to certain chemicals are also proved to be effective in reactivating some diapausing insects (Andrewartha and Birch, 1954).

Hibernation in univoltine species in a temperate climate is usually preceded by a long period of aestivation. Actual diapause may not necessarily be more intense than in multivoltine species which do not aestivate. The longer dormant period in univoltine species may be simply due to the fact that diapause-development does not take place during the hot summer months. The diapause-development is usually completed most rapidly during the autumn and winter (Zolotarev, 1947).

Diapause in insects is more recently recognized as an endocrine deficiency syndrome of the prothoracic glands (Williams, 1956) or of the corpora allata (de Wilde and Boer, 1961). In adult insects it is characterized by an inhibition in the maturation of eggs associated with corpus allatum failure (de Wilde and Boer, 1961). More recently Bowers and Blickenstaff (1966) reported that they found some inhibition of corpus allatum development in diapausing alfalfa weevil. By applying an active synthetic hormone exogenously, they were able to terminate diapause in the weevil. Using the same methods, Connin, et al. (in press) were also able to break diapause in the cereal leaf beetle.

Temperature-Development Relationship

Various mathematical formulae have been proposed and used by biologists to describe the relationship between temperature and speed of development in insects and other related animals. The relationship has been reviewed to various extents by Crozier (1926), Shelford (1929), Belehradek (1930), Uvarov (1931), Janisch (1932), Hoskins and Craig (1935), Huffaker (1944), Davidson (1944), Fry (1947), Wigglesworth (1953), Andrewartha and Birch (1954), and Messenger and Flitters (1958).

Davidson (1942 and 1944) proposed the use of the logistic equation to show the relationship between temperature and the time required for development in insects and other poikilothermic animals. The equation given below represents a form of the logistic curve originally developed by Pearl and Reed (1920).

$$\frac{1}{y} = \frac{K}{1 + e^{a - bx}}$$

1/y is the reciprocal of the time required for a given stage of an insect to develop at a given temperature x; K, a and b are constants. The calculated values for 1/y, when plotted against appropriate values for temperature (x) describe an S-shaped velocity curve, and the value for K represents the upper asymptote of the curve.

Davidson (1944) claims that this curve faithfully represents the trend of the speed of development of insects for 85 to 90 percent of the complete range of temperature at which development can go on. This formula apparently gives the most adequate empirical description of the temperature-development relationship that has so far been suggested (Wigglesworth, 1953; and Andrewartha and Birch, 1954).

Uvarov (1931) developed the following formula for calculating the theoretical threshold of development from experimental data.

$$K = \frac{dt - DT}{d - D}$$

K is the threshold of development, t and T are constant temperatures within the optimum range of development and d and D are the respective durations of the stage of insect at the constant temperatures t and T. Theoretically, the point where the velocity line or rate-of-development line crosses the temperature axis is also the threshold of development (Matteson and Decker, 1965).

Intraspecific Competition

Klomp (1964) listed the four major requisites in short supply for which the animals compete as 1) food, 2) oviposition sites, 3) space, and 4) cover.

Competition for food is regarded as the most common type of competition. It has been reported to occur among the larvae (Sang, 1949) and adults (Robertson and Sang, 1944; and Chiang and Hodson, 1950) of Drosophila. Competition for oviposition sites has been observed in Callosobruchus weevil where an increasing density of adults induces a decline in the rate of oviposition (Utida, 1941).

Competition for space has been observed in the larvae of Callosobruchus weevil where the larvae start to destroy each other when density reaches about five individuals per bean (Utida, 1942). Several authors indicated that competition for cover may occur when the number of covers or hiding places is limited during periods of unfavorable weather (Smith, 1935; Andrewartha and Birch, 1954; and deBach, 1958).

Pearl and Parker (1922) observed that the rate of reproduction in Drosophila melanogaster Meig. was highest at the lowest density and it gradually declined as the density increased. Using Chapman's (1928) data on the rate of increase in laboratory populations of Tribolium confusum Duv., Allee (1931) found that the rate of increase of population per female day was greatest at the second lowest density. The consequences of increasing intensity of competition among adult insects that are most frequently mentioned in the literature are 1) decreasing fecundity or oviposition rate, 2) increasing emigration rate, and 3) decreasing longevity.

The main causes of a decrease in the net reproduction rate with increasing adult density are due to 1) increased egg cannibalism in the adults of Tribolium sp. (Chapman, 1928; and Park, 1932 and 1933), 2) a decrease in the rate of copulation and a disturbance of the oviposition act (McLagan, 1932), and 3) an excessive contact between individuals, reduction in the amount of food available to individual animals, and conditioning of the medium by the accumulation of various metabolic waste products (Crombie, 1942).

Crombie (1944) and Andersen (1960) stated that in general an increasing competition among larval insects would result in 1) reduction of size or weight of larvae, pupae, and adults, 2) increase of the mortality rate among larvae and pupae, 3) increase or decrease in the rate of development of immature stages and of the longevity in adults, and 4) change of sex ratio.

Klomp (1964) concluded that competition is of minor significance in the regulation of density of phytophagous insects. It is almost only during heavy infestation of pests that this process is responsible for a decline in numbers.

Life Table

The use of life tables has become a common practice among population ecologists to describe and understand the population dynamics of a species. The methods for preparing such life tables are given by Deevey (1947), Morris and Miller (1954), and Southwood (1966), and further examples can be found in articles on spruce budworm populations by Morris (1963) and on winter moth by Embree (1965). Calculations for the net reproduction rate and the intrinsic rate of natural increase are described in detail by Birch (1948), Laughlin (1965), and Southwood (1966).

OBJECTIVES

As the title indicates the main objectives of this study were to investigate and obtain more complete information regarding the effects of various physical and biological factors on the reproduction, development, survival, and behavior of the cereal leaf beetle.

Included in this study are temperature-development relationship, influence of light, relative humidity, and soil factors, determination of thresholds and optimal physical environment, effect of these physical factors on induction and termination of diapause, effect of density and host plant factors, construction of life tables, and general behavior of the beetle under controlled environmental conditions.

It is hoped that the information obtained from this study would provide more complete knowledge of the species itself and it would also facilitate future quarantine and control programs.

EXPERIMENTAL PROCEDURES AND RESULTS

Influence of Physical Factors

For an effective presentation of the results, the physical factors presented in this chapter include "non-living" factors such as temperature, light, moisture, soil, and space. In this part of study, the author was mainly interested in the direct influence of various physical factors on the speed of development, fecundity, and longevity of the cereal leaf beetle.

Experiment 1. Rate of Development at Various Constant Temperatures and Thresholds for Development of Different Stages of the Cereal Leaf Beetle

Methods: In order to investigate the time-development relationship of the cereal leaf beetle at various constant temperatures and to determine the thresholds and optimal temperature range for the development of the beetle, the following procedures were employed. Fresh eggs less than 24-hour old were obtained from the cereal leaf beetle rearing program (Connin, *et al.*, 1966a). The numbers of eggs deposited on "Hudson" barley seedlings planted in plastic pots were counted and three of these pots were held in each of nine growth chambers adjusted to 48°, 58°, 67°, 76°, 80°, 85°, 90°, 95°, and 100° F. Relative humidity and light period in all growth chambers were equally set at 75 percent and 16 hours respectively. The eggs were checked daily for hatching and the numbers hatched and the number of days required for the first and last eggs to hatch were recorded.

For the larvae, a plastic pot containing about 60 four-inch tall "Hudson" barley seedlings was first caged with an ordinary lamp chimney by simply placing it on the top of the pot above the soil level. Thirty newly eclosed larvae were then transferred into each cage through an opening at the top which was later covered with a fine nylon cloth. Three pots of barley containing a total of 90 young larvae were placed in each growth chamber. The number of days required for the first and last larvae to go into pupation was recorded.

For the test of the pupal stage, thirty prepupae were placed in each lamp chimney cage with some fresh plants. Three cages were placed in each growth chamber and the days first and last prepupae entered the soil for pupation and again the days first and last new adults emerged from the soil were recorded. The number of the new adults emerged as well as that of the eggs hatched were counted to obtain percentage mortality.

The data were analyzed using Davidson's (1944) logistic formula and linear regression equation for time-development relationship. To estimate the thresholds for development of each stage, Uvarov's (1931) equation and straight velocity line method (Matteson and Decker, 1965) were used.

Results: A complete development from an egg to adult occurred at 58° F. and as high as up at 90° F. The overall results of this experiment are shown in Table 3.

At 48° F., neither eggs nor larvae completed their development. A complete kill of the eggs and larvae at 48° F. was observed after four and eleven weeks respectively. At 95° F., on the other hand, the eggs hatched but larvae did not complete their development. The thermal death point for the egg stage apparently lies between 95° and 100° F. and that for the larval stage between 90° and 95° F. A 100 percent mortality of the larvae was observed after eight weeks at 95° F. The rate of development is highest at 90° F. in this test. Mortality of all immature stages, however, is lowest at the intermediate temperatures of 76° F. and 80° F. and it gradually increases as the temperature deviates from this range.

TABLE 3.--Duration of development and mortality of the cereal leaf beetle at various constant temperatures

	Duration of Development in Days				% Mortality	
Temp. °F.	Egg	Larva	Pupa	Total	Egg	Larva and Pupa
48	No hatching	No complete development			100	100
58	18±4	27±5	47±5	92±14	74	64
67	11±2	16±2	25±3	52±7	59	34
76	7.5±1.5	13.5±1.5	18±2	39±5	28	40
80	5.5±1.5	9.5±1.5	12.5±1.5	27.5±4.5	21	47
85	5±1	8.5±0.5	10.5±0.5	24±2	38	67
90	4.5±1	8±1	10±1	22.5±2.5	40	59
95	4.5±1	No complete development			41	100
100	No hatching				100	

The data shown in Table 3 are generally in agreement with those reported by Knechtel and Manolache (1936) and Castro *et al*. (1965) in Table 2. Durations of pupal stage reported by the former, however, are significantly shorter than those in Table 3. The differences were apparently due to the difference in measurement of the duration of pupal stage. The author measured the duration of pupal stage as the period from the day prepupae enter the soil for pupation until the day they emerge as adults. Knechtel and Manolache (1936) apparently measured the true pupal period within the pupal cases.

Analyses of the Data: The reciprocal values of the days required for egg, larval, and pupal stages to develop at different constant temperatures denote the rate of development and form an S-shaped velocity curve when the values are properly plotted. Davidson's (1944) equation of

$$\frac{1}{y} = \frac{K}{1 + e^{a-bx}}$$

was used to show the relationship between temperature and the time required for development in the cereal leaf beetle. A brief explanation on this formula is already given in a section of literature review and the details on computation of K, a, and b values are given by Davidson (1944). When $\frac{1}{y}$ is multiplied by 100, the product $\frac{100}{y}$ represents average percent development of a given stage per unit time. The values of y and $\frac{100}{y}$ for each corresponding temperature are shown in Table 4.

The graphic representations of the equations derived from Davidson's formula (Davidson, 1944) for the development of egg, larval, pupal, and entire immature stages are shown in Figures 2, 3, 4, and 5 respectively. The equations used for drawing the curves, along with linear regression equations for the rate of development, are summarized in Table 5.

TABLE 4.--Number of days required for a complete development (y) and the speed of development in terms of percent development in one day (100/y) of egg, larval, pupal, and entire immature stages of the cereal leaf beetle at different constant temperatures

Temp. °F.	Egg		Larva		Pupa		All Stages	
x	y	$\frac{100}{y}$	y	$\frac{100}{y}$	y	$\frac{100}{y}$	y	$\frac{100}{y}$
58	18.0	5.556	27.0	3.704	47.0	2.128	92.0	1.087
67	11.0	9.091	16.0	6.250	25.0	4.000	52.0	1.923
76	7.5	13.333	13.5	7.407	18.0	5.556	39.0	2.564
80	5.5	18.182	9.5	10.526	12.5	8.000	27.5	3.636
85	5.0	20.000	8.5	11.765	10.5	9.524	24.0	4.167
90	4.5	22.222	8.0	12.500	10.0	10.000	22.5	4.444
95	4.5	22.222						
Varience		37.405		9.970		15.661		1.476

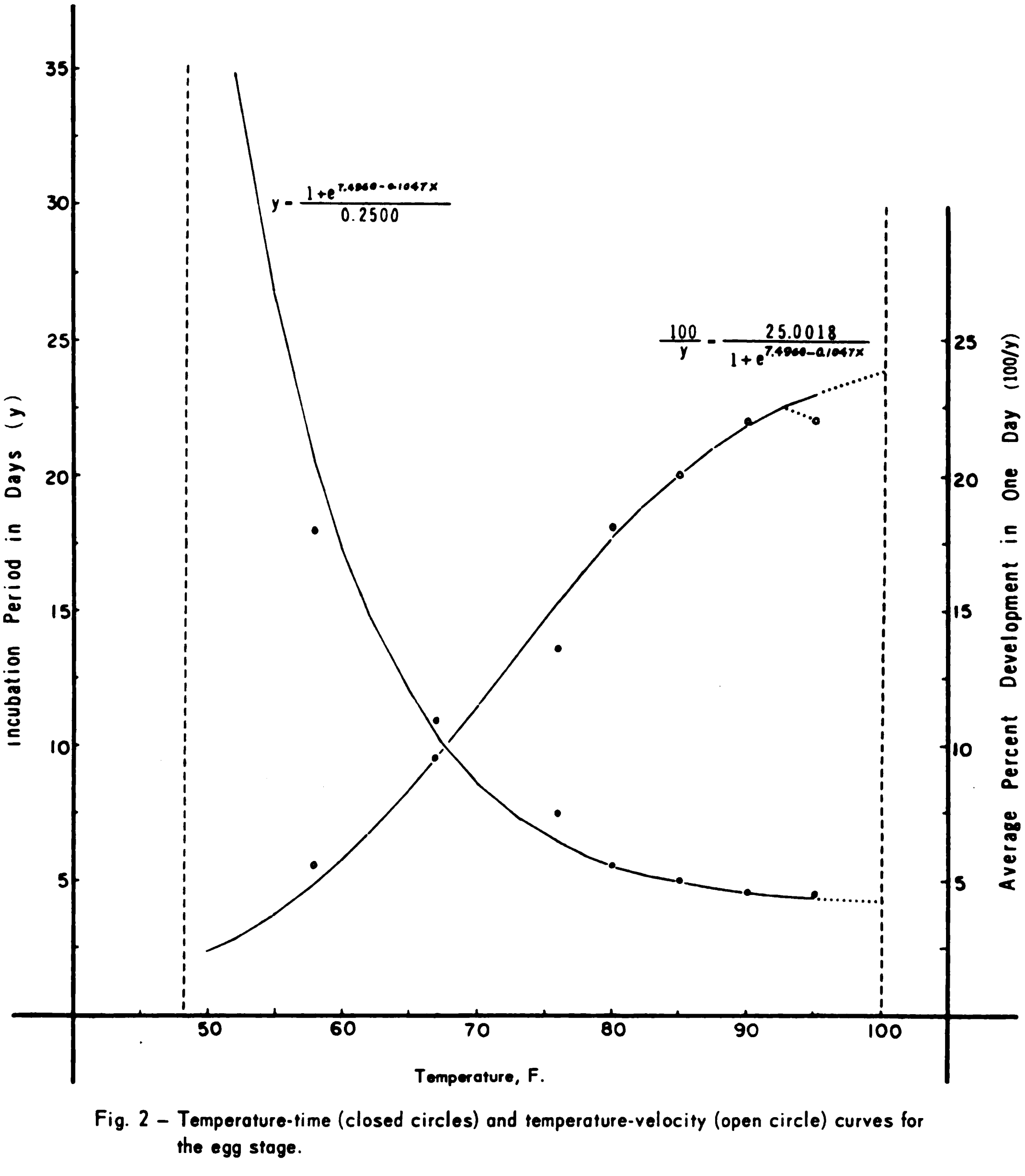

Fig. 2 – Temperature-time (closed circles) and temperature-velocity (open circle) curves for the egg stage.

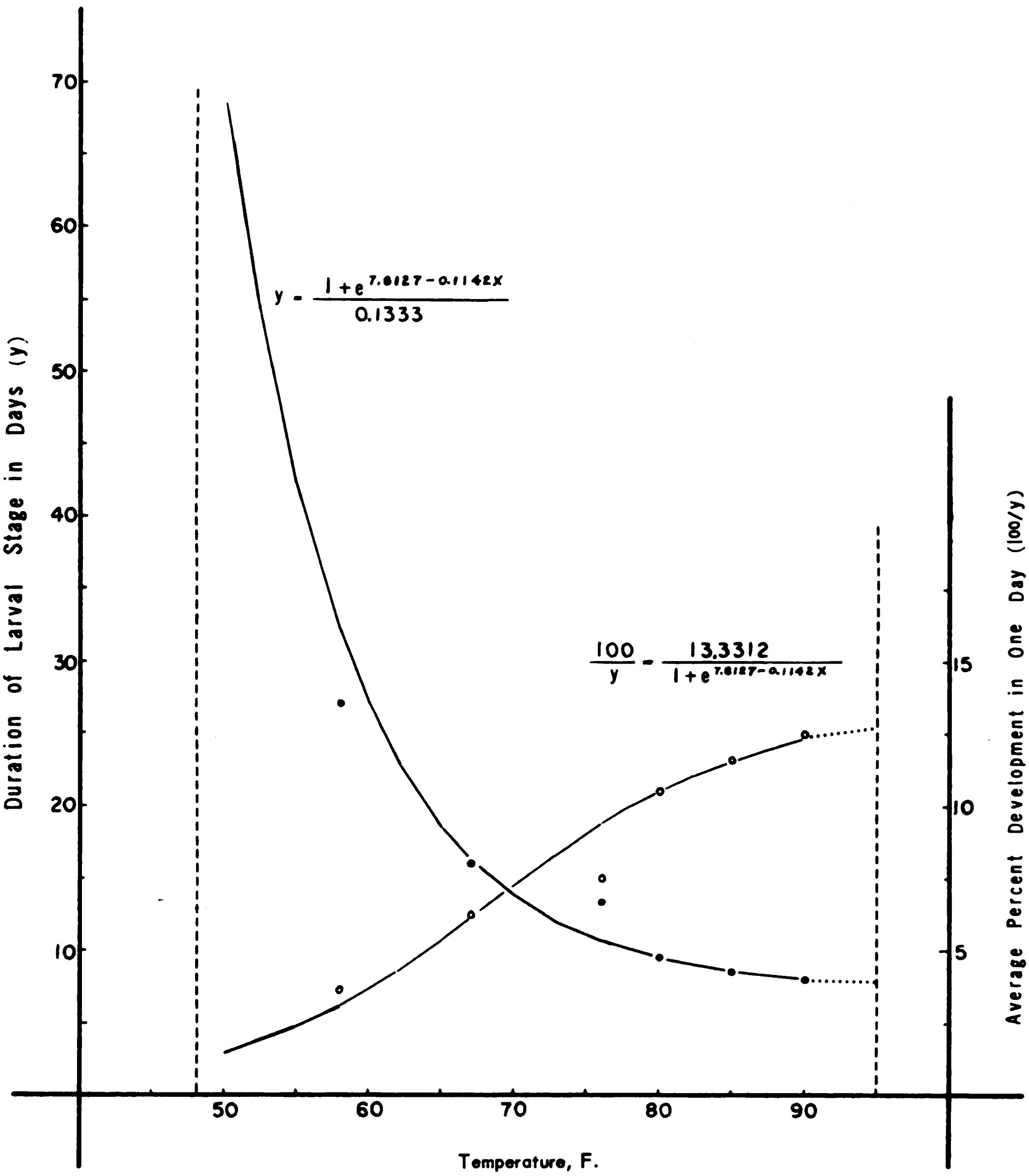

Fig. 3 – Temperature-time (closed circles) and temperature-velocity (open circles) curves for the larval stage.

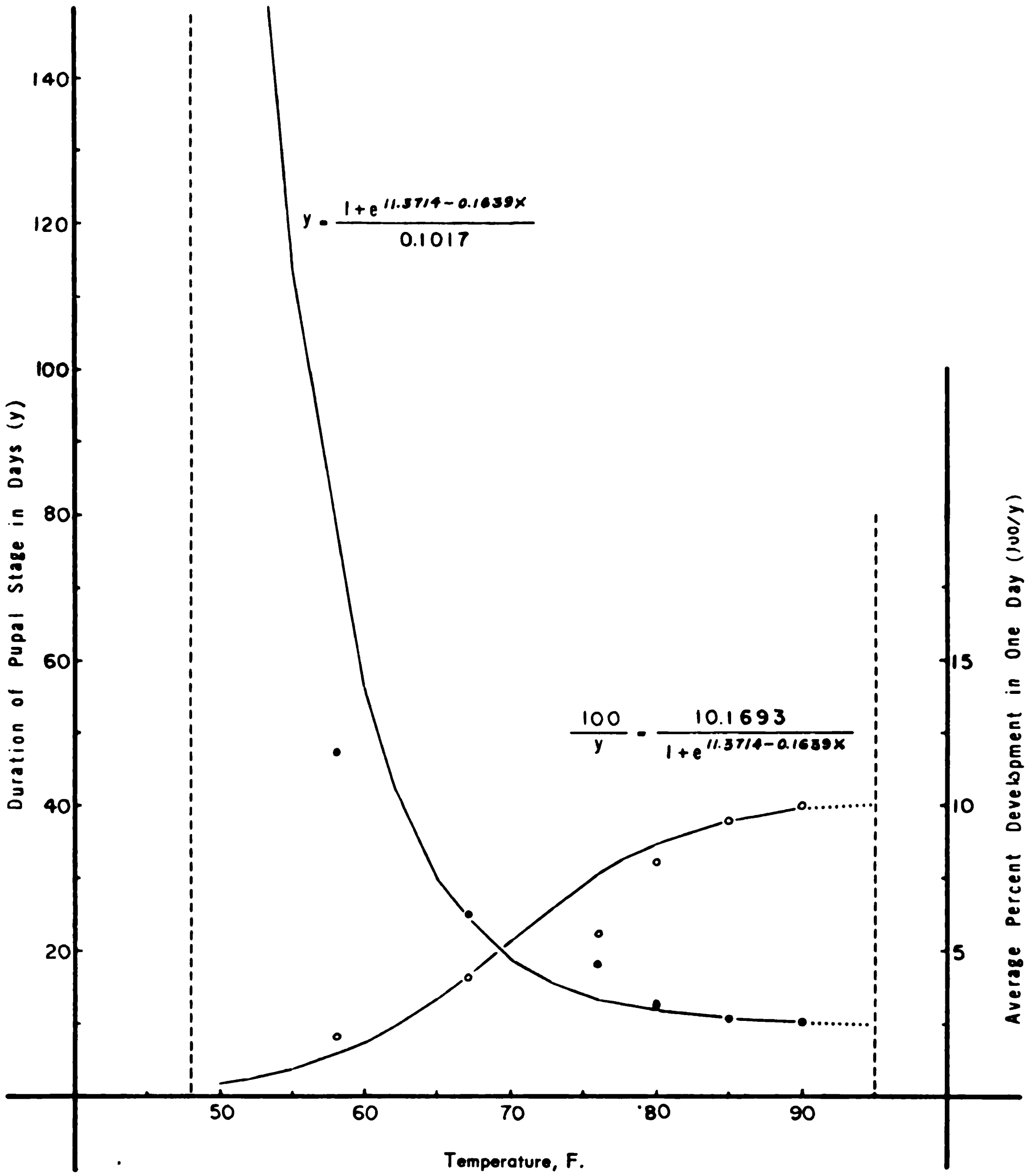

Fig. 4 – Temperature-time (closed circles) and temperature-velocity (open circles) curves for the pupal stage.

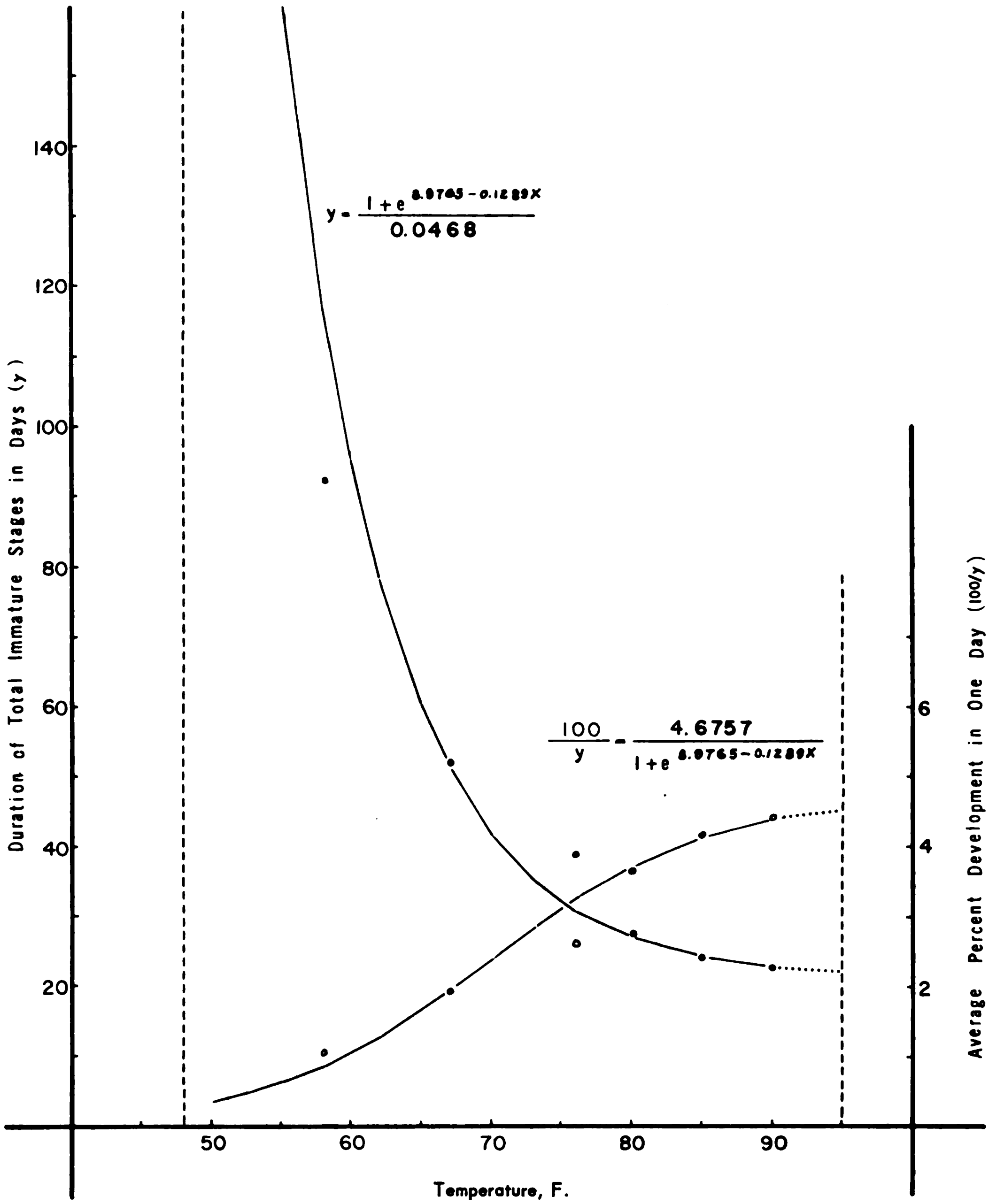

Fig. 5 – Temperature-time (closed circles) and temperature-velocity (open circles) curves for the entire immature stage.

TABLE 5.--Logistic curve and straight line equations for the development of different stages of the cereal leaf beetle

Stage	Logistic Curve		Straight Line	
	Duration	Rate	Regression Equation for the Rate	Correlation Coefficient
Egg	$y = \frac{1 + e^{7.4960 - 0.1047x}}{0.250018}$	$\frac{100}{y} = \frac{25.0018}{1 + e^{7.4960 - 0.1047x}}$	$\frac{100}{y} = 0.5473x - 26.864$	0.9890
Larva	$y = \frac{1 + e^{7.8127 - 0.1142x}}{0.133312}$	$\frac{100}{y} = \frac{13.3312}{1 + e^{7.8127 - 0.1142x}}$	$\frac{100}{y} = 0.2866x - 13.0896$	0.9792
Pupa	$y = \frac{1 + e^{11.3714 - 0.1639x}}{0.101693}$	$\frac{100}{y} = \frac{10.1693}{1 + e^{11.3714 - 0.1639x}}$	$\frac{100}{y} = 0.2628x = 13.4378$	0.9829
Total	$y = \frac{1 + e^{8.9765 - 0.1289x}}{0.046757}$	$\frac{100}{y} = \frac{4.6757}{1 + e^{8.9765 - 0.1289x}}$	$\frac{100}{y} = 0.1109x - 5.4584$	0.9844

In Figure 2, the observed points lie very closely along calculated curve lines except points at 58° and 76° F. Apparently the temperatures in these particular growth chambers were not adjusted properly. The actual temperatures could have been somewhat lower than 58° or 76° F. because the same trend is observed in Figures 3, 4, and 5 and in Table 6 which shows the deviation between observed and calculated $\frac{100}{y}$ values. The deviation is largest at 76° F. than at any other temperatures.

TABLE 6.--A comparison of observed and calculated $\frac{100}{y}$ values

	Egg Stage			Larval Stage		
Temp. °F.	Obs.	Calc.	Obs.-Calc.	Obs.	Calc.	Obs.-Calc.
58	5.556	4.867	+0.689	3.704	3.110	+0.594
67	9.091	9.557	-0.466	6.250	6.133	+0.117
76	13.333	15.327	-1.994	7.407	9.395	-1.988
80	18.182	17.672	+0.510	10.526	10.521	+0.005
85	20.000	20.056	-0.056	11.765	11.582	+0.183
90	22.222	21.833	+0.389	12.500	12.292	+0.208
	Pupal Stage			**Entire Immature Stage**		
Temp. °F.	Obs.	Calc.	Obs.-Calc.	Obs.	Calc.	Obs.-Calc.
58	2.128	1.358	+0.770	1.087	0.853	+0.234
67	4.000	4.106	-0.106	1.923	1.944	-0.021
76	5.556	7.601	-2.045	2.564	3.246	-0.682
80	8.000	8.651	-0.651	3.636	3.706	-0.069
85	9.524	9.440	+0.084	4.167	4.108	+0.059
90	10.000	9.835	+0.165	4.444	4.359	+0.086

At 95° F., the rate of development of the egg remains same as that observed at 90° F. although the curve values are constantly increasing. This would indicate that there is a break point between 90° F. and 95° F. Davidson (1942) described this break point as the peak temperature at which the embryo develops at the fastest rate. The peak temperature for the cereal leaf beetle egg is 92.5° F. As Figure 2 shows, the velocity curve is straight between 65° F. and 80° F. and this temperature range corresponds to an optimum range for the development of the cereal leaf beetle.

The rate of development in larval stage (Figure 3) is considerably lower than that in egg stage, still lower in pupal stage (Figure 4), and the lowest when entire immature stages are lumped together (Figure 5). Obviously this is because the incubation period is the shortest compared to any other stages. In general, the curves follow closely along the observed points in every stage except points at 58° and 76° F.

It was mentioned earlier that the rate-of-development or velocity curve is S-shaped. In medium range of temperatures, however, the curve is very straight. Assuming a linear relationship between temperature and the rate of development, a linear regression equation was computed for each stage (Figure 6 and Table 5).

As Figure 6 shows, all the observed points fall surprisingly close to the calculated equation lines. A further checking of the values revealed that an accurate estimation on the duration of development of a given stage could be made with these straight lines within a temperature range of 55° F. to 90° F. Below 55° F. and above 90° F., Davidson's curve lines gave more accurate estimations.

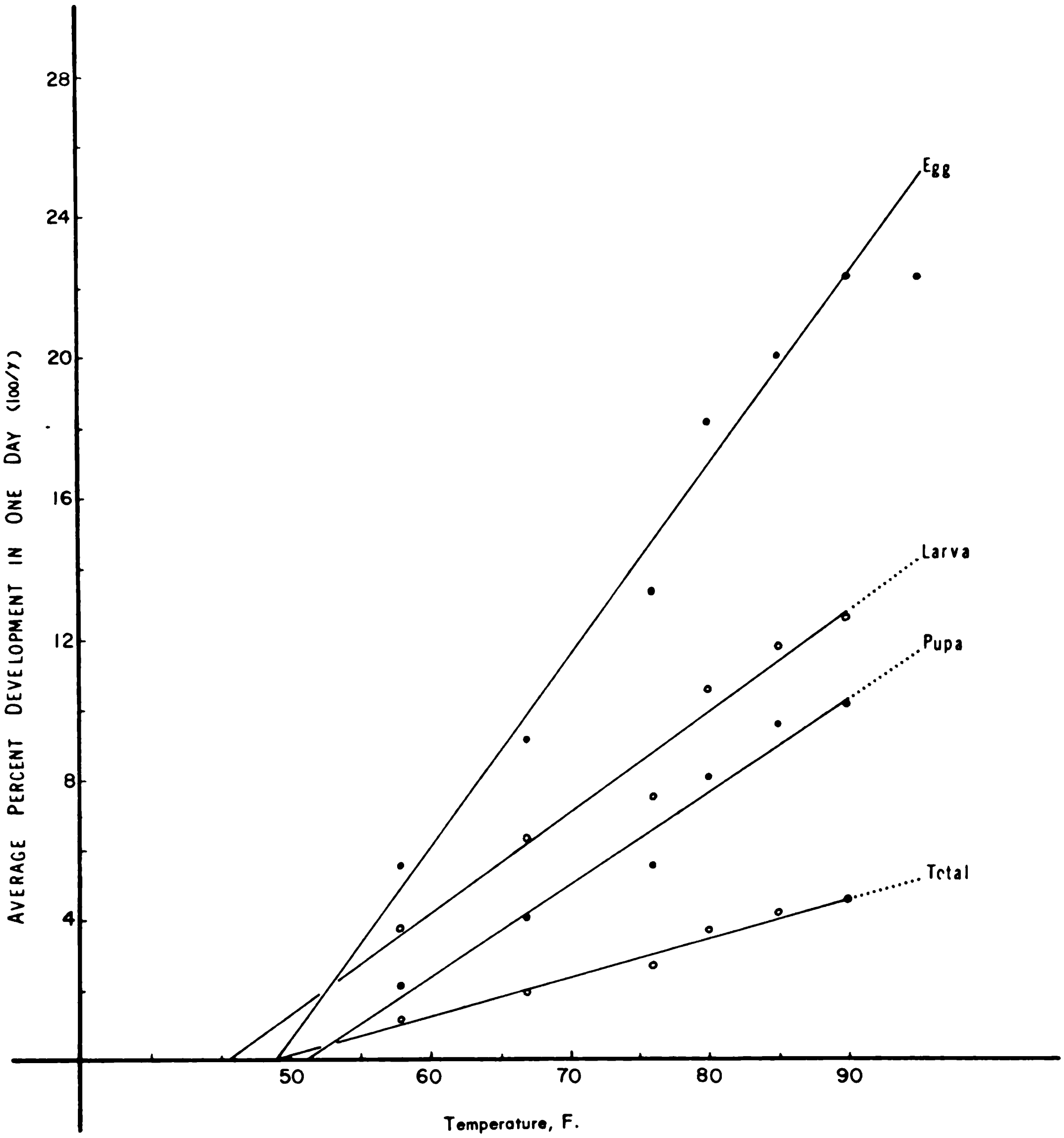

Fig. 6 – The rate of development of egg, larval, pupal, and entire immature stage at different constant temperatures.

A method for estimating threshold of development is to locate the point at which the velocity line crosses the temperature scale. The temperature at this crossing point is an estimation of the threshold (Matteson and Decker, 1965). Another method is to use Uvarov's (1931) formula, $K = \frac{dt - DT}{d - D}$. Using 67° F. for t and 85° F. for T the threshold of development for each stage was calculated. The thresholds of development of the cereal leaf beetle, using both methods, are given in Table 7.

TABLE 7.--Calculated thresholds for development of the cereal leaf beetle using two different methods

Stage	Uvarov's Equation	Velocity Line
Egg	52.0° F.	49.1° F.
Larva	46.6	45.7
Pupa	54.0	51.1
All stages	51.6	49.2

Table 7 indicates that the larval stage has the lowest threshold, the egg stage intermediate, and the pupal stage the highest threshold value for development. The threshold values obtained from Uvarov's equation, however, seems to be more closer to the actual values in the cereal leaf beetle. Knechtel and Manolache (1936) reported the thresholds for the development of the cereal leaf beetle as 53.6° F. for the egg and larva and 48.2° F. for the pupa. The threshold for the egg and larva seem to be somewhat higher while that for the pupa is considerably lower than the calculated thresholds obtained from this study.

Experiment 2. Egg Production and Development of Pupae at 80° F. and 85° F.

Methods: It was learned from previous experiment that the cereal leaf beetle is capable of developing from an egg to adult within a temperature range of 52° F. to 92.5° F. It was, however, desirable to determine an optimum temperature level at which the largest number of the beetle could be reproduced within a relatively short period of time. Two temperatures, 80° F. and 85° F., were selected to conduct this study.

In the first test, 30 adults that had been stored for 50 days at 38° F. were placed in each lamp chimney cage containing "Clintland 60" oat seedlings. Three cages with a total of 90 adults were held in each of two growth chambers adjusted to 80° F. and 85° F. In both chambers relative humidity and light period were set at 75 percent and 16 hours respectively. The plants were changed every other day and the number of eggs deposited was counted. The egg counts were made until the last female laid the last eggs.

In Test 2, the methods employed were exactly the same as in Test 1 except that the adults used in the second test had been stored at 38° F. for 90, 155, and 250 days prior to the test. Thirty adults were randomly selected from each group and they were placed separately in each lamp chimney cage. This set of three cages were then held at 80° F. and 85° F.

Until Dr. W. C. Myser of Ohio State University developed a method of sexing the adult beetles in 1966, the sexing was done by either 1) picking up mating pairs before they start laying eggs or

2) picking up a given number of pairs by their general appearance. All the beetles were dissected for sexing after they died and the number of eggs laid per replication was calculated by equating the number of females present to a predetermined number.

Survival of the pupae at 80° F. and 85° F. was also investigated. Three petri dishes, each containing 30 pupae, were placed in each growth chamber and the number of adults emerged was counted. The data were analyzed using an F-test.

Results: Table 8 shows a greater egg production at 80° F. than at 85° F. The difference, however, is not significant according to an F-test.

TABLE 8.--Egg production at 80° F. and 85° F. by the adults that had been stored for 50 days at 38° F.--Test 1

	Total number eggs laid per cage per 15 females					
Temp. °F.	I	II	III	Total	Mean	Std. dev.
80	564	2,235	1,273	4,242	1,412	686.96
85	624	210	675	1,509	503	208.23

F = 2.2590 (N. Significant)

A considerable variation in the number of eggs among three replicated cages exists and the high standard deviation values indicate the magnitude of variation. Such variation was frequently observed among the beetles that were not completely out of diapause. It can also be attributed to the individual difference existing among the beetles.

The data obtained in the second test were analyzed using a Chi-square method (Table 9). The number of eggs produced by three different groups of adults at 80° F. is significantly greater than that at 85° F. From these tests it appears that 80° F. seems to be more favorable for egg production in this beetle.

TABLE 9.--Egg production at 80° F. and 85° F. by the adults that had been stored at 38° F. for 90, 155, and 250 days--Test 2

		Total Number Eggs Laid per Cage per 15 Females			
Temp. °F.	Freq.	90	155	250	Chi^2
80	Obs.	1,005	724	452	
	Exp.	1,038.5	693.8	448.7	
	Chi^2	1.081	1.315	0.024	2.420
85	Obs.	652	383	264	
	Exp.	618.5	413.2	267.3	
	Chi^2	1.815	2.207	0.041	4.063

Chi^2 = 6.483 (Signif.)

The development of pupae was also tested at 80° F. and 85° F. The difference in the number of adults emerged at 80° F. and 85° F. is not statistically significant. The results of this test is shown in Table 10.

TABLE 10.--Development of pupae at 80° F. and 85° F.

Temp. °F.	No. New Adults Emerged per 30 Pupae I	II	III	Total	Mean	Percent Emergence
80	14	13	21	48	16	53.3
85	9	18	18	45	15	50.0

F = 0.1071 (N. Significant)

Experiment 3. Development of Larvae and Pupae at 73° F. and 80° F. on Oats and Barley

Methods: General rearing procedures for the larvae have already been described in Experiment 1. Twenty first-instar larvae were transferred into each lamp chimney cage containing about 50 seedlings of "Clintland 60" oats or "Hudson" barley. Five cages each of oats and barley were held in each of two growth chambers set at 73° F. and 80° F., 75 percent relative humidity, and 16 hours light period. The young larvae were allowed to develop inside the cage until they reached the adult stage and the number of adults emerged was counted. The data were analyzed using a Chi-square method.

Results: The experimental results are shown in Table 11.

The association between food plants and temperatures, that is the development and emergence of the beetle under any particular combination of temperature and food plant, is not significant according to a 2 x 2 Chi-square test shown in the table. Individual test, however, indicated that at 73° F. the number of adults emerged from barley was significantly higher than that from oats when a "t" test was used. It was observed in the laboratory that barley is generally more tolerant than oats against feeding damage done by larvae and adults. The amount of food, 50 plants per 20 larvae per cage, was supposed to be enough for the larvae to complete their development.

TABLE 11.--Number of adults obtained from larvae reared on oats and barley at 73° F. and 80° F.

	Oats			Barley			Total	
Temp. °F.	Obs.	Exp.	Chi^2	Obs.	Exp.	Chi^2	Obs.	Chi^2
73	33	39.23	0.989	69	62.77	0.618	102	1.607
80	42	35.77	1.085	51	57.23	0.678	93	1.763
Total	75			120			195	3.370

(N. Signif.)

The difference in adult emergence on oats and barley may be due to the following reasons. 1) The larval development was better on barley at 73° F. 2) Oat seedlings break easily from a little injury or weight and this might have caused an excessive larval movement within a cage and consequently caused a higher mortality of larvae and pupae.

Experiment 4. Egg Production under Fluctuating and Constant Temperatures

Methods: Adult beetles that had been stored for 110 days at 38° F. were used for this experiment. Ten pairs of adults were placed in each lamp chimney cage with "Clintland 60" oat seedlings. Three cages were held in the first growth chamber that was initially adjusted to 70° F. for the day and 50° F. for the night. The initial temperatures of 70° - 50° F. were selected because they approximate the mean day and night temperatures under field conditions in mid-May near Galien, Michigan. Two other cages with 20 adults in each were held in the second growth chamber that was set at a constant 80° F. Relative humidity and light period in both growth chambers were set equally at 75 percent and 16 hours respectively. The plants were changed every other day for egg counts.

Since egg production was extremely poor under fluctuating temperatures, the temperature in the first growth chamber was eventually raised to 80° F. constant after 36 days at 70° - 50° F. and again after 25 more days at 75° - 65° F.

Results: It is apparent from Table 12 that fluctuating day and night temperatures are not favorable for the female beetles to lay eggs consistently.

In the growth chamber with fluctuating temperatures, the adult beetles laid very few eggs. When temperature was raised to constant 80° F., the beetles laid as many eggs as those produced by the beetles that had been held at 80° F. from the beginning of the test. Under low fluctuating temperatures egg laying was extremely poor but the beetles survived for much longer period than those at constant 80° F. After the beetles were exposed to a constant 80° F., their total egg production nearly equalled that of the adults which had been maintained at 80° F.

During an initial 36-day period, the beetles laid a significantly greater number of eggs at constant 80° F. than at 70° - 50° F. The difference in mean egg production under these two different conditions was highly significant according to a "t" test. The difference in mean total egg production during entire testing period at constant 80° F.

and under fluctuating temperatures, however, was statistically not significant.

TABLE 12.--Egg production by overwintered adults under constant 80° F. and fluctuating day and night temperatures

Initial Temp.	Temp. °F.: Day-Night[a]	Duration in Days	No. Eggs Laid per Cage per 10 Females I	II	III	Total	Mean
Fluctuating Temp.	70 - 50	Initial 36	111	28	35	174	58
	75 - 65	Second 25	167	0	0	167	56
	80 Const.	Last 154	557	1,135	1,804	3,496	1,165
Total		215[b]	835	1,163	1,839	3,837	1,279
Constant 80° F.	80	Initial 36	1,126	1,057	---	2,183	1,092
	80	Second 25	127	411	---	538	269
	80	Last 49	90	0	---	90	45
Total		110[b]	1,343	1,468	---	2,811	1,405

[a]16-hour light and 8-hour dark periods.

[b]Also $L.D._{99}$ for the adults.

It is believed from this experiment that under normal field conditions the spring adults could survive for a considerable period of time in cold spring of Michigan and lay most of their eggs when weather, especially temperature, becomes favorable. Later observations indicated that the beetles lay eggs at constant temperature of 60° F. and fairly consistent number of eggs when temperature reaches a constant 70° F. The mean temperature of 75° - 65° F. is 70° F., and yet the egg laying at these fluctuating temperatures is poor and erratic. The slowing effect of low night temperature on sexual activity seems to be much greater than was anticipated.

Experiment 5. Mortality of Different Stages of the Beetle at Extremely High and Low Temperatures

Methods: Numerous preliminary trials were made using a small number of insects to obtain a lethal temperature range. After this range was obtained, eggs, larvae, pupae, and adults were exposed directly from room temperature to various extreme temperatures for different lengths of time. A minimum of two replications of 60 individuals was used for determining each percentage mortality value. Mortality of a given stage at room temperature was used as a check in computation of Abbott (1925) percentage mortality. Freezers for low temperatures and modified incubators for high temperatures were used in this study. Relative humidity in these cabinets ranged from 60 to 80 percent and no light was provided.

Results: Table 13 shows percent mortality of eggs, larvae, pupae, and spring and summer adults at various extreme temperatures. The eggs are the most resistant stage against cold temperatures and 100 percent mortality occurred within 2 hours at -10^{o} F. and in 4 hours at 0^{o} F. The egg stage is then followed by pupae, larvae, active summer adults, and lastly by active spring adults. Unfortunately overwintering adults have not been tested and it is quite possible that these hibernating adults might be the most resistant of all stages against the cold. Castro et al. (1965) reported that 100 percent mortality of the diapausing adults occurred after 25 days at 0^{o} F.

At higher temperatures of 110^{o} F. and 120^{o} F., inactive summer adults were most resistant, and followed by larvae, egg, pupae and

TABLE 13.--Percent mortality of egg, larva, pupa, and adult at various extreme temperatures

		Duration of Exposure															
Temp. °F.	Stage	1 min.	2	4	5	6	8	10	15	20	30	40	45	1 hr.	2	4	8
-10	Spr. Adult	10	77	100													
	Active Sum. Adult		0	23		47	73	90	100								
	Larva				13			73	100								
	Pupa							0		33		90		100			
	Egg										5			67	100		
0	Spr. Adult						0		80	100							
	Active Sum. Adult				42			59	73	79	90	100					
	Larva				11				41		93		100				
	Pupa										26			43	100		
	Egg													38	71	100	
15	Larva										0			30		60	80

TABLE 13.--Cont'd.

		Duration of Exposure														
Temp. ^{o}F.	Stage	5 min.	10	15	20	30	40	50	1 hr.	2	3	4	5	6	4 day	5
110	Active Sum. Adult					0			35	100						
	Pupa					0			33	100						
	Spr. Adult					17			70		100					
	Larva								0	40		100				
	Egg								0	13		100				
	Inactive Sum. Adult													0	97	100
120	Spr. Adult	8	90	93	100											
	Pupa			50		92			100							
	Active Sum. Adult		0		73		90	97	100							
	Egg					0			50	100						
	Larva			0		21			52		100					
	Inactive Sum. Adult											75	90	100		

active summer adults, and spring adults. In the larval stage 100 percent mortality occurred in 4 days at 110° F. and 3 days at 120° F. A complete kill of inactive summer adults was observed within 6 hours at 120° F. and in 5 days at 110° F. Mr. John T. Hayward of Plant Pest Control Division, A.R.S., USDA reported that inactive adults can be killed by a 20-minute exposure to 120° F. which is considerably shorter than the author's observation of 6 hours at the same temperature (personal communication with Mr. Hayward).

Experiment 6. Effect of Different Photoperiods on Egg Production

Methods: Two separate tests were conducted in the laboratory to determine an optimum light period for oviposition by field-collected summer adults that had been stored at 38° F. in the laboratory. In the first test six growth chambers were adjusted to give 0, 8, 12, 16, 20, and 24 hours of light. Temperature and relative humidity were set equally at 80° F. and 75 percent respectively in all the chambers. Fifteen pairs of adults which had been stored 50 days at 38° F. were placed in each lamp chimney cage with "Clintland 60" oat seedlings. Three replicated cages were placed in each growth chamber and the plants were changed every other day for egg counts.

It was learned from the first test that the egg production was highest around a 16-hour light period. For the purpose of refining the results obtained in the first test, 14-, 16-, and 18-hour light periods were used in the second test. Ten pairs of adults that had been stored at 38° F. for 77 days were placed in each lamp chimney cage. The plants were changed once every four days and the number of eggs deposited on the seedlings in each pot was counted. The test was terminated after 63 days when no further egg production was observed. The data were analyzed using an F-test.

Results: The results of the first test are shown in Table 14. Egg production was highest under a 16-hour light period.

TABLE 14.--Egg production under different photoperiods during a 56-day test period--Test 1*

Photoperiod in Hrs. per 24-hr. Cycle	No. Eggs Laid per Cage per 15 Females I	II	III	Total	Mean
0	11	12	5	28	9
8	0	2	4	6	2
12	0	34	1	35	12
16	881	1,101	968	2,950	983
20	1,040	502	413	1,955	652
24	174	0	0	174	58

F = 23.3875 (H. Significant) $LSD_{.01} = 394$

$LSD_{.05} = 277$

*Adults had been stored at 38° F. for 50 days prior to the test.

The differences between the number of eggs produced under 16 or 20 hours photoperiod and that under 0, 8, 12, or 24 hours light period were highly significant according to an F-test. The egg production under a 16-hour light period was again significantly greater than that under 20 hours of photoperiod. At least in this experiment, a 16-hour light period seems to be the most favorable region for the maximum egg production in overwintered adults. Castro, et al. (1965) have already indicated that the beetles responded more readily and produced more eggs under 16 hours than under 12 hours of photoperiod and this test confirms their results.

In the preceding test, a 16-hour photoperiod has been shown to be a close estimate of the optimum light period for the spring adults. In order to obtain more detailed information regarding the optimum photoperiod, 14, 16 and 18 hours of light periods were selected in the second test. The results are shown in Table 15 and Figure 7.

TABLE 15.--Egg production under 14, 16, and 18 hours of photoperiods during a 63-day test period--Test 2*

Photoperiod in Hrs. per 24-Hr. Cycle	No. Eggs Laid per Cage per 10 Females			Total	Mean
	I	II	III		
14	65	581	347	993	331
16	1,339	936	496	2,771	924
18	1,397	441	932	2,770	923

F = 1.8811 (N. Significant)

*Adults had been stored at 38° F. for 77 days prior to the test.

The beetles held under either 16 or 18 hours of light produced nearly three times as many eggs as those laid under a 14-hour light period. The number of eggs laid under three different light periods, however, was not significantly different from one another according to an F-test. As Figure 7 indicates the rate of oviposition was higher under 18 hours than under 16 hours of photoperiod during an initial 32-day period. This seems to indicate that a 18-hour photoperiod could be the more accurate estimation of the true optimum photoperiod for the spring adults.

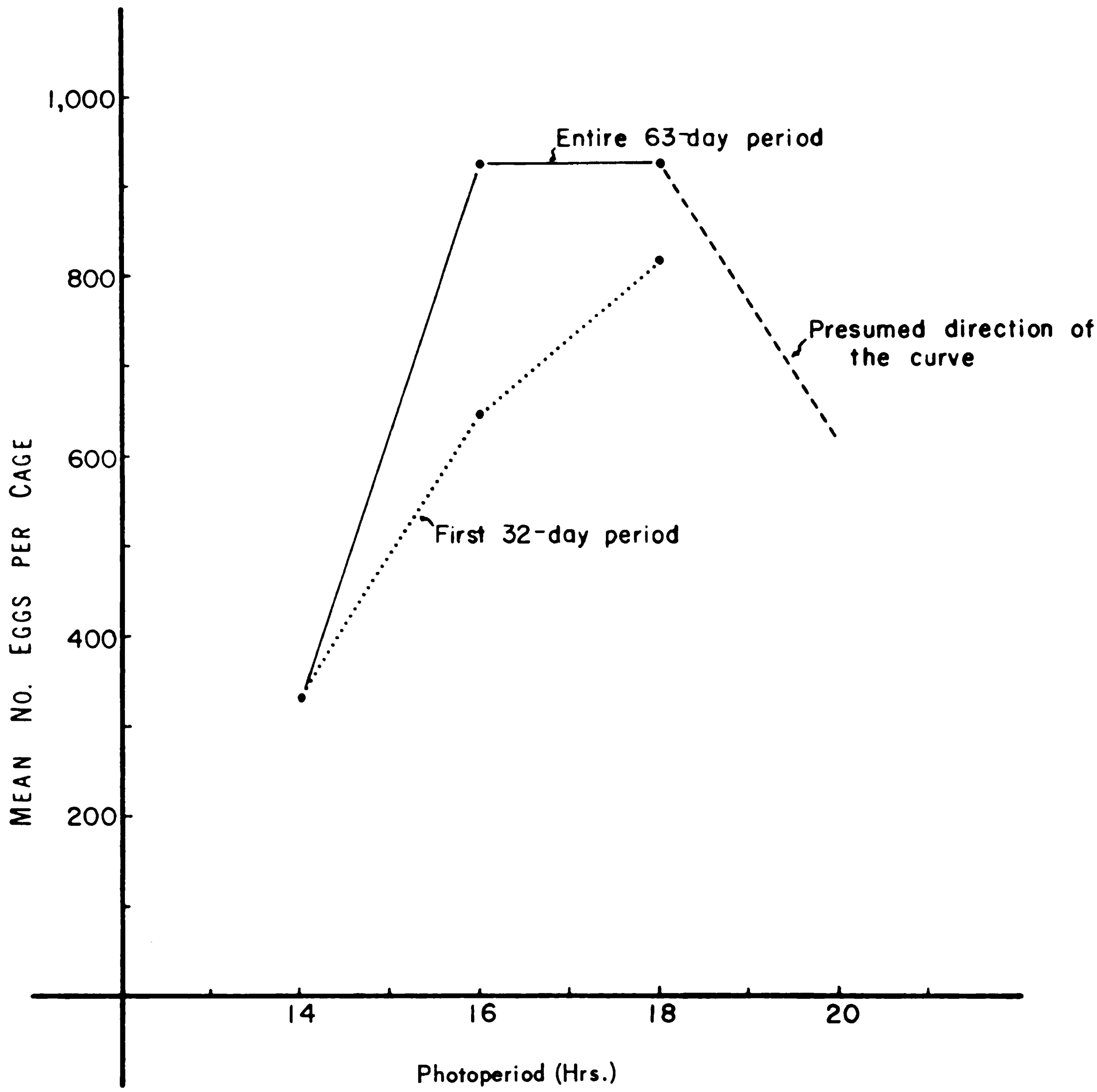

Fig. 7 – Mean total number of eggs laid per 15 females under 14, 16, and 18 hours of light periods.

Experiment 7. Development of Immature Stages under Different Photoperiods

Methods: Two pots of "Clintland 60" oat seedlings with known number of eggs were held in each growth chamber adjusted to 0, 8, 12, 16, 20, and 24 hours photoperiods, 80° F., and 65 percent relative humidity. The number of eggs hatched was later counted and percent egg-hatching was calculated.

Forty newly eclosed larvae were randomly selected from the above egg samples and were divided equally into two lamp chimney cages containing fresh "Clintland 60" oat seedlings. These two cages were held in the same growth chamber from which the larvae were taken. The number of adults emerged in each cage was later counted and it was converted to percent adult emergence.

Results: As Table 16 indicates the development of the egg stage is not directly influenced by different light periods. It is rather influenced by temperature. The egg hatching ranged from 71 percent under an 8-hour light period and 82 percent under complete darkness. The larval development, however, varied considerably. There were no apparent differences in larval and pupal growth when the light period was 16 hours or more. The adult emergence was fair under a 12-hour photoperiod, poor under 8 hours of light, and no adults emerged under complete darkness. The poor adult emergence under 0 and 8-hour photoperiods was not due to a direct effect of the short light periods on the insect growth but it is rather due to a poor plant growth under the 0 and 8-hour photoperiods. Under a 24-hour light period, the larval and pupal development seems to be slightly hastened.

TABLE 16.--Development of eggs, larvae, and pupae under different photoperiods

Photoperiod	No. Eggs Hatched/ No. Eggs Present	Percent Hatching	No. Adults Emerged/ No. Larvae Present	Percent Adult Emergence	Percent Adult Emerged from the Egg
0	111/135	82.2	0	0	0
8	96/135	71.1	8/40	20.0	14.2
12	119/163	73.0	14/40	35.0	25.6
16	123/156	78.8	17/40	42.5	33.5
20	99/161	61.5	16/40	40.0	24.6
24	142/190	74.7	16/40	40.0	29.9

Experiment 8. Egg Production during Day and Night Hours

Methods: Twenty-five adults that had been stored at 38° F. for 104 days were placed in each of three lamp chimney cages containing "Clintland 60" oat seedlings. All of the cages were held at 80° F., 75 percent relative humidity, and 16 hours photoperiod. The plants were changed twice a day, once at the end of a 16-hour light period and again at the end of an 8-hour dark period, and the number of eggs deposited on the plants was counted. The data were analyzed using a t-test.

Results: Table 17 shows that the majority of the eggs (93.1%) were laid during a 16-hour light period and only 6.9 percent of the eggs were deposited during the 8 hours of dark period.

TABLE 17.--Number of eggs laid during day and night hours for a 10-day period on oats

Repl. (day)	Day (16 hr.)	Night (8 hr.)	Total
1	178	18	196
2	248	18	266
3	266	25	291
4	265	14	279
5	263	22	285
6	252	15	267
7	233	10	243
8	218	19	237
9	209	14	223
10	175	16	191
Total	2,307	171	2,478
% Eggs	93.1	6.9	

t = 3.1255 (Significant)

A "t" test showed that the number of eggs laid during day hours is significantly greater than that deposited during night hours. The oviposition activity in this insect, therefore, is strongly affected by the presence or absence of light and most of the eggs are laid during day time.

Experiment 9. Effect of Different Relative Humidities on Egg Production

Methods: Sixty adult beetles that had been stored for 123 days at 38° F. were placed in each plastic screen cage (9-1/2 x 9-1/2 x 12 inches) with two pots of "Clintland 60" oat seedlings. Two cages were held in each of three growth chambers that were set at 45-55 percent, 70-80 percent, and 95-100 percent relative humidity. Temperature and light period in all three chambers were equally adjusted to 80° F. and 16 hours respectively. The plants were changed once every four days and the number of eggs laid was counted during the 62-day test period. The data were analyzed using an "F" test.

Results: An F-test shows that the number of eggs laid by the adults that had been exposed to different relative humidities is significantly different from each other. The least significant difference (LSD) of 175 in Table 18 indicates that the beetles that had been exposed to 95-100 percent relative humidity laid significantly greater number of eggs than the beetles that had been treated at the other relative humidities.

It was observed during the test period that some beetles remained quite inactive especially among the beetles that had been exposed to 45-55 percent relative humidity. Along with other environmental factors such as temperature and photoperiod, relative humidity seems to be an important factor affecting the reproductive activity and egg production in the spring adults.

TABLE 18.--Egg production under different relative humidities*

Percent Relative Humidity	No. Eggs Laid per Cage per 30 Females I	II	Total	Mean
45-55	0	0	0	0
70-80	88	258	346	173
95-100	972	926	1,898	949

F = 78.9322 (Significant) $LSD_{.05} = 175$

*Adults had been stored at 38° F. for 123 days prior to the test.

Experiment 10. Effect of Different Soil Moistures on Development of Pupae and Emergence of Adults

Methods: This study consists of three separate tests. In Test 1, twenty third-instar larvae were placed in each lamp chimney cage containing "Clintland 60" oat seedlings. The larvae were allowed to feed and pupate in the same cage. All the cages were held at 80° F. and 12 hours light period. The soil moisture was roughly adjusted in four different ways as follows.

1) Dry: No water was added to the soil after all the larvae entered the soil for pupation.

2) Moist: Caged plastic pots were placed in a water tub after all the larvae entered the soil. The water level in the tube was maintained up to the same level of the soil surface in the pot.

3) Wet: Caged pots were placed in the water tub from the beginning of the test.

4) Normal: The soil was watered once every three days with a watering can.

In Test 2, fifty grams of oven-dry white silica sand was poured into each 9-ounce glass jar. The sand was then mixed with a proper amount of water to give a desired percentage moisture by dry weight basis. The white sand was used simply because of its ease of handling. The moisture contents of the sand were maintained in three different levels, 0, 12.5, and 25 percent. Thirty pupae in their pupal cases were placed on the sand surface in each jar.

In Test 3, twenty pupae were suspended in the air above the sand surface by means of a piece of copper screen in each jar. Five different moisture contents, 0, 12.5, 25.0, 37.5, and 50 percent were used in this test. Both Tests 2 and 3 were conducted at 85° F. and 16 hours light period. In all three tests the number of adults emerged in each cage or jar was counted and the results were analyzed using an F-test.

Results: As shown in the third treatment (wet) of Table 19, all the mature larvae were unable to pupate in the flooded soil and consequently there was no adult emergence. The larvae in these flooded pots were found dead from the excess water in the soil.

When the soil was flooded after all the larvae entered the soil (moist), the pupation apparently took place without any appreciable mortality of the pupae. An F-test, however, indicates no significant difference in the number of adults emerged from cages with different soil moistures. Sample size used in this test seems to be too small to detect any significant differences in the number of adults emerged from different cages. However, the results of this test generally indicate that an excess water in the flooded soil is detrimental for the prepupae entering the soil for pupation. Dry and moist soil or the soil flooded after the pupal case is formed do not seem to affect the pupation process.

TABLE 19.--Development of pupae and emergence of adults from larvae that were allowed to pupate in the soil with different moisture contents--Test 1

Soil Moisture	No. Adults Emerged per 20 Larvae I	II	III	Total	Mean	Percent Emergence
Soil	7	13	2	22	7.3	29.3
Moist	7	7	7	21	7.0	28.0
Wet	0	0	0	0	0	0
Normal	8	4	5	17	5.7	22.7

F = 3.7835 (N. Significant)

The results of Test 2 shown in Table 20 are generally similar to that of the previous test.

When the data were analyzed using an F-test, the differences in the number of adults emerged from different jars were highly significant. The less the moisture content of soil, the higher was the percentage of adult emergence. There was a 100 percent mortality of the pupae in jars with 25 percent moisture. An appreciable amount of free water existed on the sand surface in these jars and this excess water was believed to be the cause for the complete kill of the pupae. The pupae used in this test were collected from the cereal leaf beetle rearing room and many pupal cases were apparently damaged during the collection process. The excess water on the sand surface then penetrated into the pupal case causing suffocation and death of the pupae.

TABLE 20.--Emergence of adults from pupae that were placed on white sand with different moisture contents--Test 2

Percent Soil Moisture	No. Adults Emerged per 30 Pupae			Total	Mean	Percent Emergence
	I	II	III			
0	22	24	19	65	21.7	72.2
12.5	7	4	11	22	7.3	24.4
25.0	0	0	0	0	0	0

F = 39.7457 (H. Significant) $LSD_{.05}$ = 6.9

As Table 21 indicates the higher relative humidity resulting from higher soil moisture content seems to favor the pupal development. The differences in the adult emergence from differently treated jars, however, are not significant according to an F-test.

The wet soil seems to be detrimental for the larvae entering the soil for pupation. Flooding of the soil during peak prepupal period, if it is feasible, may be effective for control of the summer adult population. Knechtel and Manolache (1936) reported that spring rain in May and June caused a heavy mortality to the larvae in Europe and this experiment partially acknowledges their report.

TABLE 21.--Emergence of adults from pupae that were suspended above white sand with different moisture contents--Test 3

Percent Soil Moisture	No. Adults Emerged per 20 Pupae			Total	Mean	Percent Emergence
	I	II	III			
50.0	16	18	19	53	17.7	88.3
37.5	14	20	18	52	17.3	86.7
25.0	15	15	6	36	12.0	60.0
12.5	16	17	10	43	14.3	71.7
0	16	8	8	32	10.7	53.3

F = 1.9059 (N. Significant)

Experiment 11. Effect of Different Soil Types on Oviposition

Methods: A wooden flat, 20 x 14 x 3 inches, was about two-thirds filled with regular potting soil and was visually divided into four equal sections. In the middle of each section a row of 15 "Clintland 60" oat seedlings were allowed to grow until they reached about 5 inches tall. The top one-third portions of the four sections were then filled with 1) white silica sand, 2) muck, 3) potting soil, and 4) fine sand.

Thirty adults (15 pairs) that had been stored for 128 days at 38° F. were placed in the flat and they were covered with a wooden frame screen cage with a plexiglas top (20 x 14 x 14 inches). The plants were changed daily for six consecutive days and the number of eggs deposited was counted. The data were analyzed using an "F" test.

Results: The results of this experiment are summarized in Table 22. The number of eggs deposited on oat seedlings planted in four contrasting soil types is not significantly different from one another according to an F-test.

TABLE 22.--Oviposition of eggs on oat seedlings planted in different types of soil

Soil Type	No. Eggs Laid per Day I	II	III	IV	V	VI	Total	Mean	Percent Eggs Deposited
White Sand	24	14	25	23	27	35	148	24.7	27.8
Muck	16	25	34	17	25	24	141	23.5	26.4
Potting Soil	16	27	16	22	21	34	136	22.7	25.5
Sand	14	14	9	23	26	22	108	18.0	20.3

F = 3.2874 (N. Significant)

The soil type, especially color, apparently does not exert any influence on oviposition behavior of the cereal leaf beetle as long as preferred host plants are available to the beetle.

Experiment 12. Effect of Different Soil Types on Pupation and Emergence of Adults

Methods: Three contrasting soil types, muck, fine sand, and regular potting soil in plastic pots were used for this test. For the muck and fine sand, the bottom half of the pots were first filled with regular potting soil and the upper half with muck or fine sand. "Clintland 60" oat seedlings were grown in these pots and 25 third-instar larvae were placed in each pot before they were caged with a lamp chimney. All the pots with the larvae were held at 78° F., 65 percent relative humidity, and 16 hours photoperiod. The larvae were allowed to feed and pupate in the same cage and the number of adults emerged in each cage was counted. The results were analyzed using an F-test.

Results: The number of new adults emerged in each pot is shown in Table 23. The adult emergence was generally poor in all three soil types. Although the difference in the number of adults emerged among three different soil types was statistically not significant, a loose muck soil seems to be more favorable for pupation than the other two soil types. From the regular potting soil, which contained the largest amount of clay, only 8 adults emerged.

TABLE 23.--Development of pupae and emergence of adults from larvae in different types of soil

Soil Type	No. Adults Emerged per 25 Larvae				Total	Mean	Percent Emergence
	I	II	III	IV			
Muck	4	4	6	19	33	8.3	33.0
Sand	0	0	8	6	14	3.5	14.0
Potting Soil	2	1	3	2	8	2.0	8.0

F = 1.667 (N. Significant)

From the author's own experience, the ideal soil type for pupation of the cereal leaf beetle seems to be the one which is a relatively light and well aerated soil. A soil which contains a large amount of clay has a high water adsorbing capacity during the rainy season and would become hardened during the drought season. This type of soil would obviously drown and suffocate the beetle when it is wet and would block the way out for newly formed adults when dry.

In Galien, Michigan where the heaviest infestation occurs, the soil is generally heavy yet the beetle population remains at high level. The primary reason would be probably due to a relatively dry weather in the area during pupation period. In the case of dry heavy soil, the prepupae enter the soil following the stem of host plant or through cracks or any openings on the soil surface. As long as weather remains relatively dry the cracks would remain opened and the new adults would emerge without any difficulty through the same route they chose when entering the soil as prepupae. The amount of precipitation during the peak pupation period would undoubtedly alter the physical property of the soil and this in turn seems to influence the abundance of beetle population.

The soil type and soil moisture also influence the soil temperature. A heavy wet soil would absorb the solar heat more readily than a light dry soil. It was demonstrated in an earlier test that the cereal leaf beetle pupae could not develop at 95° F. and above in the laboratory. In the field, however, the soil temperature can reach 128° F. at the surface (Ruppel et al. 1965), and yet the pupae somehow managed to survive and the new adults emerge. The temperature is usually highest at the soil surface but it seems to drop considerably 1 to 2 inches below the soil surface where the beetles pupate.

The author believes that the soil type along with soil moisture and soil temperature can influence the distribution and abundance of the cereal leaf beetle. A sandy-loam type soil that is well aerated and stays relatively dry during the pupation period would definitely favor the population increase in the cereal leaf beetle. Unfortunately the soil types that are apparently favorable for the cereal leaf beetle are also desirable types for most of the agricultural crops.

Experiment 13. Effect of Different Cage Sizes on Egg Production

Methods: This study consists of two separate tests. In Test 1, field-collected summer adults that had been stored for 100 days at 38° F. while in Test 2 field-collected active spring adults were used. In both tests three different types of cages 1) a lamp chimney (3 inches in diameter at the bottom and 7 inches in height), 2) a "medium" plexiglas cage (8 x 8 x 12 inches), and 3) a "large" plexiglas cage (15 x 15 x 15 inches) were used.

Thirty adults (15 pairs) were placed in each cage along with a pot of "Clintland 60" oat seedlings. All the cages were held in the rearing room at 76° F., 75 percent relative humidity, and 16-hour photoperiod. The plants were changed every other day and once every three days in Tests 1 and 2 respectively and the number of eggs deposited was counted. Test 1 was terminated after 23 days while Test 2 continued for 37 days. The results were analyzed by means of an "F" test.

Results: Table 24 shows the results of the two tests during the first 23-day period. An F-test indicated no significant difference in the number of eggs produced in cages of three different sizes in both tests.

In Test 1, however, the small lamp chimney cage seems to be more effective in inducing earlier and more consistent egg production in the case of overwintering adults. In a small cage the beetles are kept close to the food plants and potential mates while they are fully exposed to the light. Under such conditions, the beetles seem to become more quickly reactivated. The same effect with lamp chimney cage has been previously reported by Castro (1964).

TABLE 24.--Effect of different cage sizes on egg production by field-collected beetles stores in the laboratory and by field-collected active spring adults[a]

Adults	Cage Size	No. Eggs Laid per Cage per 15 Females I	II	Total	Mean
Laboratory-stored adults[b]	Lamp Chimney	911	645	1,556	778
	Medium	8	457	465	233
	Large	391	131	522	261
Field-collected spring adults[c]	Lamp Chimney	254	580	834	417
	Medium	611	779	1,390	695
	Large	521	639	1,160	580

F = Not significant in both tests.

[a]Test ended after 23 days.

[b]Adults had been stored at 38° F. for 100 days prior to the test.

[c]Adults had been collected in April in the field.

In the second test with field-collected active spring adults, more eggs were laid in medium and large cages than in small lamp chimney cages. For the active adults a larger space is apparently needed for their various activities.

Influence of Biological Factors

The biological factors cited in this chapter include all the "living organisms" such as predators, other individuals of the same species, and host plants. Interactions occurring within and between these organisms were studied. The results of the studies and analyses of the results are presented below.

Experiment 14. Effect of Different Plant Densities on Development of Larvae through to the Adults

Methods: The number of "Clintland 60" oat seedlings grown in plastic trays of 5 x 8 x 2-1/2 inches in size was adjusted to 5, 10, 20, 40, and 80 plants per tray. The soil in each tray was first covered with plaster of Paris and then with white silica sand following the method of Connin, *et al*. (1966b).

Twenty newly eclosed larvae were placed in each tray and they were caged with a plexiglas cover of 5 x 8 x 11 inches in size. Three replications were made for each treatment and all the cages were held at 80° F., 70 percent relative humidity, and 16 hours photoperiod. The number of adults emerged in each cage was recorded and the data were analyzed using an F-test.

Results: The number of adults emerged from cages with original ratios of 0.25 and 0.50 larvae per plant was significantly greater than those emerged from the other three groups of cages. The difference is highly significant according to an F-test (Table 25).

When the larval density in terms of the number of larvae per seedling was transformed into logarithm and percent mortality into probit, an apparent linear relationship was obtained. The details of the transformations and a graphic representation are shown in Table 26 and Figure 8. According to the equation, $LD_{.50}$ occurs at the original density of 0.5 larvae per plant or two 4-inch tall seedlings per larva.

TABLE 25.--Effect of different larval densities on development of larvae through to the adults

No. Larvae per Plant	No. Adults Emerged per Cage per 20 Larvae			Total	Mean	Percent Survival
	I	II	III			
0.25	20	8	16	44	14.7	73
0.50	16	16	10	42	14.0	70
1.00	0	2	9	11	3.7	18
2.00	2	0	0	2	0.7	3
4.00	0	0	0	0	0	0

F = 9.5337 (H. Significant) $LSD_{.01}$ = 11.1

$LSD_{.05}$ = 7.6

TABLE 26.--Relationship between larval density and mortality

No. per Cage						
No. Plants	No. Larvae	No. Plants per Larva	No. Larvae per Plant (x)	Log 10(x)	Percent Mortality	Probit: Percent Mortality (y)
80	20	4	0.25	0.398	26.7	4.378
40	20	2	0.50	0.699	30.0	4.476
20	20	1	1	1.000	81.7	5.904
10	20	0.5	2	1.301	96.7	6.838
5	20	0.25	4	1.602	100	7.854

Regression equation: Probit $y = 3.0938x + 2.7962$

Correlation coefficient: $r = 0.9795$

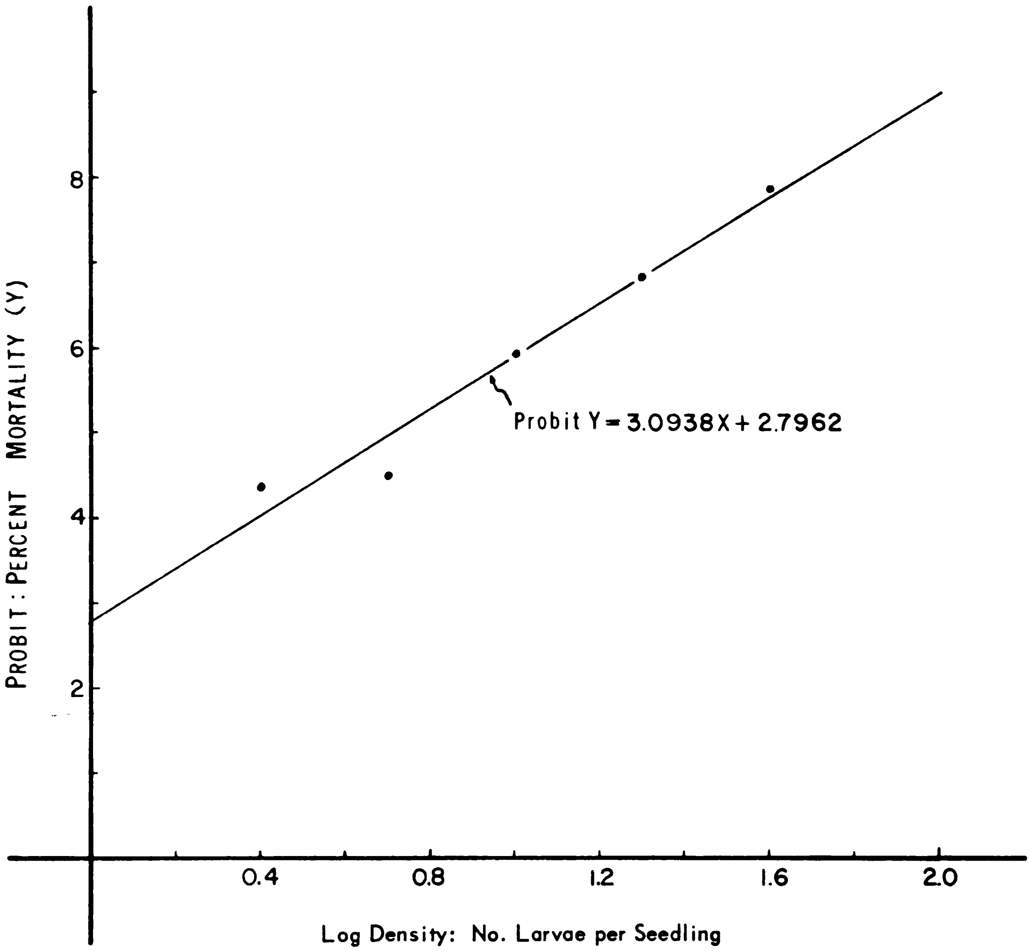

Fig. 8 – Relationship between larval density and mortality.

The results of this study may not hold true under field conditions because the plants usually outgrow the larvae. It was observed in the field that young oat seedlings carrying more than one larva per seedling were still able to grow. In the laboratory, however, original number of at least two 4-inch tall seedlings per larva have to be provided at the beginning of the test to bring 50 percent of the first-instar larvae through to the adult stage.

There were considerable individual differences among the larvae. Certain individuals fed vigorously and were able to reach the adult stage even at an original density of two larvae per seedling or 0.5 plants per larva. Intraspecific competition, in this sense, exists among the larvae of the cereal leaf beetle especially when the amount of food is limited. No cannibalistic behavior was observed among the larvae even at the highest density.

Klomp (1964) stated that competition for food is generally regarded as the most common type of competition. Sang (1949) reported occurrence of such competition among the larvae of _Drosophila_. Crombie (1944) and Andersen (1960) listed increase of the mortality rate among larvae and pupae as one of the consequences resulting from an increasing competition among larval insects. Figure 8 clearly shows the increase in mortality among the larvae with decrease in the amount of available food per larvae.

Experiment 15. _Effect of Different Adult Densities on Egg Production_

Methods: Three separate tests were involved in this study. General procedures employed in all three tests were about the same. Fifty 4-inch tall "Clintland 60" oat seedlings per pot and thirty

4-inch tall "Hudson" barley seedlings per pot were used in Test 1 and Tests 2 and 3 respectively. Adults used in Test 1 had been stored at 38^{o} F. for 110 days prior to the test while those used in Tests 2 and 3 were collected in the field in late April of 1966 when a few eggs were observed.

Five different adult densities, 10, 20, 30, 40, and 50 adults per lamp chimney cage were chosen for this study. Each cage contained an equal number of males and females. All prepared cages were held in the rearing room at 78^{o} F., 75 percent relative humidity, and 16-hour light period. The plants were changed every other day in Test 1 and 2, and once every four days in Test 3. The number of eggs deposited on the plants and the mortality of the adults were recorded. The data were analyzed using an F-test.

<u>Results</u>: The total number of eggs laid per cage and per female in three tests are shown in Tables 27, 28, and 29. The differences in egg production at five different adult densities were not statistically significant in all three tests according to an F-test.

An earlier observation indicated that an active spring adult required at least one 4-inch tall fresh seedling supplied in every two to three days for an adequate feeding and oviposition. Theoretically with an average of 50 seedlings per pot and two-day renewal intervals, 50 adult beetles could be supported without hindering their egg laying capacity as far as plant factors, the amount of available food and oviposition sites, are concerned.

The adults used in Test 1 had been stored in the laboratory and were unmated prior to the test. Table 27 shows that the total egg production per cage as well as that per female is highest at the

TABLE 27.--Total number of eggs laid per cage and that per female at different adult densities--Test 1[a]

Measure	No. Adults per Cage[b]	No. Eggs per Cage or per Female I	II	Total	Mean
Per Cage	10	838	631	1,469	735
	20	1,229	2,190	3,419	1,710
	30	736	1,259	1,995	998
	40	1,142	1,403	2,545	1,273
	50	1,126	552	1,678	839
Per Female[c]	10	209	179	388	194
	20	197	330	527	264
	30	150	159	309	155
	40	83	136	219	110
	50	112	34	146	73

F = 3.3466 (N. Significant)

[a]Field-collected summer adults after 110 days of storage at 38° F. Plants changed every other day. Test ended after 106 days.

[b]1:1 sex ratio.

[c]Egg counts based on female days.

TABLE 28.--Total number of eggs laid per cage and that per female at different adult densities--Test 2[a]

Measure	No. Adults per Cage[b]	No. Eggs per Cage or per Female I	II	Total	Mean
Per Cage	10	376	666	1,042	521
	20	854	539	1,393	697
	30	1,387	1,047	2,434	1,217
	40	389	913	1,302	651
	50	823	551	1,374	687
Per Female[c]	10	100	224	324	162
	20	160	164	324	162
	30	159	131	290	145
	40	32	106	138	69
	50	64	52	116	58

F = 2.5611 (N. Significant)

[a]Field-collected active spring adults collected in April, 1966. Plants changed every other day. Test ended after 42 days.

[b]1:1 sex ratio.

[c]Egg counts based on female days.

TABLE 29.--Total number of eggs laid per cage and that per female at different adult densities--Test 3[a]

Measure	No. Adults per Cage[b]	No. Eggs per Cage or per Female I	II	Total	Mean
Per Cage	10	478	842	1,320	660
	20	637	1,209	1,846	923
	30	779	696	1,475	738
	40	453	238	691	346
	50	527	467	994	497
Per Female[c]	10	112	213	325	163
	20	119	150	269	135
	30	92	90	182	91
	40	56	18	74	37
	50	37	51	88	44

F = 4.5802 (N. Significant)

[a]Field-collected active spring adults collected in April, 1966. Plants changed once every four days. Test ended after 72 days.

[b]1:1 sex ratio.

[c]Egg counts based on female days.

second lowest density and original density of 20 beetles per cage. An increase in fecundity of the female with an increase in density from 10 to 20 adults per cage is probably due to a stimulatory effect resulting from a slight crowding in the case of unmated adults. At the lowest density the total egg production per cage is lowest of all but the fecundity of individual females is second highest. As the density increases from 20 to 30, 40, and 50 adults per cage, the fecundity of females decreases accordingly. This decrease in fecundity is not due to the shortage of food, but apparently due to overcrowding of the adults in a limited area and space.

The fecundity of females in Test 1 is generally higher than that in Tests 2 and 3 at all density levels. The reason is simply that the laboratory-stored adults survived much longer than the field-collected spring adults that had been exposed to variable natural environment prior to the test and brought into a confined laboratory conditions. With the unmated laboratory-stored adults and about 50 seedlings with two-day renewal intervals, the optimum density level of the adults for the maximum egg production seems to be 20 adults per lamp chimney cage that has approximately seven square inches of area and 49 cubic inches of space.

The adults used in Tests 2 and 3 were active spring adults collected in the field. They had apparently mated in the field. The only difference in Tests 2 and 3 was the renewal period of food plants. Fresh seedlings were supplied every other day in Test 2 and once every four days in Test 3. The largest egg production per cage was observed at the medium density of 30 adults per cage in Test 2 and at 20 beetles per cage in Test 3. The egg production per female was greatest at the two lowest density levels in Test 2 and at the lowest density in Test 3. The fecundity of females gradually decreases as the density increases up to 40 adults per cage and then it seems to level off. The fecundity is generally higher in Test 2 than in Test 3 except at the lowest density. This difference is obviously due to the difference in renewal period of food plants in the two tests. A slight crowding did not seem to favor egg production with the adults that had already mated prior to the test.

Surprisingly, even under highly crowded conditions, only a few eggs deposited on the plants were damaged by the adults. The damage to the eggs was purely accidental and no egg cannibalism was observed in this insect. The main causes of the decrease in fecundity with increased density level seem to be: 1) a proportional reduction in the amount of available food and oviposition sites and 2) a disturbance of copulation and oviposition act resulting from more frequent body contact. A decrease in the rate of copulation, a disturbance of the oviposition act, an excessive contact between individuals, and reduction in the amount of food available to individual insects have been reported by McLagan (1932) and Crombie (1942) as contributing to reduced egg production by *Tribolium* sp.

Survival of the adults was slightly better at the lowest density than at the rest of the densities in all tests. However, no significant adult mortality due to high adult density was observed. The majority of females died after mating and laying eggs. A few adults survived for much longer period than the others. These surviving ones were found to be either males or unmated females. A small number of field-collected spring adults in Test 3 survived for over 100 days; beyond the 10th of August. These beetles, therefore, may be able to survive for two years as was reported by Megalov (1927) and Hodson (1929) in Europe.

Experiment 16. Amount of Feeding, Egg Production, and Survival of the Adults on Oats and Barley

Methods: This study consists of two separate tests. Adult beetles that had been stored at 43° F. for 65 days and two different host plants, "Clintland 60" oats and "Hudson" barley were used in Test 1. Thirty adults were placed in each lamp chimney cage containing 30 seedlings of oats or barley. Three replicated cages were made for each host plant. Three additional caged pots without beetles were also prepared for oats and barley and these pots were later used as check pots for computation of the amount of feeding. All the pots were then held in a growth chamber adjusted to 80° F., 70 percent relative humidity, and 16 hours light period.

The plants were changed once every two to three days and the number of eggs deposited on the plants and that of live and dead beetles in each cage were counted. The amount of feeding on fresh weight basis was calculated by measuring the difference in the weight of seedlings that had been fed and that had not been fed by the beetle. The seedlings were cut uniformly at the level of soil surface for weighing.

In Test 2, the same two varieties of oats and barley were used to test oviposition preference by field-collected active spring adults. Three pots each of oats and barley were placed side by side in an oviposition cage (14-1/2 x 16 x 16 inches) that contained about 150 adults. Exactly twenty-five 4-inch tall seedlings were left in each pot. The oviposition cage was held at 78° F., 75 percent relative humidity, and 16 hours light period. The plants were changed daily for ten consecutive days for egg counts and the data were analyzed using a t-test.

Results: The results of this experiment are shown in Table 30. The amount of feeding and the egg production were greater in oats than in barley. The differences, however, are not statistically significant according to a t-test.

The total amount of feeding per beetle was 860 mg. in oats and 466 mg. in barley on fresh weight basis. The amount also includes the water loss and other weight losses due to the feeding damage. The method employed, however, would satisfy the preliminary nature of this test. The amount of feeding per beetle roughly corresponds to eight oat and 5.4 barley seedlings of 4-1/2 inches in height.

More than twice as many eggs were laid on oats as on barley even though the difference is not statistically significant. More detailed study on oviposition preference between oats and barley were conducted in the second test. The egg production seems to be correlated with the amount of feeding. Table 30 also indicates that a greater number of eggs was laid between the 10th and 20th days in this group rearing. The mortality of adults at the end of a 45-day test period is much higher in oats than in barley. This is probably due to a greater egg production on oats than on barley since the majority of adults usually die when oviposition activity ceases.

The total amount of leaf tissue consumed per beetle in this test is considerably lower than that estimated by Castro *et al*. (1965). Their report of 25.9 mg. of leaf tissue per beetle per day is nearly

TABLE 30.--Amount of feeding, egg production, and survival of cereal leaf beetles stored at 43° F. for 65 days

	Oats			Barley		
Days	Amt. Fed per Beetle (mg.)	No. Eggs per Cage	Percent Survival	Amt. Fed per Beetle (mg.)	No. Eggs per Cage	Percent Survival
5	62	18.7	88.9	30	9.0	91.1
10	146	97.7	80.0	114	51.0	88.9
15	207	243.3	70.0	110	123.7	86.7
20	151	166.7	63.9	66	70.7	83.3
25	76	127.3	57.8	37	65.5	76.7
30	47	88.3	51.1	63	50.0	72.2
35	68	72.0	47.8	29	23.0	68.9
40	78	42.0	42.2	17	19.0	66.7
45	25	11.0	38.9	--	5.0	61.7
Total	860	867.0		466	416.8	
	8 seedlings per beetle	56.4 eggs per female		5.4 seedlings per beetle	22.6 eggs per female	

twice as much as 13.2 mg. per beetle per day in this test. The reasons for such difference could be due to the facts that 1) the beetles used in this test were not as active as field-collected spring adults and 2) the feeding activity sharply decreased after the 20th day.

In the second test the adult beetles were exposed to both oats and barley in the same cage. The results of the test are presented in Table 31.

TABLE 31.--Oviposition of eggs on oats and barley by female cereal leaf beetles

Host	Days 1	2	3	4	5	6	7	8	9	10	Total	Mean
Oats	172	200	166	152	119	89	265	140	65	95	1,463	146.3
Barley	115	129	131	128	88	44	204	86	47	87	1,059	105.9

t = 8.5885 (H. Significant)

A t-test showed a highly significant difference between the number of eggs deposited on oats and that laid on barley. The number of eggs on oats is consistently higher than that on barley throughout a ten-day test period. This test clearly indicates that the cereal leaf beetle prefers oats to barley for oviposition. The results confirm the report made by Wilson and Shade (1964a) that oats are preferred to barley or wheat for oviposition.

<u>Experiment 17</u>. <u>Oviposition of Eggs on Oat Seedlings with Different Heights and Densities</u>

<u>Methods</u>: "Clintland 60" oat seedlings of three different size groups, 2, 4, and 6 inches in height, were obtained by adjusting the

planting date. For each size group, three pots containing different numbers of seedlings were prepared. In the first pot 25 seedlings, in the second 50, and in the third pot 75 seedlings were allowed to remain. All nine pots were placed in an oviposition cage that contained about 150 active spring adults. The cage was held at 78° F., 75 percent relative humidity, and a 16-hour light period. The plants were changed daily for five consecutive days and the number of eggs laid was counted. The data were analyzed using a Chi-square test.

Results: The results on the number of eggs deposited on plants with different combinations of height and density are shown in Table 32.

TABLE 32.--Oviposition of eggs on oat seedlings of different heights and densities

No. Plants per Pot	Plant Height in Inches			Total
	2	4	6	
25	98	95	98	291
50	78	82	96	256
75	74	159	111	344
Total	250	336	305	891

Chi^2 = 1.57 (N. Signif.)

A Chi-square test indicated no significant difference in the number of eggs found among different combinations of plant height and density. However, the total number of eggs deposited on plants with 75 seedlings per pot and that on 4-inch tall plants are slightly greater than the others. Castro, *et al.* (1965) reported that more eggs are deposited on 4 to 4-3/4-inch tall seedlings than on smaller or taller plants. This experiment, however, does not show any definite relationship between plant height and egg deposition.

Experiment 18. Effect of Different Male-Female Combinations on Egg Production

Methods: This study consists of two separate tests. Adults used in Test 1 were field-collected summer adults that had been stored at 38° F. for 130 days and were not mated prior to the test. The beetles used in Test 2 were field-collected active spring adults and most of them had probably mated before they were collected for the test.

Six different sets of female-male combinations, 4/1, 2/1, 1/1, 1/2, 1/4, and 1/0 were used in both tests. The first set of 4/1 indicates that four females and one male were placed together in a lamp chimney cage containing about 30 "Clintland 60" oat seedlings. Each set was replicated twice in both tests. All the cages were then held at 78° F., 75 percent relative humidity, and a 16-hour light period. The plants were changed every other day until the last female died and the number of eggs deposited was recorded. The data were analyzed using a F-test.

Results: Table 33 and 34 show the results of the first and second tests respectively. The results on the total number of eggs laid per combination and that per female for field-collected beetles that had been stored in the laboratory and field-collected active spring adults are presented graphically in Figure 9.

The difference in the number of eggs laid per female in cages with different male-female combinations is statistically not significant in both tests. The total egg production per combination as well as

TABLE 33.--Fecundity of females in cages with different male-female combinations

No. Female/Male per Cage	No. Eggs Laid per Female per Cage I	II	Total	Mean
4/1	37	46	83	41.5
2/1	170	152	322	161.0
1/1	631	505	1,136	568.0
1/2	172	98	270	135.0
1/4	43	38	81	40.5
1/0	129*	--	129	129.0

F = 15.1431 (N. Significant)

*All eggs did not hatch.

TABLE 34.--Fecundity of field-collected active spring females in cages with different male-female combinations

No. Female/Male per Cage	No. Eggs Laid per Female per Cage I	II	Total	Mean
4/1	190	157	347	173.5
2/1	195	204	399	199.5
1/1	127	127	254	127.0
1/2	240	126	366	183.0
1/4	122	355	477	238.5
1/0	42	211	253*	126.5

*Normal eggs.

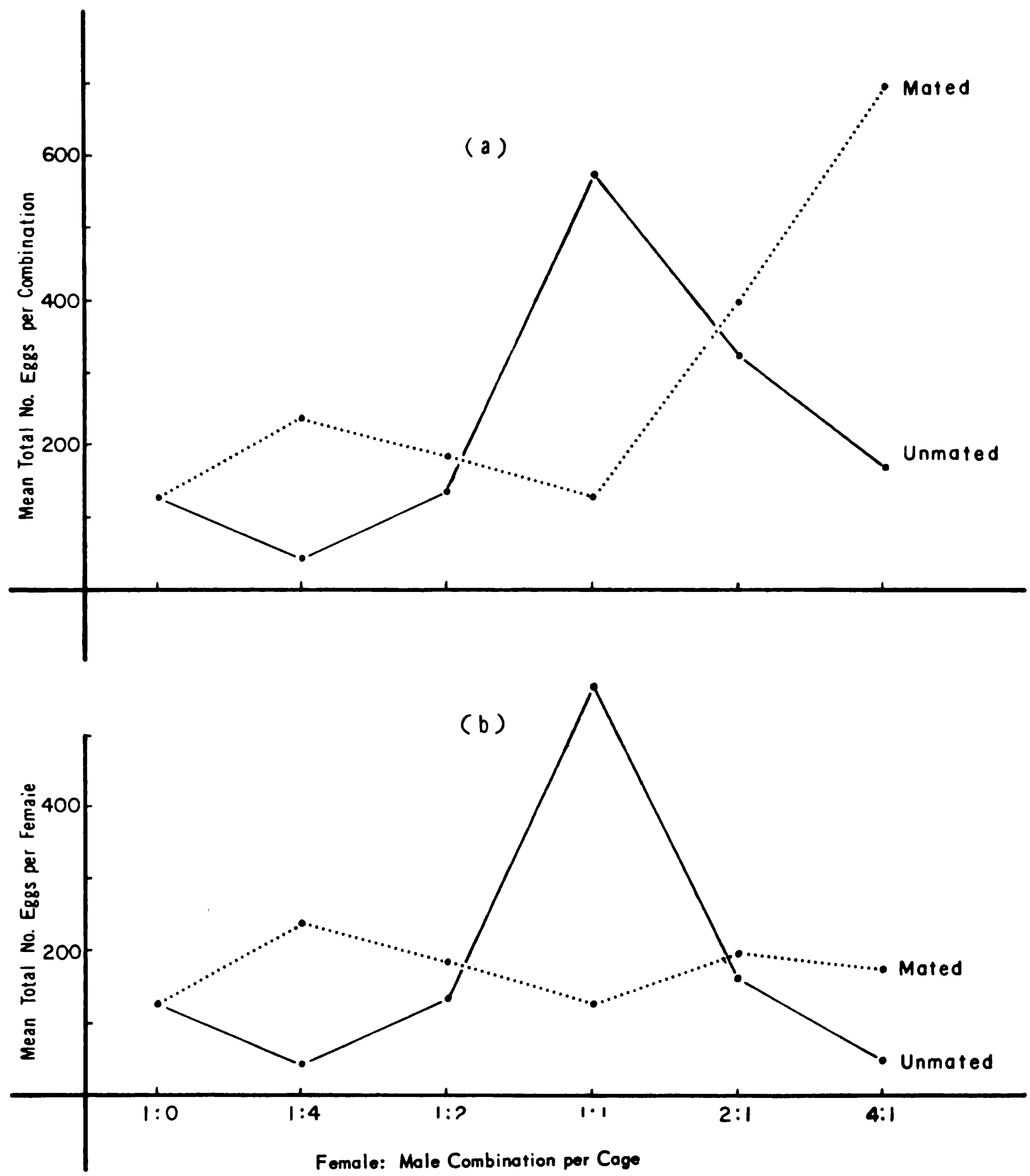

Fig. 9 – Mean total number of eggs produced per combination (a) and that per female (b) in cages with different male-female combinations in previously mated and unmated adults.

that per female for unmated adults, however, are largest in cages containing one female and one male (Table 33). Fecundity of the female decreases as the ratio deviates from 1/1 to either direction.

The trend of the curves shown in Figure 9 is difficult to explain. One logical explanation for a gradual decline in fecundity in 2 female/1 male and 4 female/1 male combinations compared to that in 1 female/1 male could be as follows. Possibly only one female out of 2 or 4 females in 2/1 and 4/1 combinations did mate and the total number of eggs laid per cage actually represents the number of eggs laid by one female and not by all 2 or 4 females. The reason for a decline in egg production with increased males in 1 female/2 male and 1 female/4 male combinations is probably due to a decreased chance for an effective copulation from disturbances by other males or there might have been an excessive copulation of a single female by 2 or 4 males in the same cage. A total of 129 eggs were produced by an unmated virgin female but none of them hatched. Mr. Patrick Brennan of Michigan State University also reported his observation on unfertile eggs produced by virgin females (personal communication).

The trend observed in the second test with field-collected spring adults is quite different from that in the first test. The egg production per female remained steady in all cages while the number of eggs produced per cage increased with the increase in the number of females from 1 to 2 to 4 (Figure 9). An average of 126 eggs were produced by an isolated female. All of the eggs were normal in viability.

A further investigation on the influence of different sex ratios on fecundity of individual females is needed. The results obtained from this experiment, however, seem to indicate that different sex-ratio combinations in a given unit area or space prior to the initial mating influence the fecundity of individual females while it is no longer influential in the mated females.

Experiment 19. Predation of Cereal Leaf Beetle Eggs by a Native Coccinellid Predator

Methods: Eight pots of oats containing fresh cereal leaf beetle eggs were obtained from the rearing program (Connin, *et al.*, 1966a). The number of eggs in each pot was counted and the plants were caged with lantern globes. One coccinellid was placed in the first cage, two in the second, four in the third, and eight coccinellids in the fourth cage. Each treatment was replicated twice and the number of eggs remaining in each cage was counted after 1, 2, and 4 days.

Results: A native coccinellid predator, *Coleomegilla maculata lengi* Timberlake, has been reported as an important predator of the cereal leaf beetle eggs in Michigan by Castro (1964) and Castro, *et al.* (1965). The results of this experiment are shown in Table 35.

A single coccinellid consumed an average of 19 to 76 eggs during the first 24-hour period. The table indicates that the number of eggs consumed per coccinellid per unit time decreased as the time progressed from 1 to 2 to 4 days and also with a gradual increase in the number of coccinellids per cage from 1 to 2, 3, and 4. In both situations the number of eggs available per coccinellid gradually decreased thus causing a decline in predation efficiency by the coccinellid.

Even with a single coccinellid per cage, 82 to 86 percent egg predation occurred in 4 days. With 2 and more coccinellids per cage 88 to 99 percent egg loss was observed in 2 days and 99 to 100 percent egg consumption by the coccinellid in 4 days. C. maculata lengi Timberlake seems to be an efficient predator of the cereal leaf beetle eggs under a confined laboratory condition. It was also observed to feed on the cereal leaf beetle larvae in the laboratory when no other prey was available. Its feeding habit of possessing a variety of prey, however, would certainly reduce its efficiency as a predator of the cereal leaf beetle eggs under field conditions.

TABLE 35.--The number and percent of cereal leaf beetle eggs consumed by a coccinellid predator

Days after Test Began		No. Coccinellid per Cage 1 I	1 II	2 I	2 II	3 I	3 II	4 I	4 II
1	A	125	139	74	79	42	42	23	24
	B	44	76	48	56	21	32	19	22
	C	35	55	65	71	51	76	84	93
2	A	81	63	26	23	21	10	4	2
	B	17	14	25	20	16	9	3	1
	C	49	65	99	96	89	97	97	97
4	A	54	49	1	3	5	1	1	1
	B	46	24	1	2	4	1	1	1
	C	86	82	100	99	99	100	100	100

A: No. eggs available per coccinellid.

B: No. eggs consumed per coccinellid per given period.

C: Percent eggs consumed per coccinellid per given period (cumulative).

Diapause Studies

According to Castro *et al.* (1965) the cereal leaf beetle seems to have only one generation a year with an obligatory diapause under field conditions in Michigan. This implies that the beetle remains inactive for nearly nine months a year in the field.

To find a means by which the newly emerged summer adults can be prevented from going into diapause or the diapausing beetles can be quickly reactivated for continuous reproduction is essential for rearing of the beetle in a year-round research program. The author was mainly interested in the influence of physical factors, especially temperature and photoperiod, on the induction and termination of diapause in this insect.

Experiment 20. Egg Production by Newly-Emerged Summer Adults After a Brief Exposure to Extreme Temperatures

Methods: Newly emerged summer adults were treated in the following ways.

A. -10° F. for 1 minute followed by 120° F. for 2 minutes.

B. -10° F. for 1 minute.

C. 120° F. for 2 minutes.

D. 120° F. for 2 minutes followed by -10° F. for 1 minute.

T. No treatment (80° F. constant).

Each treatment consisted of two replicated lamp chimney cages each containing 20 summer adults (10 pairs). After the treatments were made all the beetles were held at 80° F., 75 percent relative humidity, and 16-hour photoperiod during a 60-day test period. The "Clintland 60" oat seedlings used as host plants were changed once every four days, the number of eggs deposited was counted, and the data were analyzed using an F-test.

Results: As Table 36 indicates the egg production in all treatments is extremely irregular and inconsistent.

TABLE 36.--Egg production by newly emerged summer adults after a brief exposure to extreme temperatures[a]

Treatment[b]	No. Eggs per Cage per 10 Females I	II	Total	Mean	Days First Eggs Observed
-10°/120° F.	0	470	470	235	50
-10° F.	470	0	470	235	25
120°/-10° F.	203	0	203	101.5	37
120° F.	103	0	103	51.5	54
80° F. Const.	0	1	1	0.5	60

F = 0.3846 (N. Significant)

[a]Test terminated after 60 days.

[b]Exposure time: 1 minute at -10° F. and 2 minutes at 120° F.

Egg production by the treated adults seems to be somewhat higher than the untreated adults at 80° F. constant. An F-test, however, indicated no significant difference in egg production among the five different treatments. The first eggs were observed in 25 to 60 days depending on the treatment. In general, the total egg production in all treatments was low and inconsistent, and no conclusive evidence of continuous and consistent egg laying by newly emerged adults could be observed from this experiment. All the eggs laid by the newly emerged adults were normal in a sense that the percentage hatching is comparable to that of the eggs laid by spring or overwintered adults.

Experiment 21. Egg Production by Field-Collected Summer Adults After a 30-Day Storage Period at Low Temperatures

Methods: The summer adults used in this experiment were collected in the field on July 18, 1965. The beetles were exposed to different low temperatures for varying period of time as follows.

A. 38^{o} F. for 15 days followed by 32^{o} F. for 10 days followed by 15^{o} F. for 5 days.

B. 38^{o} F. for 15 days followed by 32^{o} F. for 15 days.

T. 38^{o} F. constant for 30 days.

Thirty adults (15 pairs) were placed in each of three replicated cages for each treatment. Following the treatments all the cages were held at 80^{o} F., 75 percent relative humidity, and 16-hour photoperiod. The plants (oats) were changed every other day and the number of eggs laid was counted. The data were analyzed using an analysis of varience method.

Results: The results presented in Table 37 show that the egg production in this experiment is considerably greater and more consistent than that observed in previous experiment. This is probably caused by the fact that the beetles used in this experiment had been subjected to low temperatures for 30 days prior to the test.

The mean egg production in all treatments is not significantly different from one another when the data were analyzed using an F-test. Castro, *et al*. (1965) reported that the hibernating beetles were successfully induced to reproduce by subjecting them to temperatures of 25^{o} - 43^{o} F. for 15 - 25 days prior to exposing them to higher temperatures of 75° - 80° F. In fact, some of the newly emerged adults can be induced to lay eggs even without subjecting them to low temperatures as it is shown in Tables 43, 44 and 45. An important question is how soon the beetles would start laying eggs and how consistently they would lay. The egg production in this experiment is obviously low compared to that by spring adults and other beetles that had been stored at 38° F. for more than 30 days (Table 38).

TABLE 37.--Egg production by field-collected summer adults after an exposure to low temperatures for 30 days[a]

Cold Treatment	No. Eggs Laid per Cage per 15 Females			Total	Mean	First Eggs Laid in Days
	I	II	III			
38°/32°/15° F.[b]	761	744	228	1,733	578	7
38°/32° F.[c]	480	564	643	1,687	562	8
38° F.[d]	360	209	992	1,561	520	11

F = N. Significant

[a]Test ended after 30 days.

[b]15 days at 38° F., 10 days at 32° F., followed by 5 days at 15° F.

[c]15 days at 38° F. followed by 15 days at 32° F.

[d]Entire 30 days at 38° F.

Experiment 22. Egg Production by Summer Adults after Varying Periods of Storage at 38° F.

Methods: This experiment consists of two separate tests. In Test 1, the summer adults stored at 38° F. were taken out after 30, 50, 70, 90, 130, 160, and 220 days. Fifteen pairs of adults were placed in each lamp chimney cage and two replicated cages were made for each storage group. All the cages were then held at 80° F., 75 percent relative humidity, and 16 hours light period. "Clintland 60" oat seedlings were used as host plants and they were changed every other day for egg counts. The data on cumulative egg production during the first 30-day period were analyzed using an F-test.

In Test 2, only one pair of beetles were kept in each lamp chimney cage. The beetles used in this test had been stored at 38° F. for 50, 80, 110, and 130 days prior to the test. Also used in this test were field-collected overwintering adults and active spring adults that had been collected in the field in February and April respectively. The number of eggs laid during their life time was recorded. All the rest of the procedures were exactly the same as in Test 1. The data were analyzed using an F-test and 95 percent confidence limits for the mean egg production per female for each group were calculated.

<u>Results</u>: Table 38 shows the total egg production per cage per 15 females that had been held at 38° F. for varying periods of time.

As the storage period increases from 30 to 90 days the egg production also increases accordingly. The peek egg production is at 90 days of storage at 38° F. and the egg production gradually decreases from there on. An F-test indicates that the mean egg production is significantly different from one another at the 5 percent level. The least significant difference (LSD) also shows that the egg production at 90 days is significantly greater than that at 30 days at the 1 percent level and that at 50 and 70 days at the 5 percent level. The egg production at 90, 130, 160, and 220 days is not significantly different from one another. Active spring adults collected in the field laid as many eggs as the others that had been stored at 38° F. for 130, 160, and 220 days. From this analysis it is apparent that at least 90 days of storage period seems to be required for the summer adults to complete their diapause development at 38° F.

TABLE 38.--Egg production by summer adults after varying periods of storage at 38° F.

Storage at 38° F. in Days	No. Eggs per Cage per 15 Females		Total	Mean	Days First Eggs Laid
	I	II			
30	361	222	583	292	11
50	515	358	873	437	13
70[a]	570	831	1,401	701	7
90	1,263	1,983	3,246	1,623	6
130	1,632	848	2,480	1,240	5
160[a]	1,620	1,218	2,838	1,419	4
220[a]	1,026	1,134	2,160	1,080	5
Spr. Adults[b]	1,383	1,044	2,427	1,213	3

F = 4.3846 (Significant) $LSD_{.01}$ = 1,265

$LSD_{.05}$ = 835

[a]Laboratory-reared adults. All the rest were field-collected and laboratory-stored adults.

[b]Field-collected active spring adults collected in early April. Data not included in analysis.

In order to obtain more detailed information regarding egg laying capacity of individual females, the beetles were raised singly or in pairs in lamp chimney cages. The results of this test shown in

Table 39 are generally in agreement with those observed in previous test with group culture (Table 38).

Again the egg production increases with an increase in storage period up to 110 days and it declines with further increase in the storage period. The largest egg production was observed among the beetles that had been stored for 110 days at 38^{o} F. and one female in this group produced 1,251 eggs during a 153-day period. This may well be considered as an exceptional case but it indicates a high reproductive potential possessed by this species.

Although the multiple mating is the rule in this species (Castro, et al., 1965; and Merino, 1966), a single mating seems to be generally sufficient for a full production of eggs. One isolated female after the first mating laid 465 eggs while another female produced 629 eggs. In the latter egg hatching was normal in the first 400 eggs, the rest of over 200 eggs were unfertilized, and egg laying stopped completely after laying a total of 629 eggs. By reintroducing an active male into the cage the same female laid additional 293 eggs of normal viability. This observation generally confirms an earlier report made by Dr. R. F. Ruppel of Michigan State University that there were no significant differences between the number of eggs nor the viability of the eggs produced by the isolated females and isolated pairs (unpublished data in 1965). The multiple matings, however, seem to increase egg production as well as viability in some females that survive for a long period of time.

One isolated virgin female laid 129 sterile eggs without mating while the other female did not lay eggs until an active male was introduced into the cage. Normally the female beetles produce about 35

TABLE 39.--Total number of eggs laid by isolated pairs and isolated females

Storage at 38° F. in Days	No. Eggs Laid per Female per Cage							Total	Mean	Days First Eggs Laid	Longevity of Female in Days
	I	II	III	IV	V	VI	VII				
50	165	87	74	71	40	37	14	488	69.7	4-8	15-21
80	219	133	--	--	--	--	--	352	176.0	5	16-18
110	1,251	922[a]	815	465	303	34	--	3,790	631.7	5-22	63-153
130	631	505	92	--	--	--	--	1,228	409.3	3	10-36
Winter Adult[b]	480	405	350	271	214	182	--	1,902	317.0	4-10	30-39
Spring Adult[c]	256	232	100	84	49	40	--	761	126.8	--	8-43

F = 4.7625 (H. Significant) $LSD_{.01} = 414.4$

$LSD_{.05} = 305.8$

[a]Female isolated after the first mating. Egg laying ceased after laying 629 eggs. By reintroducing an active male, additional 293 eggs were laid.

[b]Overwintering adults collected on February 9, 1966 near Galien, Michigan.

[c]Active spring adults collected in early April of 1966 near Galien, Michigan.

percent of unfertilized eggs even in the presence of males in the laboratory (Table 46).

The data presented in Table 39 were analyzed using an F-test. The differences in the mean egg production by adults of different age groups are highly significant. As the least significant difference (LSD) values indicate the adults after 110 days of storage at 38° F. laid significantly greater number of eggs than those laid by winter and spring adults and by the adults that had been stored at 38° F. for 50 and 80 days. The difference between the mean egg production by the adults stored for 110 days and that by the adults after a 130-day storage period is not statistically significant.

Table 40 shows 95 percent confidence limits for the mean egg production per female. According to the table the adults that had been stored for 110 days are capable of laying from 163 to 1,099 eggs per female while active spring adults would lay from 28 to 225 eggs per female. A wide range in the number of eggs produced by different individuals is reflected in exceptionally large coefficient of variation values. Such high values could be resulted from individual differences in egg-laying capacity and longevity and also due to inadequate samples. Ninety-five percent confidence limits for the mean egg production by the adults that had been stored at 38° F. for 80 and 130 days are not presented in Table 40. The calculated values for these two groups of adults did not reasonably represent the mean egg production because of small number of samples.

The mean egg production of 126 eggs per female in the spring adults is slightly higher than 96 eggs per female reported by Dr. R. F. Ruppel of Michigan State University (unpublished data). The

TABLE 40.--Ninety-five percent confidence limits for mean egg production per female

Storage at 38^{o} F. in Days	No. Eggs per Female: Range	Mean	Coefficient of Variation	95% Confidence Limit
50	14-165	69.7	70.4	$24.2 < \mu < 115.2$
80	133-219	176.0	34.5	Omitted
110	34-1,251	631.7	70.6	$163.6 < \mu < 1{,}099.8$
130	92-631	409.3	41.9	Omitted
Winter Adult	182-480	317.0	36.3	$196.1 < \mu < 437.9$
Spring Adult	40-256	126.8	73.9	$28.5 < \mu < 252.2$

latter's report of 96 eggs per female, however, was based on his field data. Castro, *et al.* (1965) reported that approximately from 150 to 400 eggs per female is expected in the laboratory. In Europe, it is reported that one female lays up to 50 eggs during a season in England (Hodson, 1929), 50 to 150 eggs in Rumania (Knechtel and Manolache, 1936), and 100 to 150 eggs in Italy (Venturi, 1942).

The oviposition rate of the female was relatively constant during an entire egg-laying period. The rate of 15 to 20 eggs per female day is most common. One female produced up to 27 eggs in a 24-hour period. These figures are generally higher than those reported by Hodson (1929) of 1 to 3 eggs a day, by Knechtel and Manolache (1936) of 2 to 18 eggs, by Venturi (1942) of 3 to 12 eggs, and by Borg (1959) of 5 to 6 eggs per female day in Europe.

Diapause development in the cereal leaf beetle seems to be completed by the end of December under field conditions in Michigan. Hibernating adults collected in the field in early January of 1966 for an observational purpose became immediately active and produced a considerable number of eggs in the laboratory. Tables 38 and 39 seem to indicate that once their diapause development is completed younger adults are more vigorous and produce more eggs than older ones whose reproductive activities are hindered and delayed by unfavorable environmental conditions. Therefore, in the region where spring comes early in March the adults would lay more eggs and the subsequent larval damage would be much more severe than in the region where spring comes late in May. Even in a given region, the same phenomenon would be observed in a particular year when spring comes early. Kadocsa (1916) reported that early warm spring favors population build-up in this species.

Experiment 23. Mortality of Field-Collected Summer Adults Stored at 38^{o} F.

Methods: A large number of summer adults were collected in the field during their peak emergence period. Two hundred adults were placed in each plastic container (3-1/2 x 3-1/2 x 3 inches) with a piece of moist paper towel. These containers were then stored for later uses in a cooler that was adjusted to 38^{o} F. The date the beetles entered the cooler, the date they were removed from the cooler, and the number of dead and live beetles were all recorded in a record book.

The collection and maintenance of the beetles were done by Messrs. David L. Cobb and George Lawson of Entomology Research Division, USDA, and Mr. John C. Arnsman of Michigan State University. The writer is grateful for their aid in furnishing valuable data for this study.

Over 180-day period, a total of 264 containers having 52,800 beetles have been examined. For each duration-of-storage category a median duration in days was calculated and this was converted to logarithm (X). The conversion was made because the observed points fit better on a semi-log scale. The percentage mortality for each category was converted to Probit using a table provided by Finney (1952). A linear regression equation was calculated from logarithm time (X) and Probit mortality (Y).

Results: The time-mortality relationship at a constant storage temperature of 38^{o} F. for field-collected summer adults is shown in Table 41 and Figure 10. All observed points fall very closely to the empirical regression line. $LD_{.50}$ was calculated to be 146.8 days at

TABLE 41.--Observed and calculated time-mortality relationship for summer adults stored at constant 38° F.

Duration at 38° F. in Days	No. Containers Examined	Median Duration in Days (X)	Log. X	Percent Mortality	Probit: Mortality (Y)
52	2	52.0	1.7160	3.0	3.12
67-80	9	76.1	1.8814	12.0	3.83
82-100	15	94.2	1.9741	18.7	4.11
104-120	21	108.1	2.0338	32.2	4.54
121-140	40	133.7	2.1261	37.8	4.69
141-150	39	144.6	2.1602	43.4	4.83
151-169	41	159.8	2.2036	62.6	5.32
171-180	57	174.9	2.2428	64.7	5.38
181-189	40	183.7	2.2641	65.0	5.39
Total	264		18.6021		41.21

Regression equation: Probit $Y = 4.2102X - 4.1221$
Correlation coefficient: $r = 0.9911$

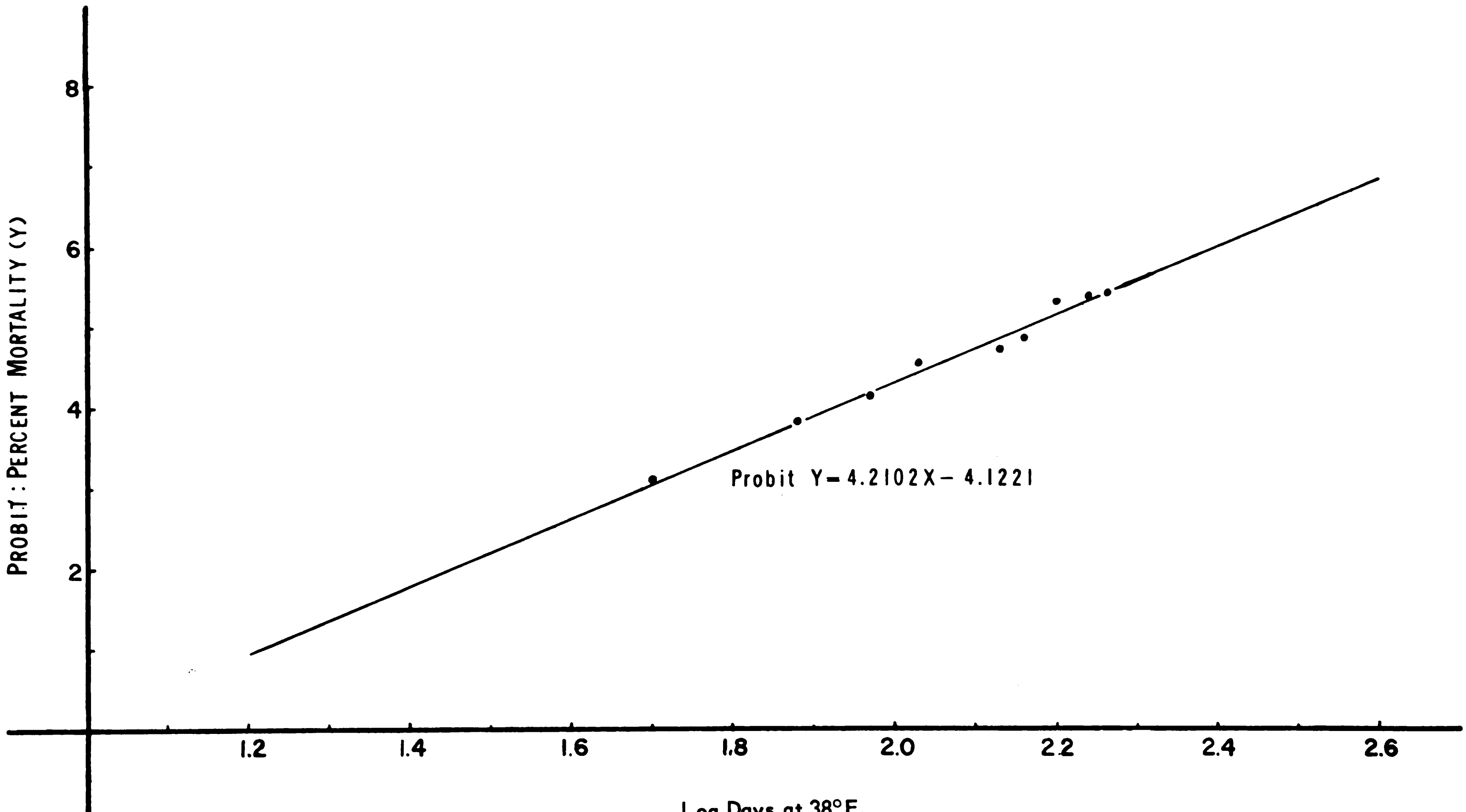

Fig. 10 – Observed and empirical time-mortality relationship for the summer adults stored at constant 38° F.

38° F. and other time-mortality values obtained from the equation are presented in Table 42.

TABLE 42.--Empirical time-mortality values for summer adults stored at 38° F.

Percent Mortality	Duration at 38° F. in Days	Percent Mortality	Duration at 38° F. in Days
5	59.7	50	146.8
10	72.8	55	157.2
15	83.3	60	168.6
20	92.6	65	181.2
25	101.5	70	195.5
30	110.2	75	212.2
35	118.9	80	232.6
40	127.7	85	258.7
45	137.1	90	295.9

The survival of the adults at 38° F. seems to be better than that at 43° F. Castro, _et al._ observed 30 percent mortality after 90 days and 50 percent after 160 days of storage at 43° F. Their percent mortality figures are obviously higher than those shown in Table 42 during the same periods of storage at 38° F. The higher percent mortality at 43° F. seems to be due to a higher metabolic activity than at 38° F. A further investigation is obviously needed to improve the present storage methods so that a greater number of summer adults could be preserved for much longer period of time.

Experiment 24. Egg Production by Field-Collected Summer Adults under Different Photoperiods

Methods: The summer adults used in this test were collected in the field in early July. Thirty beetles (15 pairs) were placed in each lamp chimney cage and two of these cages were placed in each of six growth chambers that were adjusted to 0, 8, 12, 16, 20, and 24 hours light periods. All the chambers were maintained at 80° F. and 75 percent relative humidity. "Clintland 60" oat seedlings used as host plants were changed every other day for egg counts. The data were analyzed using an analysis of varience method.

Results: The total egg production per cage during a 38-day period and the mortality of adults at the end of the test period are shown in Table 43.

The table indicates that the egg laying pattern under different photoperiods is very much similar to that observed in the overwintered adults (Table 14). The largest egg production was again observed in the region of 16 hours photoperiod. Under a constant 12-hour light period no eggs were produced by the beetle. The results of this experiment confirms an earlier report made by Castro, et al. (1965). The differences in the mean egg production by the adults under different photoperiods, however, were not significant according to an F-test. Use of three or more replicated cages could have yielded more conclusive results.

The summer adults collected on the same day did not lay eggs in a large screen cage (15-1/2 x 15-1/2 x 15-1/2 inches) even under the favorable environmental conditions. The author was unable to detect the causes for such difference in reproductive activity in the

TABLE 43.--Egg production by newly emerged summer adults under different photoperiods during a 38-day test period

Photoperiod in Hrs. per 24-Hr. Cycle	No. Eggs Laid per Cage per 15 Females					
	I	II	Total	Mean	First Eggs Laid in Days	% Adult Mortality after 38 days
0	116	43	159	80	6	60.5
8	104	13	117	59	4	76.7
12	0	0	0	0	--	21.7
16	484	140	624	312	10	38.4
20	0	57	57	29	30	28.4
24	0	0	0	0	--	22.7

F = N. Significant

summer adults. The amount of light penetrating through the cage or the light intensity inside the cage as well as the cage size as was demonstrated in Experiment 13 (Table 24) seem to have at least some effect on inducing egg production in the summer adults. Even in the small lamp chimney cages the adults are extremely sluggish and all the activities remain at the minimum level.

Experiment 25. Egg Production by Newly-Emerged Summer Adults for Three Successive Generations under Different Photoperiod Combinations

Methods: The eggs laid by summer adults under a 16-hour light period in a preceding experiment were divided into three groups. The first group of eggs was held under 12-hour light period, the second group under 24-hour light, and the third group under a 16-hour light period. Temperature and relative humidity in all three growth chambers were maintained equally at 80^{o} F. and 75 percent respectively. Larvae hatching from these eggs were allowed to develop in each growth chamber until they reached the adult stage.

All the newly emerged adults under 12- and 24-hour light periods were immediately transferred to a constant 16-hour light period. Thirty adults (15 pairs) were placed in each lamp chimney with fresh "Clintland 60" oat seedlings. The plants were changed every other day and the number of eggs laid and adult mortality were recorded. When the first generation adults started laying eggs in their respective cages, the eggs were transferred back to 12- and 24-hour light periods under which the parent beetles were raised. As in the first generation, the immature stages were raised under 12-, 24-, and 16-hour light periods and all the adults were held under a constant 16-hour photoperiod.

The same procedure was used for the third generation and the data were analyzed using a Chi-square test.

Results: The overall results of egg production by three continuous generations of summer adults are shown in Table 44. The first generation adults were raised from the eggs laid by field-collected summer adults. Only those eggs that were laid early in their egg laying period were kept for further investigation on possible non-diapausing strain of the beetle.

TABLE 44.--Egg production by newly-emerged summer adults for three continuous generations under different light periods

Photoperiod in Hrs. per 24-Hr. Cycle	No. Eggs Laid per 15 Females per Generation			Total	50% Mortality of Females in Days
	1st	2nd	3rd		
12-16[a]	2,104	730	312	3,146	56-41-44[b]
24-16	1,077	532	435	2,044	30-53-53
16 Const.	640	167	11	818	89-61-60
Total	3,821	1,429	758	6,008	

Chi-square = 302.1006 (H. Significant)

[a]Immature stages raised under 12-hour light and adults under 16-hour light period.

[b]Percent mortality for the 1st, 2nd, and 3rd generations respectively.

A Chi-square test indicates that there are significant differences in the number of eggs produced by three successive generations under three different light period combinations. The table shows that the egg production decreases from one generation to the next and also with a change in light period from a 12-16-hour combination to 16-hour constant. The adults seem to be more responsive to the changing light period than to the constant photoperiod. General vigor of the newly-emerged adults seems to decrease from one generation to the next. Even though some of the adults did oviposit viable eggs, all of the adults remained very much inactive throughout entire test period. A non-diapausing strain of the beetle did not exist, at least in a sample of population dealt in this experiment.

Experiment 26. Egg Production by Field-Collected Summer Adults in Laboratory and Natural Environment with and Without Hiding Places

Methods: The summer adults used in this experiment were collected in the field near Galien, Michigan on July 11, 1966. Fifteen pairs of adults were placed in each of six lamp chimney cages. Hiding places were provided in three cages by means of a piece of rolled paper towel that was suspended above the soil surface. No hiding places were provided in the other three cages. All six cages were then held at 78° F., 75 percent relative humidity, and a 16-hour light period.

Another set of six cages with and without hiding places were held outside the laboratory building in East Lansing, Michigan until October 11, 1966. "Clintland 60" oat seedlings used as host plants were changed once every four days for egg counts and the data were analyzed using a t-test.

Results: Table 45 shows quite obvious results of this experiment. The summer adults collected on the same day from the same field produced eggs in the laboratory but not in the natural environment during the period from July 11 through October 11, 1966.

TABLE 45.--Egg production by the summer adults in the laboratory and natural environment with and without hiding places

Environment	Hiding Place	No. Eggs Laid per Cage per 15 Females I	II	III	Total
Laboratory	With	0	124	45	169
	Without	5	61	82	148
Outdoor	With	0	0	0	0
	Without	0	0	0	0

With _vs._ without hiding places: t = 0.4000 (N. Significant)

Laboratory _vs._ Outdoor: t = 2.5046 (Significant)

Castro, _et al._ (1965) reported that egg production by summer adults can be more easily induced in lamp chimney cages with a minimum of hiding places. As the table indicates the egg production was not influenced by the presence or absence of hiding places. Even under the laboratory conditions a considerable variation in the numbers of eggs produced among replicated cages, especially between the first and the remaining two replicates. Such variation could be caused by difference in the number of females that were actually laying eggs and also by the individual differences existing among the females. Approximately 10 percent of the adults remained moderately active in all cages even when hiding places were provided. The author's observation indicated that there was a small proportion of summer adults that were a little more active than the others and these active adults seemed to be the ones that produced eggs. Egg production by these small number of adults, however, was extremely irregular and inconsistent.

The difference in egg production between indoor and outdoor cages was apparently due to the differences in environmental conditions. Day length gradually decreased from 13:56 hours on August 15 to 12:31 hours on September 15. This range of light period is apparently not favorable for the summer adults to produce eggs (Expt. 24). Average minimum temperature also decreases considerably from 55.7° to 47.2° F. respectively on the same dates.

The existence of the second generation under field conditions has been reported by Averin (1914) in Russia and by Wilson and Toba (1963) in Indiana. The author's experimental results, however, show that the presence of the second generation in Michigan is very unlikely. A univoltine life cycle with an obligatory diapause reported by Castro *et al*. seems to be the rule in this species.

Life Tables and Net Reproduction Rates

Experiment 27. Age-Specific Life and Fertility Tables Based on Laboratory and Field Mortality Data

Methods: This experiment was conducted in the laboratory that was adjusted to 78° - 80° F., 65 - 75 percent relative humidity, and 16-hour light period. The first table is a generalized life table of the beetle under present rearing conditions in the laboratory. The table form was adapted from Morris (1963) and Embree (1965). A total of 3,987 eggs were examined for hatchability. These eggs were obtained from the rearing room. Only 670 larvae were kept for further studies because of handling difficulties. The larvae, pupae, and newly emerged adults were reared in lamp chimney cages. Inactive summer adults were stored at 38° F. for 90 days following the method developed by

Connin, et al. (1966a). The age interval was divided into six distinct categories, egg, larva, pupa, newly emerged active adult, diapausing adult, and post-diapause active adult. The number of individuals dying during each age interval and possible causes for the mortality were investigated.

The second and third tables were constructed to estimate the net reproduction rate for the laboratory and field populations of the cereal leaf beetle. The table form was adapted from Birch (1948) and Laughlin (1965). The second table was constructed for the laboratory population of adults stored at 38° F. for 90 days. Information on the mortality rate was derived from the first table. The age-specific fertility column (m_x) was computed by multiplying a constant 0.324 to each of the corresponding numbers in preceding f_x column. The constant 0.324 was derived by dividing the survival rate of the eggs 0.648 by two. Assuming 1:1 sex ratio, 32.4 percent of the eggs laid per female would be the fertile female eggs.

The third table was made for the field population. The active spring adults were collected in April of 1966 in the field before they started laying eggs. The adults were brought into the laboratory and their fecundity and survival were recorded under the laboratory conditions. An important assumption made before constructing this table was that the survival and fecundity rates observed in the laboratory approximate sufficiently close to that would occur in the field. The following estimates on survival rates of different stages of the beetle at Galien, Michigan were made by Dr. R. F. Ruppel of Michigan State University (unpublished data in 1965).

Stage	Survival Rate
Egg	0.50
Larva	0.34
Pupa	0.91
Summer Adult	0.85
Overwintering Adult	0.40

Table 48 is based on Dr. R. F. Ruppel's estimates on the survival rates under field conditions in 1965 season. A constant used in this table was 0.25 which is exactly one-half of 0.50, the survival rate of the egg stage in the field. In other words, only 25 percent of the total number of eggs laid by a female would be viable female eggs. The net reproduction rate (Ro), the rate of multiplication in one generation, was calculated by simply adding all $l_x m_x$ values.

Results: Table 46 shows a generalized age-specific life table for the cereal leaf beetle that was reared under the laboratory conditions of 78° - 80° F., 65 - 75 percent relative humidity, and a 16-hour light period (Connin, *et al.*, 1966a). It was assumed that a 90-day storage period at 38° F. is necessary to break a diapause in this beetle.

As the table indicates the highest mortality was observed in the egg and pupal stages. Most of the egg mortality was simply due to the production of unfertilized eggs by female beetles and it accounted for about 35 percent of the total number of eggs produced by the females. Approximately 24 percent mortality occurred in the larval stage from unknown causes. The larval mortality could have been due to a mishandling of young larvae and a slightly crowded condition in small cages. Thirty-nine percent of prepupae entering the soil for pupation

TABLE 46.--Age-specific life table for the cereal leaf beetle under laboratory conditions

x Age Interval	l_x No. Alive at Beginning of x	d_xF Factor Responsible for d_x	d_x No. Dying During x	100 d/l d_x as Percentage of lx	S_x: $(\frac{l_{x+1}}{l_x})$ Survival Rate Within x
Egg	1,000	Unfertilized and unknown	352	35.2	0.648
Larva	648	Unknown	155	23.9	0.761
Pupa	493	Premature pupation and unknown	192	39.0	0.610
Active Summer Adult	301	Unknown	54	17.9	0.821
Diapausing Adult	247	Low moisture, mold, and unknown	45	18.6	0.813
Active Spring Adult	202				

died during the pupal stage. One positive cause for the mortality was premature pupation. Most of these partially developed prepupae constructed complete pupal cases in the soil but failed to make further development into pupae and adults inside the pupal cases.

During a two-week feeding period, about 18 percent of the newly emerged summer adults died from unknown causes. Again the confined caged condition could be one of the contributing factors for the mortality. There was a negligible number of deformed adults that died in a few days after emergence. The mortality of diapausing adults during a 90-day storage period was approximately 19 percent. The mortality in the diapausing adults was mainly due to low moisture level and development of molds in storage containers. Some of the dead adults contained unidentified species of nematodes. Starting with a convenient number of 1,000 eggs, 202 post-diapause adults can be obtained under the present laboratory conditions.

Table 47 shows net reproduction rate for the laboratory population under above mentioned rearing conditions. The table is based on the mortality data given in previous table (Table 46) and a 90-day diapause period at 38^{o} F. was assumed. It indicates that the laboratory population increases about four times in one generation. The net reproduction rate, however, can be increased to some extent by 1) improving rearing techniques and 2) shortening of diapause period using a synthetic hormone reported by Connin _et al_. (in press), for instance.

Table 48 was constructed using the field mortality data prepared by Dr. R. F. Ruppel (unpublished data in 1965). Active spring adults collected in the field in early April were brought into the laboratory and the survival and fecundity rates of the females were measured in

TABLE 47.--Age-specific life and fertility table for the cereal leaf beetle that had been stored at 38^{o} F. for 90 days in the laboratory

x	l_x	f_x	$m_x (= f_x \times 0.324)$	$l_x m_x$
Age Interval in Weeks	Survival Rate of the Female at Age x	No. Male and Female Eggs laid at x	No. Fertile Female Eggs Laid per Female at x	Total No. Fertile Female Eggs Laid in each x
0	1.000			
0-4	0.301	Immature stages		
4-6	0.247	Post-emergence feeding period		
6-19	0.202	Storage period at 38^{o} F.		
19-20	0.199	1.08	0.350	0.070
20-21	0.189	17.54	5.683	1.074
21-22	0.174	24.34	7.886	1.372
22-23	0.148	18.79	6.088	0.901
23-24	0.101	1.92	0.622	0.063
24-25	0.066	3.25	1.053	0.069
25-26	0.056	9.04	2.929	0.164
26-27	0.047	5.63	1.824	0.086
27-28	0.040	7.00	2.268	0.091
28-29	0.035	4.00	1.296	0.046
Total		92.59	29.999	$3.936 = R_o$

TABLE 48.--Age-specific life and fertility table for the field population using field mortality data

x Age Interval in Weeks	l_x Survival Rate of the Female at Age x	f_x No. Male and Female Eggs Laid at x	$m_x (= f_x \times 0.324)$ No. Fertile Female Eggs Laid per Female at x	$l_x m_x$ Total No. Fertile Female Eggs Laid in each x
0	1.000			
0-8	0.158	Immature stages		
8-10	0.134	Post-emergence feeding period		
10-50	0.054	Diapause period		
50-51	0.048	24.55	6.138	0.295
51-52	0.034	32.20	8.050	0.274
52-53	0.023	40.85	10.213	0.235
53-54	0.014	35.17	8.793	0.123
54-55	0.007	7.74	1.935	0.014
Total		150.51	35.129	$0.941 = R_o$

the laboratory. Assuming the survival and fecundity rates in the laboratory approximate sufficiently close to those under the field conditions and the mortality data reported by Dr. R. F. Ruppel are relatively accurate, then the calculated net reproduction rate for the field population in Galien area is close to one. This means the population size remained very much constant in one generation from 1965 to 1966.

The calculated net reproduction rate of .941 for the field population is surprisingly low. It is, however, quite possible that the field population is actually increasing from one generation to the next but the reproduction rate is underestimated because of following reasons. 1) The adult sample used in this test did not adequately represent the field population, 2) the survival and fecundity rates were more or less suppressed in laboratory cages, 3) an excessive handling of the adults, or 4) the survival rates made by Dr. R. F. Ruppel could have been somewhat too low. Obviously more studies on this phase of population dynamics are needed.

General Behavior and Sex Ratio

Laboratory observations showed that the overwintered adults require a minimum of 55° F. for an effective initial mating and oviposition. This observation confirms the report made by Castro, *et al.* that the adults seem to be active when the temperature reaches 55° F. or more. Knechtel and Manolache (1936) reported 50° F. as the threshold for the initiation of adult activity in spring. The flight of overwintered adults was observed when temperature reached about 68° F. in the laboratory. Hodson (1929) and Balachowsky's (1963) reports of flight occurring at 62° F. seems to be somewhat lower while 72° F. reported by Castro, et al. (1965) is slightly higher than the author's observation at about 68° F.

The adult beetles after 110 days of storage at 38° F. laid their first eggs in 4 days at 80° F., in 10 days at 70° F., and in 16 days at 60° F. The egg laying was extremely poor and irregular at 60° F. while it was fairly consistent at 70° F. Multiple mating seems to be the rule in this species as was mentioned by Castro, et al. (1965) and Merino M. (1966). The existence of definite homosexual behavior in the males has been reported by Merino M. (1966) but the author was unable to observe any mating activity taking place between two males. Frequently an active male mounts on the back of another male, attach its protruded aediagus below the elytra of the second male, and remain in that position for some time. Some other males even mount on dead beetles. Homosexual behavior in this sense does exist in this beetle, however.

Oviposition behavior of the female beetle is as follows. Prior to oviposition the female while maintaining the sternum about 2 mm. above the leaf surface moves up and down apparently to locate a proper oviposition site. When the egg laying is imminent the female lowers the abdominal tip to the leaf surface and an egg is deposited. If the leaf width is relatively narrow as in oats (3-4 mm.), the ovipositor falls right on the midvein of the leaf. This is the main reason why majority of the eggs are deposited along the midvein in oats.

The first egg is usually laid on the basal portion of the leaves. The female upon depositing the first egg moves about 1/2 inches up and deposits the second one, and so on only when the plant is in single-leaf stage. When the second leaf appears the female tends to deposite the eggs in a chain-like fashion one right next to the other near the lower portion of the first leaf. The eggs are usually laid singly or in pairs when enough host plants are provided. One female was observed to lay four eggs in a row one after another in the laboratory in about five minutes. It requires from approximately 30 seconds to two minutes from pausing for oviposition to actual deposition of an egg. If the female continues to lay eggs at this speed, the rate would be from 30 to 120 eggs per hour. The highest oviposition rate observed in the laboratory, however, was 27 eggs a day per female and normally from 10 to 20 eggs per female per day during the peak oviposition period. These figures are generally higher than those reported by European workers. It has been stated that one female in spring lays 1 to 3 eggs a day in England (Hodson, 1929), 3 to 12 eggs in Italy (Venturi, 1942), 5 to 6 eggs in Sweden (Borg, 1959), and 2 to 18 eggs per day in Rumania (Knechtel and Manolache, 1936).

Experiment 28. Distribution of Eggs on Oat and Barley Seedlings

Methods: Thirty active spring adults were placed in each lamp chimney cage containing "Clintland 60" oats or "Hudson" barley. The seedlings were at a growth stage where the second leaf was about to emerge at the time of the test. Three replicated cages were used for each host plant. All the cages were held at 80° F., 75 percent relative humidity, and 16 hours photoperiod. The plants were changed every other day and the number of eggs deposited on the apical, central, and basal third of the leaves and those occurring singly, in twos, in threes, in fours, and in fives or more were recorded.

Tests with oats and barley were conducted at different times using different beetles and a direct comparison of the original data for oats and barley could not be made. However, the percentage figures for one host could be reasonably compared with those for the other host plant.

Results: As Table 49 indicates over 99 percent of the eggs were deposited on the upper leaf surface in oats while about 62 percent of the eggs were laid on the same leaf surface in barley. Nearly 68 percent of the eggs were laid singly and about the same proportion of the eggs were deposited on the basal third of the upper leaf surface in oats. In barley, on the other hand, about 45 percent of the eggs occurred singly and also on the basal third of the upper leaf surface.

The proportion of eggs laid on the lower leaf surface was considerably greater in barley than in oats. A Chi^2 test showed that significantly greater number of eggs were laid singly and on the basal third of the two host plants. This experiment clearly demonstrates that the female beetles prefer the basal third of oat and barley leaves for oviposition. The reason why the female beetles prefer a particular leaf surface or portion of a particular host plant is not clearly understood. Oviposition behavior of the females seems to be at least partly influenced by density of the beetle and physical conditions of the host plant. The physical conditions include such factors as stage of growth, condition of the leaf surface, and width and shape of the leaves. At higher density, the basal third of the plants are usually less damaged compared to the upper portion of the leaves and the oviposition activity of the female is less disturbed at the basal region of the plant.

TABLE 49.--Number and percent eggs occurring in different numbers on different parts of oat and barley leaves

Host	No. Eggs in a Row and Part of Leaves	Upper Leaf Surface		Lower Leaf Surface	
		No. Eggs	% Eggs	No. Eggs	% Eggs
Oats	1	1,254	67.86	10	0.54
	2	444	24.03	2	0.11
	3	93	5.03	0	0
	4	40	2.16	0	0
	5 or more	5	0.27	0	0
	Basal 1/3	1,255	67.91	1	0.05
	Central 1/3	446	24.13	7	0.38
	Apical 1/3	135	7.31	4	0.22
	Chi-square = 22.7142 (H. Significant)				
Barley	1	485	44.62	342	31.46
	2	154	14.17	76	6.99
	3	30	2.76	0	0
	4	0	0	0	0
	5 or more	0	0	0	0
	Basal 1/3	489	44.99	179	16.47
	Central 1/3	152	13.98	168	15.45
	Apical 1/3	28	2.58	71	6.53
	Chi-square = 11.3247 (H. Significant)				

The author's results are generally in agreement with those reported by Castro, et al. (1965). The latter's figures are slightly lower than those in Table 49 in that they found 98.5 percent of the eggs on the upper surface of oat leaves, about 60 percent on the basal third, and about 42 percent occurring singly on the upper leaf surface.

Experiment 29. Oviposition Behavior of Female Beetles on Oats with Different Leaf Postures

Methods: It was learned from the preceding experiment that the cereal leaf beetle lays a greater number of eggs on the upper surface than on the lower surface of oat leaves. This experiment was intended to see if such oviposition pattern would be influenced by different leaf postures.

Exactly 25 "Clintland 60" oat seedlings of the same age group were allowed to grow in each plastic pot of four inches in diameter. The following pretest treatments were made on the oat seedlings in three pots. 1) Leaves in the first pot were slightly broken at the base and maintained horizontally to show their upper surface up, 2) leaves in the second pot were also maintained horizontally but with their upper surface down, 3) leaves in the third pot were maintained in normal upright posture.

Above three pots were then placed in an oviposition cage that contained actively ovipositing spring adults. The oviposition cage was held at 78° F., 70 percent relative humidity, and a 16-hour photoperiod. The plants were changed daily for four consecutive days and the number of eggs deposited on the upper and lower surfaces of the leaves was counted. The data were analyzed using an F-test.

Results: Table 50 shows that different leaf postures did not influence oviposition behavior of the female beetles.

TABLE 50.--Oviposition of eggs on upper and lower surfaces of oat leaves with different leaf postures

Leaf Posture	Leaf Surface	No. Eggs Deposited I	II	III	IV	Total	Mean
Horizontal, Upper Surface Up	Upper	18	27	21	31	97	24.3
	Lower	4	0	1	0	5	1.3
Horizontal, Upper Surface Down	Upper	32	31	16	76	155	38.8
	Lower	0	0	0	0	0	0
Normal	Upper	23	24	10	31	88	22.0
Upright	Lower	1	0	0	0	1	0.3

Leaf posture: F = 2.0566 (N. Significant)

Leaf surface: F = 34.7238 (H. Significant)

No matter how the leaves stand and what direction the upper surface of the leaves are facing, a significantly greater number of eggs are laid on the upper leaf surface than on the lower surface in oats. The difference in the number of eggs deposited on the upper leaf surface and that on the lower leaf surface is highly significant according an F-test.

Experiment 30. Sex Ratios of Laboratory-Reared and Field-Collected Adults

Methods: A total of 1,781 spring and summer adults, laboratory-reared and field-collected, were dissected under a binocular scope for sex determination. The laboratory-reared spring adults denote the beetles that had been stored at 38° F. for 145 days after their emergence from the soil. A Chi^2 test was used to analyze the data.

Results: Table 51 shows the results of analyses on the sex ratio of four different samples.

TABLE 51.--Sex ratios of field-collected and laboratory-reared spring and summer adults

Source of Samples	Stage of Adults	No. Female	No. Male	Total	Chi^2
Field	Spring	361	239	600	24.806 (H.S.)
	Summer	139	116	255	1.579 (N.S.)
Laboratory	Spring	179	156	335	1.579 (N.S.)
	Summer	322	269	591	4.753 (Signif.)

The number of females is consistently greater in all four samples. The sex ratio of field-collected spring adults and laboratory-reared summer adults was found to be significantly different from one to one while that of the other two samples was proved to be not significant according to the Chi^2 test. The reason why the former two samples contained significantly greater number of females is not known. It could be due to a "chance" factor such as a particular time of collection or due to a larger sample size than the other two samples.

DISCUSSION

As in any other living organisms the development and multiplication of the cereal leaf beetle are directly and indirectly influenced by the conditions of physical and biotic environments in which they live.

Hibernating adults seem to be most resistant to cold temperatures since they were able to survive for a prolonged period of time at 38° F. (Table 42) while the eggs and larvae were unable to survive at 48° F. for more than 5 and 11 weeks respectively. Diapause development can take place at moderately low temperatures above 32° F. Castro _et al._ (1965) reported that the winter survival of the overwintering adults was highest at the ground level and no adults survived at 4 feet above the ground. Mr. M. S. Gomulinski of Michigan State University (personal communication) has reported that the temperature at the snow-covered ground level near Galien, Michigan stayed around 32° F. during winter months and did not drop far below 32° F. The author observed a considerable number of adults under the roadside grasses in early March of 1964 when the temperature was still too low for the adults to fly. Mr. John T. Hayward of Plant Pest Control Division, USDA, found a large number of overwintering adults in the litter and duff samples near Galien, Michigan in late winter of 1967 (unpublished report).

Based on these observations the most probable site for the majority of overwintering adults seems to be the ground level that is covered with snow. The layer of snow on the ground serves as an effective barrier and insulator and it protects the beetles from direct exposure to the excessive cold during winter months. The adults overwintering under the loose bark or inside the cracks of fence post would be directly exposed to freezing winter temperatures and a prolonged exposure to such extreme temperature would eventually result in a high overwintering mortality. The adults were unable to hang on to the plants when temperature was dropped to 38° F. in the laboratory.

The heaviest winter mortality is expected in the area where there is only a little snow on the bare ground and temperatures stay well below 32° F. during the winter months. The winter kill will be relatively low in the area where winter temperatures stay around 32° F. and the right amount of moisture or free water is available to the beetle. Diapause development in the cereal leaf beetle seems to be completed by the end of December under the field conditions in Michigan because the mating and oviposition activities of the overwintering adults collected in January was comparable to those of active spring adults in the laboratory (Table 39). Diapause development, therefore, apparently proceeds during late fall and early winter months as was indicated by Zolotarev (1947).

Fecundity of the female seems to be highest immediately after the completion of its diapause development and it decreases gradually as time progresses (Tables 39 and 40). In the area where the spring comes early, the individual female would lay more eggs than in the area where the winter is long and a cold spring prevails. In the former area an early warm spring would certainly favor the population build-up as was mentioned by Kadocsa (1916) while in the latter area the maximum fecundity or egg production cannot be realized.

The overwintering beetles become active during the first warm days in spring (Castro _et al_. 1965). From the author's observation it is believed that at least some mating can take place in the overwintering site before they move into the green grasses or other cultivated crops. In the laboratory the mating often preceded the feeding activity. The flight of the spring adults occurred freely when temperatures reached 68° F. or more in the laboratory. The flight temperatures of 62° F. reported by Hodson (1929) and 62.6° F. by Balachowsky (1963) seem to be a little lower than that observed by the author.

The multiple mating without any noticeable courtship behavior is reported as the general rule in this beetle (Castro _et al_., 1965 and Merino, 1966). In the laboratory one female that was isolated from the male after the first mating laid up to 629 eggs in an isolated cage (Table 39). Out of 629 eggs about 400 eggs were normal in hatchability and the rest of the eggs were all unfertilized. When a male beetle was introduced into the cage, the same female laid an additional 293 eggs and the hatchability gradually increased. Another female laid 465 eggs in an isolated cage. From these observations it was concluded that the single mating would be sufficient for average females to lay normal eggs during their life time. The multiple mating, however, apparently stimulates oviposition activity and increases hatchability of the eggs in some cases. There is a report of homosexual mating behavior in this beetle (Merino, 1966) but the author was unable to detect any incidence of actual copulation by two males.

An active male would mount on the back of another male, insert its aedeagus below the elytra of the other male, and remain in that position for some time. Such apparent copulatory behavior between two males can be frequently observed upon a close look at the pair of the beetles.

A complete cessation of movement by the larvae and adults occurred at 38^{o} F. but a slight movement was observable at 45^{o} F. in the laboratory. A noticeable feeding activity was observed at 50^{o} F. but it was negligible at 45^{o} F. A minimum of 55^{o} F. seems to be required for an effective initial mating and egg deposition and at least of 70^{o} F. for more consistent egg deposition.

The reproductive activity of the spring adults is largely influenced by temperature, photoperiod, and relative humidity. The egg production by the spring adults is greater at 80^{o} F. than at 85^{o} F. (Table 9) and the optimum temperature for the spring adults seems to be 80^{o} F. The mean monthly temperatures recorded at Eau Claire Station in Michigan are 48.3^{o} F. in April, 59.1^{o} F. in May, and 69.5^{o} F. in June. Majority of the eggs are laid in May (Castro _et al._, 1965) and it is quite reasonable to assume that most of them are laid during day light hours when temperature modified by the sunlight are sufficiently high.

Photoperiod also influences the egg production in the spring adults. The range of photoperiod favorable for the spring adults is between 16 and 18 hours but the maximum oviposition rate was observed under 18 hours of light period in the laboratory. Under field conditions, however, the photoperiod never reaches 18 hours. Photoperiods in Kalamazoo, Michigan are recorded as 13:22 hours on April 15, 14:36 hours on May 15, and 15:16 hours on June 15 and majority of the eggs are laid between 14 and 15 hours of light period in the field. As Table 15 shows egg production under 14-hour photoperiod is relatively low compared with that under 16 and 18 hours of light periods. From the fact that the spring adults lay most of their eggs during day hours (Table 17) and the largest quantity under 16-18 hours indicate that the cereal leaf beetle is definitely a diurnal and long-day insect.

The egg production by the spring adults is also significantly influenced by relative humidity. The higher the relative humidity the greater was the egg production in the laboratory test. The mean relative humidities are recorded at South Bend, Indiana as 57% in April, 46% in May, and 48% in June at 12:00 noon and those recorded at Muskegon, Michigan are 58, 56, and 60% respectively at 1:00 P.M. These data might be considerably different from the actual values that can be expected near the micro-environment the cereal leaf beetle chooses. The relative humidity as well as temperature in the actual habitat of the beetle are expected to be somewhat higher than those recorded at weather stations. The mean percipitation is highest (4.18 inches) in June and followed by 4.08 inches in May and 3.88 inches in April according to the data recorded at Eau Claire, Michigan.

The optimum environmental conditions, therefore, seem to be 80^{o} F., 18 hours of photoperiod, and 95 percent of relative humidity. Obviously such ideal constant conditions will never be attained in nature and consequently a full reproductive potential of the cereal leaf beetle will not be realized. The beetles may, in fact, be more adaptive under such "sub-optimum" conditions of natural field environment. The "sub-optimum" conditions could allow more flexibility and this would provide a selective advantage for the maintenance of entire population.

Fecundity and longevity of individual females vary widely. Laboratory reared females after 110 days of storage period at 38° F. laid from 34 to 1,251 eggs with an average of 631 eggs per female. Field collected summer adults after 130 days at 38° F. deposited from 92 to 631 eggs with an average of 409 eggs per female. Overwintering adults collected on February 9, 1966 in the field and stored for 48 days at 38° F. produced from 182 to 480 eggs with an average of 317 eggs. Lastly active spring adults collected on April 28, 1966 when a few eggs were observed in the field laid from 40 to 256 eggs with an average of 126 eggs per female under laboratory conditions of 80° F., 16-hour photoperiod, and 75 percent relative humidity (Table 40). Average number of eggs per female per season is reported as 50 by Hodson (1929), 50 to 150 by Knechtel and Manolache (1936), and 100 to 150 by Venturi (1942). The author's observation of 40 to 256 eggs per female for active spring adults includes the figures presented by above European workers. A report by Castro *et al.* of 150 to 400 eggs per female per season at 80° F. is also confirmed by the author's laboratory test.

Individual females in isolated cages in the laboratory laid from 5 to 27 eggs per female per day with an average of about 20 eggs during their peak oviposition period. These figures are expected to be somewhat lower under the field conditions. The rates of oviposition in terms of the number of eggs laid per female per day are reported as 1 to 3 by Hodson (1929), 2 to 18 by Knechtel and Manolache (1936), 3 to 12 by Venturi (1942), and 5 to 6 by Borg (1959) (Table 2).

Temperature seems to be the most important single factor which directly controls the development of the cereal leaf beetle. The maximum range of temperature for the development of the immature stages of the cereal leaf beetle seems to be between 54^{o} F. and 92.5^{o} F. where 54^{o} F. is the threshold for the development of pupae and 92.5^{o} F. the peak temperature at which the embryo develops at the fastest rate. Thresholds for the development of egg, larva, pupa, and entire immature stage were calculated using Uvarov's (1931) formula and a straight velocity line method used by Matteson and Decker (1965). Based on the authors repeated tests, the thresholds obtained using Uvarov's formula seem to be more accurate than those obtained by the latter method (Table 7). Knechtel and Manolache (1936) reported the thresholds of development as 53.6^{o} F. for the egg and larva and 48.2^{o} F. for the pupa. According to the author's observation and calculation the pupal stage requires a higher threshold for the development than the egg or larval stage and this is exactly the opposite to the data presented by Knechtel and Manolache (1936).

Both Davidson's (1944) logistic curves and straight velocity lines were used to express the relationship between temperature and the rate of development of the egg, larva, pupa, and entire immature stage. The reciprocals of the days required for the development of a given stage form an S-shaped velocity curve when they are properly plotted. Observed points follow very closely to the Davidson's logistic curves throughout most of the temperature range except at 95° F. for egg stage and 76° F. for all stages (Figures 2, 3, 4, and 5). At 95° F., the rate of development of the embryo has slowed down because of an excessively high temperature. Temperature in a growth chamber that was supposed to be set at 76° F. apparently had not been maintained properly. When the linear regression equation was used, the observed points does not follow as close to the calculated lines as in the case of Davidson's logistic curves (Figure 6). However, computation involved is much simpler in the case of straight lines and they still give considerably accurate estimates on the rate of development of a given stage throughout most of the temperature range.

The author's data on duration of the development of different stadia at various constant temperatures are generally in agreement with those reported by Castro *et al.* (1965) at 80° F. and by Knechtel and Manolache (1936) except the duration of pupal stage (Table 2). The latter's report on the pupal stage of 5 days at 30° C. (86° F.) and 6 days at 26° C. (78.8° F.) seems to be more accurate estimation of the pupal period since the author's observation was based on the period from the day a prepupa enters the soil for pupation until the day it emerges from the soil as an adult.

It was observed in the laboratory that the cereal leaf beetle eggs cannot develop at constant temperature of 48° F. One hundred percent mortality of the eggs occurred within 5 weeks while a complete kill of the larvae was observed in 11 weeks at 48° F. This would indicate that a high percentage of egg mortality can be expected in the field if an early warm spring is followed by a prolonged cold spell of

48^{o} F. or below. Mr. M. S. Gomulinski of Michigan State University has reported a high mortality of early eggs in the field in April of 1966 mainly due to a long unseasonably cold weather that followed immediately after a brief warm days in early spring.

Among the four major requisites listed by Klomp (1964) food and oviposition sites seem to be the two main items for which the cereal leaf beetle competes under certain laboratory conditions. Adult beetles compete for both food and oviposition sites while the larvae compete mainly for food. With laboratory stored adults the greatest egg production was observed at the second lowest density of 20 adults per lamp chimney cage with about 50 seedlings that were changed every other day (Table 27). As the adult density increased the fecundity of the individual female decreased. As Crombie (1942) mentioned a slight crowded density of 20 adults per cage might have caused some stimulatory effect on the rate of oviposition than at the lowest density of 10 beetles per cage.

In spring adults that were collected in the field after mating had already occurred, the fecundity of the individual female was highest at the lowest density of 10 adults per cage with 30 seedlings that were changed every other day (Table 29). As the density increased from 10 to 20, 30, 40, and up to 50 adults per cage, the fecundity of the female gradually declined. The fecundity was slightly lower in cages where the plants were changed once every 4 days than in cages where the plants were changed every other day. The main causes for the decline in egg production with increasing adult density seem to be due to 1) a reduction in the amount of food available to individual beetle, 2) a reduction in the oviposition sites, and 3) an excessive contact between individual beetles. Similar phenomena were observed in *Tribolium* sp. by Chapman (1928), Park (1932 and 1933), McLagan (1932), and by Crombie (1942).

The total number of eggs produced per cage, however, was greatest at the density of 30 adults per cage when the plants were changed every other day and at 20 adults per cage when the plants were changed once every 4 days. An active spring adult was observed to destroy one 4-inch tall seedling in two days. It is estimated that at least one 4-inch tall seedling per adult per day is required for the beetle to feed and deposit an adequate number of eggs.

The amount of food available has a direct effect on the development and survival of the larvae. The mortality rate among the larvae increased with an increase in the larval density (Figure 8). At least two 4-inch tall seedlings are necessary for a newly hatched larva to complete its development (Tables 23 and 24). The rate of feeding in terms of the amount of feeding per individual per unit time is more intense in adults than in larvae. The larvae, however, cause more damage to the plants than the adults in the long run simply because they become more numerous than the adults in the field. Dr. R. F. Ruppel of Michigan State University estimated the actual yield reductions in spring grains at 15 to 35 percent in Michigan (unpublished data).

The development and survival of the pupae are apparently influenced by soil type and soil moisture. A light, well aerated but slightly moist soil is apparently preferred to the heavy and wet soil for pupation in the cereal leaf beetle (Tables 19 and 23). This soil factor may have a significant effect on the distribution of the cereal leaf beetle in a given geographical area.

Mesnil (1931) stated that some new adults emerge in summer while others stay in the soil until the following spring. Vassiliev (1913) and Vereshtchagin (1914), on the other hand, reported that all of the new adults stay within the pupal cases in the soil until the following spring. The author has examined numerous pupal cases during his entire study period and was unable to detect any sign of new adults remaining in their pupal cases. Those remaining in the pupal cases were either dead or deformed ones.

Exposures of different stadia of the beetle to various temperatures and photoperiods have failed to prevent the majority of summer adults from going into diapause. Egg laying by the summer adults, however, can be induced by keeping the beetles in a lamp chimney cage at 80° F., 16-hour photoperiod, and 75 percent relative humidity. This has been already demonstrated by Castro, et al. (1965). The beetles even in the lamp chimney cage remained quite inactive throughout the oviposition period and the egg production was extremely irregular and inconsistent. These eggs were apparently produced by a small number of females. Mr. T. L. Burger of Plant Pest Control Division, ARS, USDA at Niles, Michigan reported that he and his colleague have successfully isolated a non-diapausing strain of the cereal leaf beetle from the eggs laid by field-collected summer adults (personal communication). Further systematic studies on genetic constituents of natural population and a critical evaluation of adaptive value of the non-diapausing individuals are needed.

De Wilde and Boer (1961) stated that diapause in adult insects is generally characterized by an inhibition in the maturation of eggs associated with corpus allatum failure. More recently Kumararaj (1964) and Hoopingarner, *et al*. (1965) reported that the gonads of the female beetle do not normally develop until they have been subjected to a low temperature. Their statements do not seem to hold true, at least in some females that lay eggs without having a low temperature treatment.

From these observations and experimental results (Table 45), the cereal leaf beetle seems to be a univoltine species with an obligatory diapause in the adult stage. The existence of the second generation reported by Averin (1914) in Russia and Wilson and Toba (1963) under field conditions in Indiana is very unlikely to occur under natural conditions in Michigan. Certain field-collected active spring adults (Expt. 15) laid eggs in July and survived well beyond the 11th of August in the laboratory. The author did observe small larvae in early August in the field but it is hard to believe that they were the second generation. Dr. D. L. Haynes of Michigan State University also reported that he observed numerous spring adults, eggs, and larvae in a late-planted oat field in late June and July in 1967 near Galien, Michigan (personal communication). The surest way of breaking diapause in the cereal leaf beetle is to hold the beetle at 38° F. for at least 90 days. Another promising method by which diapause can be readily terminated would be the use of a synthetic juvenile hormone described by Connin, *et al*. (in press).

The summer adults seem to be highly sensitive to high temperatures. A 50 percent mortality of the adults occurred after a 7-day exposure to 100° F. A 100 percent mortality of the inactive adults occurred within 6 hours at 120° F. A high temperature treatment in this sense may be useful in disinfestation of grains and hay crops unless the cost involved is too high or the quality of the crops changes appreciably from such treatment.

After a brief feeding period the summer adults show a photonegative behavior and go into an inactive stage (Castro, et al., 1965). The main factor that induces such hiding behavior seems to be heat, a combined effect of temperature and photoperiod (sunlight). Many beetles reappear in the fall when the weather becomes cool (personal communication with Mr. M. S. Gomulinski). The summer adults seem to go into aestivation in July, move in and out during cool days in the fall, and then move into hibernation sites.

An age-specific life table was constructed for the beetle under laboratory conditions (Table 46). This type of life table is very unrealistic because such natural mortality factors as parasitization, predation, and winter kill are not included in this table. However, it still provides some valuable information regarding the survival and mortality of the beetles in their different stages of development. Based on a fertility table for the beetles that had been stored for 90 days at 38° F., the cereal leaf beetle multiplies about 4 times per generation under laboratory conditions (Table 47). The net reproduction rate can be increased to some extent by more careful handling of the insect. The net reproduction rate for the field population was estimated at .941 (Table 48). If we assume all the mortality data are accurate and the field-collected spring adults reproduced faithfully in the laboratory as they would in the field, the figure indicates more or less a steady density level. It is, however, quite probable that the low net reproduction rate of .941 could be due to the underestimation of mortality and fecundity rates and possibly due to a continued aerial application of insecticides during the past several years.

The distribution of the cereal leaf beetle is highly spotty and shows an extremely discrete pattern under natural conditions (Castro, et al., 1965). Such distributional pattern seems to be influenced by a micro-climate or micro-environment of a given habitat. The density of the beetle often varies widely among closely adjacent fields. The major factors which seem to influence the distribution and abundance of the cereal leaf beetle are 1) natural enemies, 2) availability of preferred host plants, 3) temperature, 4) photoperiod, 5) moisture, 6) wind, 7) soil, and 8) presence or absence of woodlots in the vicinity.

As in any other phytophagous species natural enemies would regulate the population density of the beetle and distribution of preferred host plants would largely determine the distribution of the beetle itself. Such climatic factors as temperature, photoperiod, moisture, and wind have a direct influence on the development and survival of the beetle by one factor modifying the others. The beetle activities are strongly affected by the wind. The adult beetles usually stay low near the ground on windy days and the population density is generally low in open windy fields. The wind also influences the distribution of the beetle. It carries the beetles for a considerable distance (Wilson and Ruppel, 1964; and Shade and Wilson, 1964). Soil type and moisture would have a significant effect on the survival of the pupae. Woodlots would serve as an effective aestivation and hibernation sites as well as an effective windbreak.

All the experiments presented in this paper were conducted in the laboratory. The author hopes that the results of these experiments would serve as the basis for future field experiments. Continuous studies are obviously needed especially in the area of population dynamics of this species. All mortality factors should be analyzed more carefully and precisely in order to establish more complete life tables under various conditions. A close evaluation of native enemies, orientation system, soil factors, and perfection of artificial media are highly desirable and would certainly facilitate future studies.

SUMMARY

During a two and a half year period from 1964 to 1966, a series of laboratory studies were conducted on the reproduction, development, and survival of the cereal leaf beetle under various specific conditions.

A large number of field-collected summer adults were stored at 38° F. for the year round rearing and research purposes in the laboratory (Connin, et al., 1966a). A linear regression equation was calculated to express the relationship between the storage period at 38° F. in days and the mortality of the beetle. A $LD_{.50}$ for the summer adults stored at 38° F. was found to be 146.8 days (Table 42).

Diapause development in the summer adults seems to be completed after 90 days at 38° F. The largest egg production was obtained with the beetles that had been stored for 90 days at 38° F. Those which had been held at 38° F. for 70, 50, and 30 days or 130, 160, and 220 days laid progressibly fewer number of eggs (Table 38). Diapause development of the field populations appears to be completed by the end of December since the beetles collected in early January immediately started laying eggs when they were held under the laboratory conditions.

The initial flight by the overwintered adults occurred freely at 68° F. in the laboratory. A minimum of 55° F. seems to be required for an effective initial mating and egg deposition in the overwintered adults. The egg laying was extremely irregular at 60° F. but it became

more consistent at 70° F. Laboratory observations showed that the adults and larvae were completely immobilized at 38° F. but they were able to hang on to the plants at 45° F. without any noticeable feeding activity.

Random samples of field-collected spring adults and laboratory-reared summer adults contained a significantly greater number of females than males. The sex ratio in the samples of field-collected summer adults and laboratory-reared spring adults was not significantly different from 1:1 (Table 51).

With unmated adults (unmated before the test), the egg production, total number as well as per female, was greater in a lamp chimney cage containing 1 female and 1 male than in cages with 2/1, 4/1, 1/2, or 1/4 female/male combinations. With already mated adults (already mated before the test), however, the total egg production per female remained about the same in all female/male combinations and the total egg production per combination increased almost proportionately with an increase in the number of females from 1 female/1 male to 2 females/ 1 male and to 4 females/1 male. An unmated female laid as high as 631 eggs and the mated female laid up to 355 eggs per female. A virgin female laid 129 eggs but none of them hatched. A sex ratio of 1:1 prior to the mating seems to be ideal in the cereal leaf beetle (Table 34).

A laboratory reared female after 110 days of storage period at 38° F. laid up to 1,251 eggs during her life time of 153 days. The female was kept with a male in a lamp chimney cage at 80° F., 75 percent relative humidity, and 16 hours photoperiod. Another female, isolated from the male after the first mating, ceased egg laying activity after depositing 629 eggs. Approximately the first 400 eggs, however, were normal and the rest of the eggs were all unfertilized. When the same female was paired with a male it laid an additional 293 eggs and the hatchability of the eggs increased gradually up to the normal level of 65 percent. The oviposition rate remained fairly constant throughout oviposition period (Table 39).

Field-collected active spring adults that were collected on April 28, 1966 when a few eggs were observed in the field laid from 40 to 256 eggs per female in an isolated cage in the laboratory. Considerable individual differences as to fecundity and longevity were observed among the adults of the same age group. A 95 percent confidence limit for the mean egg production per female was calculated to be from 163 to 1,099 eggs for the beetles that had been stored for 110 days at 38^{o} F. and from 28 to 225 eggs for the field-collected active spring adults (Table 40).

The cereal leaf beetle laid a significantly greater number of eggs at 80^{o} F. than at 85^{o} F. (Table 9). In another test, the survival of the larvae and pupae at 73^{o} F. and 80^{o} F. was not significantly different (Table 11). The egg laying was extremely poor under fluctuating day and night temperatures of 70^{o}-50^{o} F. and 75^{o}-65^{o} F. A maximum egg production was apparently attained when the temperature was maintained at 80^{o} F. constantly (Table 12).

The largest egg production was observed under the photoperiodic range of 16 to 18 hours, when the temperature was maintained at 80^{o} F. and relative humidity at 75 percent. The rate of oviposition, however, was higher under 18 hours than that under 16 hours of photoperiod (Tables 14 and 15).

Over 93 percent of the eggs were laid during 16-hour light period and only 7 percent during 8-hour dark period. Thus oviposition activity in the cereal leaf beetle seems to be confined only during day light hours (Table 17). The photoperiod, however, had no influence on the development of the immature stages (Table 16).

The overwintering adults were more readily reactivated and laid a significantly greater number of eggs at the high relative humidity of 95 to 100 percent than at 70 to 80 or 45 to 55 percent (Table 18). The optimum environmental conditions for the development of the cereal leaf beetle, therefore, seem to be 80° F., 18 hours of photoperiod, and 95 percent of relative humidity.

The wet soil containing free water seems to have a detrimental effect on the prepupae entering the soil for pupation. Although a slightly moist soil seems to favor the general pupation process, a complete development of the pupae can take place in a completely dry soil (Tables 19, 20, and 21).

Soil type and color did not seem to affect oviposition behavior of the female (Table 22). The soil type, however, seems to be an important factor affecting the pupation process in the cereal leaf beetle (Table 23). A light, well aerated soil seems to be the best soil type for the development of the pupae.

A small lamp chimney cage seems to be more effective than a larger cage in inducing earlier and more consistent egg laying in overwintering adults. For active spring adults the egg production was higher in a larger cage than in a lamp chimney cage (Table 24).

"Clintland 60" oats are apparently preferred to "Hudson" barley for feeding and oviposition by the cereal leaf beetle. The adult beetles, after 65 days of cooling period at 43° F., consumed an average of eight 4 1/2-inch tall oat seedlings and 5.4 barley seedlings per beetle when they were exposed to either oats or barley during 45-day test period. During the same period, the same beetles laid 56 eggs per female on oats and only 22 eggs per female on barley (Table 30). These figures seem to underestimate the actual figures because diapause development in this beetle was apparently not completed at the time of the test.

In another test, when the beetles were exposed to both oats and barley and were given a choice of host plants, they laid a consistently greater number of eggs on oats than on barley and the difference was statistically highly significant (Table 31). The female seems to prefer slightly rough and concaved leaf surface and the leaf width of about 3 to 4 mm. for oviposition.

A slightly larger number of eggs was deposited on 4-inch tall seedlings than on 2- or 6-inch tall seedlings and more eggs were found in a pot with 75 seedlings than in a pot with either 50 or 25 seedlings. The differences, however, were statistically not significant (Table 32).

In "Clintland 60" oats over 99 percent of total eggs laid were deposited on upper surface of the leaves and 68 percent of them were found on basal third of the leaves. In "Hudson" barley, however, only 62 percent of the eggs were deposited on upper surface and 45 percent of them were found on basal third of the leaves. Nearly 68 percent of the eggs occurred singly on the upper surface of the oat leaves and 44 percent of the eggs were deposited singly on the same surface of the barley leaves (Table 49). The cereal leaf beetle laid significantly greater number of eggs on the upper surface of the leaves in oats regardless of the posture of the leaves (Table 50).

A single spotted lady beetle, Coleomegilla maculata lengi Timberlake, consumed up to 76 cereal leaf beetle eggs in 24-hour period. A nearly 100 percent egg predation occurred with a pair of lady beetles in 4 days when the lady beetles were confined in a lamp chimney cage containing an average of 152 cereal leaf beetle eggs (Table 35).

In the laboratory test, the cereal leaf beetle was able to complete its development within a temperature range of 58° F. to 90° F. The eggs, larvae, and pupae were unable to complete their developments at 48° F. (Table 3). The calculated thresholds of development, according to Uvarov's (1931) equation, are 52.0° F. for the egg, 46.6° F. for the larva, 54.0° F. for the pupa, and 51.6° F. for the entire immature stage (Table 7). A complete development of the eggs occurred at 95° F. but not at 100° F. The larvae and pupae developed normally at 90° F. but not at 95° F. The peak temperatures at which the embryo develops at the fastest rate was 92.5° F. A more complete range of temperature for the development seems to be between 54° F. and 92.5° F.

A complete development from an egg to adult requires an average of 92 days at 58° F., 52 days at 67° F., 28 days at 80° F., and 23 days at 90° F. Both Davidson's (1944) logistic and linear regression equations were used to express the relationship between temperature and the rate of development in egg, larval, and pupal stages (Table 5).

Intra-specific competition among the larvae and adults was tested in the laboratory. However, no aggressive behavior was observed among the larvae and adults. At least two 4-inch tall oat seedlings are required for a larva to complete its development (Table 26). A decrease in fecundity of the females was observed as the adult density increased. The egg production was highest in the laboratory stored beetles at the initial density of 20 adults (10 females) per lamp chimney cage with 50 fresh seedlings changed every other day. For field-collected active spring adults, the greatest egg production was obtained at the lowest density of 10 adults (5 females) per lamp chimney cage with 30 fresh plants changed every two to four days. The former (20 adults) laid as high as 330 eggs per female while the latter (10 adults) laid 213 to 224 eggs per female (Tables 27, 28, and 29).

The egg is the most resistant stage against extremely cold temperatures of 0° and -10° F. and it is followed by pupae, larvae, active summer adults, and lastly by active spring adults. A 100 percent egg mortality occurred within 2 hours at -10° F. and in 4 hours at 0° F. Although diapausing adults have not been tested in the laboratory, it is most likely that the hibernating adults might be the most resistant of all stages against the cold since the inactive summer adults can withstand a considerable period of time at 38° F. At high temperatures of 110° F. and 120° F., inactive summer adults were most resistant. It is then followed by larvae, eggs, pupae and active summer adults, and spring adults. A complete kill of the inactive adults was observed within 6 hours at 120° F. and in 5 days at 110° F. (Table 13).

Under normal conditions both in the laboratory and field, the cereal leaf beetle seems to have an obligatory diapause. Under field conditions it seems to have a single generation per year and all of the newly emerged summer adults go into diapause after a brief feeding period (Castro, et al., 1965). Even in the laboratory, when the newly emerged summer adults were held in a large screen cage (15-1/2 x 15-1/2 x 16 inches) with hiding places in it, all the adults hid inside the hiding places after the post-emergence feeding period regardless of the environmental conditions. When the summer adults were kept in a small lamp chimney cage, they started laying eggs after varying period of time. The egg laying is generally irregular and the egg production is extremely low (Table 45).

The egg laying by the summer adults, however, seems to be influenced to some extent by 1) age of the beetle after emergence, 2) temperature, 3) photoperiod, and 4) light intensity. The newly emerged adult seems to be the most responsive stage for a continuous reproduction. As the summer adults get older the induction of egg laying becomes harder. Exposures of eggs, larvae, pupae, and new adults to various combinations of 12, 16, and 24 hours of photoperiods have failed to prevent the summer adults from going into diapause.

A brief exposure of the summer adults to extremely low temperatures of 0^{o} or -10^{o} F. and to extremely high temperatures of 110^{o} or 120^{o} F. did not favor the egg production in the summer adults (Table 36). The number of eggs produced by the summer adults that had been exposed for 30 days to $38^{o}/32^{o}/15^{o}$ F. or $38^{o}/32^{o}$ F. was not significantly different from that produced by the adults exposed for 30 days to 38^{o} F. constantly (Table 37).

Some of the early eggs laid by the summer adults were sampled and reared under different photoperiods of 12, 16, and 24 hours for three successive generations without subjecting them to a cold temperature. The egg production gradually decreased and no apparent non-diapausing strain was detectable from the samples (Table 44).

An age-specific life table was constructed for the laboratory population of the cereal leaf beetle. Starting with an imaginary number of 1,000 eggs, 301 new adults were obtained and 202 adults survived after 2 weeks of post-emergence feeding period and 90 days of cold storage period at 38° F. The 202 adults are comparable to the spring adults and these are ready to mate and lay eggs of the following generation. The apparent mortality was highest in the pupal stage (39.0%) followed by the egg stage (35.2%) and the larval stage (23.9%) (Table 46).

Based on an age-specific life and fertility table for the beetle that had been stored at 38° F. for 90 days, the net reproduction rate was calculated to be 3.936 under the laboratory rearing conditions (Table 47). Using a similar table for the field-collected spring adults, applying field mortality data prepared by Dr. R. F. Ruppel (unpublished data), the net reproduction rate of the field population was estimated at .941 (Table 48). Assuming all the data on fertility and survival of the female and mortality of various developmental stages of the beetle are sufficiently correct, the laboratory population can multiply about four times per generation while the field population remains relatively constant.

LITERATURE CITED

Abbott, W. B. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265-7.

Allee, W. C. 1931. Animal aggregations, a study in general sociology. Univ. Chicago Press, Chicago. 431 pp.

Andersen, F. S. 1960. Competition in populations consisting of one age group. Biometrics. 16:19-27.

Andrewartha, H. G. 1952. Diapause in relation to the ecology of insects. Biol. Rev. 27:50-107.

Andrewartha, H. G. and L. C. Birch. 1954. The distribution and abundance of animals. Univ. of Chicago Press, Chicago. 782 pp.

Averin, V. G. 1914. Review of the pests noticed in the Govt. of Charkov during 1913. pp. 10-65. (Cited from Hodson, 1929.)

Balachowsky, A. S. 1963. "The family Chrysomelidae". Entomologie appliquee a l'agriculture. Masson et Cia., Paris. Vol. 2.

Balachowsky, A. S. and L. Mesnil. 1935. "Lema melanopa L." Les insectes nuisibles aux plantes cultivees. Est. Busson, Paris. pp. 788-95.

Beck, S. D. and W. Hanec. 1960. Diapause in the European corn borer, Pyrausta nubilalis (Hübn.). J. Insect Physiol. 4:304-19.

Belehradek, J. 1930. Temperature coefficients in biology. Cambridge Phil. Soc. Biol. Rev. 5:30-58.

Birch, L. C. 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. 17:15-26.

Borg, A. 1959. The grain leaf beetle, a common harmful insect. Vaxtskyddsnotiser. 23(5):63-7. (From an English translation by M. K. Lien.)

Bowers, W. S. and C. C. Blickenstaff. 1966. Hormonal termination of diapause in the alfalfa weevil. Science. 154(3757):1673-4.

Castro, T. R. 1964. Natural history of the cereal leaf beetle Oulema melanopa (Linnaeus) and its behavior under controlled environmental conditions. Ph.D. thesis, Mich. State Univ. 121 pp.

Castro, T. R. and G. E. Guyer. 1963. Notes on the biology, distribution, and potential importance of Oulema melanopa (L.) in the Mid-West. Abst. in Proc. North Central Branch, Entomol. Soc. Amer. 18:74.

Castro, T. R., R. F. Ruppel, and M. S. Gomulinski. 1965. Natural history of the cereal leaf beetle in Michigan. Mich. Agr. Expt. Sta., Mich. State Univ. Quart. Bull. 47(4):623-53.

Chapman, R. N. 1928. The quantitative analysis of environmental factors. Ecology. 9:111-22.

Chiang, H. C. and A. C. Hodson. 1950. An analytical study of population growth in Drosophila melanogaster. Ecol. Monogr. 20: 173-206.

Connin, R. V., D. L. Cobb, J. C. Arnsman, and M. S. Gomulinski. 1966a. Laboratory rearing of the cereal leaf beetle, Oulema melanopa (L.). Entomol. Res. Div., A.R.S., U.S.D.A. Special Rpt. W-222.

________. 1966b. Plaster of Paris as an aid in rearing insects pupating in the soil. J. Econ. Entomol. 59(6):1530.

Connin, R. V., O. K. Jantz, and W. S. Bowers. In press. Termination of diapause in the cereal leaf beetle. J. Econ. Entomol.

Crombie, A. C. 1942. The effect of crowding upon the oviposition of grain-infesting insects. J. Exptl. Biol. 19:311-40.

________. 1944. On intraspecific and interspecific competition in larvae of graminivorous insects. J. Exptl. Biol. 20:135-51.

Crozier, W. J. 1926. On curves of growth, especially in relation to temperature. J. Gen. Physiol. 10:53-73.

Davidson, J. 1942. On the speed of development of insect eggs at constant temperatures. Aust. J. Exptl. Biol. & Med. Sci. 20:233-9.

________. 1944. On the relationship between temperature and the rate of development of insects at constant temperatures. J. Anim. Ecol. 13:26-38.

DeBach, P. 1958. The role of weather and entomophagous species in the natural control of insect populations. J. Econ. Entomol. 51:474-84.

Deevey, E. S. 1947. Life tables for natural populations of animals. Quart. Rev. Biol. 22:283-314.

Dickson, R. C. 1949. Factors governing the induction of diapause in the oriental fruit moth. Ann. Entomol. Soc. Amer. 42:511-37.

Ditman, L. P., G. S. Weiland, and J. H. Guill. 1940. The metabolism in the corn earworm. III. Weight, water, and diapause. J. Econ. Entomol. 33(2):282-95.

Embree, D. G. 1965. The population dynamics of the winter moth in Nova Scotia, 1954-1962. Mem. Ent. Soc. Can. 46. 57 pp.

Favinger, J. J. 1962. A new insect pest: cereal leaf beetle, Oulema melanopa (L.). Outdoor Indiana. 6(5):2-4.

Finney, D. J. 1947. Probit analysis, a statistical treatment of the sigmoid response curve. Cambridge Univ. Press.

Fry, F. E. 1947. Effects of the environment on animal activity. Univ. Toronto Stud. Biol. 55:1-62.

Gallun, R. L. and R. F. Ruppel. 1963. Cereal leaf beetle resistance studies. Agr. Res. Serv., U.S.D.A. Special Rpt. W-178, 36 pp.

Grison, P. 1947. Developpement sans diapause des chenilles de Euproctis phaeorrhaea, L. (Lep. Liparides). Compt. rend. Acad. Sci., Paris. 225:1089-90. (Cited from Wigglesworth, 1953).

Guyer, G. E. 1962. The bionomics of Oulema melanopa (L.) on small grains in Michigan. Abst. in Bull. Entomol. Soc. Amer. 8(3):162.

Harvey, W. R. 1962. Metabolic aspects of insect diapause. Ann. Rev. Entomol. 7:57-80.

Hodson, W. E. H. 1929. The bionomics of Lema melanopa L. (Criocerinae) in Great Britain. Bull. Ent. Res. 20(1):5-14.

Hoopingarner, R. A., S. Kumararaj, and A. L. French. 1965. Gametogenesis and radiation effects in the cereal leaf beetle, Oulema melanopa. Ann. Entomol. Soc. Amer. 58(6):777-81.

Hoskins, W. M. and R. Craig. 1935. Recent progress in insect physiology. Physiol. Rev. 15:525-96.

Huffaker, C. B. 1944. The temperature relations of the immature stages of the malarial mosquito, Anopheles quadrimaculatus, with a comparison of a developmental power of constant and variable temperatures in insect metabolism. Ann. Ent. Soc. Amer. 37:1-27.

Janes, R. L. and R. F. Ruppel. 1965. Cereal leaf beetle control. Mich. State Univ. Ext. Bull. 443. 4 pp.

Janisch, E. 1932. The influence of temperature on the life history of insects. Trans. Roy. Ent. Soc. London. 80:137-68.

Kadocsa, G. 1916. Criocerus melanopa (Lema melanopa) injurious to oats and barley in Hungary. Mthly. Bull. Agr. Intel. Dis. 7(2):312-4. (Abst. in Rev. Appl. Entomol. 4:350.)

Kadocsa, J. Undated. The red-necked barley beetle, its life history and control. (From an English translation by G. Angelet.)

Kevan, D. K. M. 1944. The bionomics of the neotropical cornstalk borer, Diatraea lineolata Wlk. (Lep. Pyral.) in Trinidad. Bull. Ent. Res. 35:23.

Klomp, H. 1964. Intraspecific competition and the regulation of insect numbers. Ann. Rev. Entomol. 9:17-40.

Knechtel, W. K. and C. I. Manolache. 1936. Biological observations on L. melanopa in Rumania. Anal. Inst. Cerc. Agron. Roman. 7:186-208. (Abst. in Rev. Appl. Entomol. 24:678.)

Kogure, M. 1933. The influence of light and temperature on certain characters of the silkworm, Bombyx mori. J. Dept. Agr. Kyushu Imp. Univ. 4:1-93.

Kumararaj, S. 1964. Gametogenesis and radiation effects on the reproductive tissue of Oulema melanopa (L.). M.S. Thesis. Mich. State Univ. 77 pp.

Laughlin, R. 1965. Capacity for increase: a useful population statistic. J. Anim. Ecol. 34:77-91.

Lees, A. D. 1955. The physiology of diapause in arthropods. Cambridge Univ. Press, Cambridge. 151 pp.

Manolache, C. 1932. On the occurrence of L. melanopa in Rumania in 1931. Pub. Soc. Nat. Romania, 10. 4 pp. (Abst. in Rev. Appl. Entomol. 20:340.)

Matteson, J. W. and G. C. Decker. 1965. Development of the European corn borer at controlled constant and variable temperatures. J. Econ. Entomol. 58:344-49.

McLagan, D. S. 1932. The effect of population density upon the rate of reproduction with special reference to insects. Proc. Roy. Soc. London, B. 111:437-54.

Megalov, V. A. 1925. Report of the work of the Entomological Division for 1920-1925. Saratov Reg. Agr. Expt. Sta. 31 pp. (Abst. in Rev. Appl. Entomol. 14:29.)

________. 1927. Lema melanopa L., a pest of oats, barley, and other graminaceous plants. Ibid. 29 pp. (Abst. in Rev. Appl. Entomol. 15:643.)

Mesnil, L. 1931. Contribution a'l'etude de trois coleopteres nusibles aux cereales. Ann. Epiphyties. 16(3-4):190-208. (Abst. in Rev. Appl. Entomol. 19:658.)

Merino M., L. G. 1966. Studies of the sexual behavior of the adult cereal leaf beetle, Oulema melanopus (L.). M.S. Thesis, Mich. State Univ. 25 pp.

Messenger, P. S. and N. E. Flitters. 1958. Effect of constant temperature environments on the egg stage of three species of Hawaiian fruit flies. Ann. Entomol. Soc. Amer. 51:109-19.

Morris, R. F. 1963. The dynamics of epidemic spruce budworm populations. Mem. Ent. Soc. Can. 31. 332 pp.

Morris, R. F. and C. A. Miller. 1954. The development of life tables for the spruce budworm. Can. J. Zool. 32:283-301.

Park, T. 1932. Studies in population physiology: The relation of numbers to initial population growth in the flower beetle, Tribolium confusum Cuval. Ecology. 13:172-81.

________. 1933. Studies in population physiology. II. Factors regulating initial growth of Tribolium confusum populations. J. Exptl. Zool. 65:17-42.

Pearl, R. and S. L. Parker. 1922. On the influence of density of population upon the rate of reproduction in Drosophila. Proc. Nat. Acad. Sci. 8:212-19.

Pearl, R. and L. J. Reed. 1920. On the rate of growth of the population of the United States since 1790 and its mathematical representation. Proc. Nat. Acad. Sci., Washington. 6:275-88.

Robertson, F. W. and J. H. Sang. 1944. The ecological determinants of population growth in Drosophila cultures. I. Fecundity of adult flies. Proc. Roy. Soc. London, B. 132:258-77.

Ruppel, R. F. 1964. Biology of the cereal leaf beetle. Proc. North Central Branch, Entomol. Soc. Amer. 19:122-4.

Ruppel, R. F. and T. R. Castro. 1964. Cereal leaf beetle; life cycle, biology and damage. Abst. in 16th Illinois Custom Spray Oper. Training School. Urbana, Illinois. pp. 46-7.

Ruppel, R. F., David L. Cobb, and Melvin S. Gomulinski. 1965. Control of cereal leaf beetle pupae. Mich. Agr. Expt. Sta., Mich. State Univ. Quart. Bull. 47(3):328-31.

Sang, J. H. 1949. The ecological determinants of population growth in a Drosophila cultures. III. Larval and pupal survival. Physiol. Zool. 22:183-202.

Sengupta, G. C. and B. K. Behura. 1957. On the biology of Lema praeusta Fab. J. Econ. Entomol. 50(4):471-4.

Shade, R. E. and M. C. Wilson. 1964. Population build-up of the cereal leaf beetle, Oulema melanopa (L.), in Indiana and the apparent influence of wind on dispersion. Agr. Expt. Sta., Purdue Univ. Res. Prog. Rpt. 98.

Shelford, V. E. 1929. Laboratory and field ecology. Williams and Wilkins Co., Baltimore. 608 pp.

Smith, H. S. 1935. The role of biotic factors in the determination of population densities. J. Econ. Entomol. 28:873-98.

Southwood, T. R. E. 1966. Ecological methods with particular reference to the study of insect populations. Methuen and Co., Ltd., London. 277-310.

Squire, F. A. 1940. On the nature and origin of the diapause in Platyedra gossypiella, Saund. Bull. Ent. Res. 31:1-6.

Urquijo, P. 1940. Una plaga de Lema melanopa entrigos de Galicia. Bol. Pat. Veg. Ent. Agr. 9:147-163.

U. S. Department of Agriculture. 1958. A leaf beetle (Lema melanopa L.) in "Insects not known to occur in the United States." Coop. Econ. Insect Rept. 8(3):47-8.

________. 1962. Survey reports on cereal leaf beetle. Ibid. 12(32):881.

________. 1963. Coop. Plant Pest Cont. Programs, Fiscal Year 1963. Plant Pest Cont. Div., A.R.S., U.S.D.A. pp. 8-9.

________. 1964. Coop. Plant Pest Cont. Programs, Fiscal Year 1964. ibid. pp. 9-10.

________. 1965. Coop. Plant Pest Cont. Programs, Fiscal Year 1965. ibid. p. 7.

________. 1966a. Coop. Plant Pest Cont. Programs, Fiscal Year 1966. ibid. p. 8.

________. 1966b. Coop. Programs, Central Region, Fiscal Year 1966. ibid. pp. 7-14.

________. 1967. Coop. Econ. Insect Rpt. ibid. 17(31):720.

Utida, S. 1941. Studies on experimental population of the Azuki bean weevil, Callosobruchus chinensis (L.). IV. Analysis of density effect with respect to fecundity and fertility of eggs. Mem. Coll. Agr. Kyoto Univ. 51:1-26.

________. 1942. Studies on experimental population of the Azuki bean weevil, Callosobruchus chinensis (L.). VII. Analysis of the density effect in the preimaginal stage. Mem. Coll. Agr. Kyoto Univ. 53:19-31.

Uvarov, B. P. 1931. Insects and climate. Trans. Roy. Entomol. Soc. London. 79:1-248.

Vassiliev, E. V. 1913. The chief remedies against the larvae and beetles of Lema melanopa L., a pest of summer grown grain. Studies from Expt. Sta. All-Russian Soc. Sugar-Ref. 1912, Kiev. pp. 1-2. (Abst. in Rev. Appl. Entomol. 1:479.)

Venturi, F. 1942. La Lema melanopa L. (Coleoptera, Chrysomelidae). Redia. 22:11-86.

Vereshtchagin, B. 1914. Experiments on the control of Lema melanopa L. in Bassarabia. Agr. Bassarabia. 19. 4 pp. (Abst. in Rev. Appl. Entomol. 4:218.)

Way, M. J. and B. A. Hopkins. 1950. The influence of photoperiod and temperature on the induction of diapause in Diataraxia oleracea L. (Lepidoptera). J. Exptl. Biol. 27:365-6.

Wigglesworth, V. B. 1953. The principles of insect physiology. Methuen and Co. Ltd., London. 546 pp.

Wilde, J. de. 1962. Photoperiodism in insects and mites. Ann. Rev. Entomol. 7:1-26.

Wilde, J. de and J. A. de Boer. 1961. Physiology of diapause in the adult Colorado beetle II. Diapause as a case of pseudo-allatectomy. J. Insect Physiol. 6:152-61.

Wilde, J. de, C. S. Duintjer, and L. Mook. 1959. Physiology of diapause in the adult Colorado beetle. I. The photoperiod as a controlling factor. J. Insect Physiol. 3:75-85.

Williams, C. M. 1956. Physiology of insect diapause. X. An endocrine mechanism for the influence of temperature on the diapausing pupa of the cecropia silkworm. Biol. Bull. 110:201-18.

Wilson, M. C. and R. F. Ruppel. 1964. Airplane trapping of the cereal leaf beetle and the meadow spittlebug. Agr. Expt. Sta., Purdue Univ. Res. Prog. Rpt. 110. 7 pp.

Wilson, M. C. and R. E. Shade. 1964a. Adult feeding, egg deposition and survival of larvae of the cereal leaf beetle on seedling grains. Agr. Expt. Sta., Purdue Univ. Res. Prog. Rpt. 97. 7 pp.

________. 1964b. The influence of various gramineae on weight gains of post-diapause adults of the cereal leaf beetle, Oulema melanopa (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Amer. 57(6):659-61.

________. 1966. Survival and development of the cereal leaf beetle, Oulema melanopa (Coleoptera: Chrysomelidae), on various species of Gramineae. Ann. Entomol. Soc. Amer. 59(1):170-3.

Wilson, M. C. and H. Toba. 1963. Cereal leaf beetle. Coop. Econ. Insect Rpt. 13(33):942.

Yun, Y. M. 1964. Laboratory and field studies of insecticides for control of the cereal leaf beetle. M.S. Thesis. Mich. State Univ. 76 pp.

Yun, Y. M. and R. F. Ruppel. 1964. Toxicity of insecticides to a coccinellid predator of the cereal leaf beetle. J. Econ. Entomol. 57(6):835-7.

Zolotarev, E. K. 1947. Diapause and development of puape of the Chinese oak silkworm (Antheraea pernyi Guer.). Zool. Zhur. 26:539. (Cited from Lees, 1955.)