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SOME EFFECTS OF TEMPERATURE ON THE RATE
OF INCUBATION OF EGGS OF THE PALE WESTERN
CUTWORM (AGROTIS ORTHOGONIA MORR.)

by

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SOME EFFECTS OF TEMPERATURE
ON THE RATE OF INCUBATION OF EGGS OF
THE PALE WESTERN CUTWORM
(AGROTIS ORTHOGONIA MORR.)

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by

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PREFACE

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CONTENTS

PREFACE	iii
ABSTRACT	v
INTRODUCTION	1
REVIEW OF LITERATURE	3
Insect Embryology	4
Temperature and Humidity	8
LIFE HISTORY OF THE PALE WESTERN CUTWORM	11
DESCRIPTION OF THE EGG	13
MATERIALS AND METHODS	13
RESULTS	18
THE ORDER OF MORPHOLOGICAL DEVELOPMENT IN THE EMBRYO OF AGROTIS ORTHOGONIA MORR.	18
A KEY TO EMBRYOLOGICAL DEVELOPMENT AT 30° C.	25
The 30° C. Key	25
THE EFFECT OF TEMPERATURES WITHIN THE DEVELOPMENTAL RANGE	26
Additional Incubation Tests at 30° C.	26
The Effect of Median Temperatures on the Rate of Development	29
THE EFFECT OF ABNORMAL TEMPERATURES	33
Tests Conducted at 35° C. and 40° C.	33
Some effects of Incubating Eggs at 2° C. and 5° C.	37
The Effect of Exposing Eggs to 0° C.	44
Some Effects of Below Freezing Temperatures on Development	47
DISCUSSION	50
Distorted Embryos	50
Infertile Eggs	53
The Criterion of Fully Developed Eggs	54
Future Investigations	55
SUMMARY AND CONCLUSIONS	56
LIST OF REFERENCES	57

ABSTRACT

A study of the late embryological development in the egg of Agrotis orthogonia Morr. is described. From the order of morphological development, as viewed through a binocular dissecting microscope, a key to the rate of incubation at 30° C. has been composed. It is shown that the eggs of this species will incubate and hatch at 10° C., 15° C., 20° C., 25° C., and 30° C. At 35° C. and 40° C. normal hatch fails to occur.

Information is included on the partial development which occurs at 2° C. and 5° C. The effects of transferring these partially incubated eggs to the median temperatures after diverse periods of exposure are also shown. The results of storing freshly deposited eggs at 0° C., -5° C., -10° C., -15° C., and -20° C. for various lengths of time are illustrated by the data.

The discussion includes some possible reasons why the material reacted in the observed manner. Some practical applications of the data are mentioned.

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INTRODUCTION

One of the most serious pests of grain crops in Saskatchewan and Alberta is the pale western cutworm, Agrotis orthogonia Morr. This insect is a native of the prairie regions, but it was not until 1911 that damage of economic importance was reported by Gibson (13, 14). The species was first described by H. K. Morrison (26) in 1877, although Gibson (16) later gave it the common name of the pale western cutworm. Since 1911 periodic outbreaks have caused severe losses to farmers of Saskatchewan and Alberta, as well as the northern prairie areas of the United States.

Each year a forecast of the expected pale western cutworm infestation is made. This forecast is based on several of the previous years data and observations:-

1. The number of wet days occurring during the larval feeding period.
2. A survey of larval damage.
3. The state of the soil surface at the time of the moth flight.
4. The moth survey.

On several occasions all these factors have favoured an increase in cutworm population for the following spring, and yet the expected outbreak has failed to materialize. It is possible that this is due to one or more field conditions affecting embryological development. For this reason it was decided that it would be desirable to know something of the developmental stages of the pale western cutworm egg.

There are several other reasons why this project was undertaken. Field deposited eggs are quite often examined. Providing the approximate incubation temperature is known, an estimate of the time of oviposition may be made if the developmental stage can be recognized. An idea of the time of larval emergence might also be given. Egg storage in the laboratory is essential if off-season work is to be carried on normally. The most recent data indicate that eggs at some stages of development store more successfully than at others. Certain temperatures are also more favour-

able to egg storage. Egg dissections in the laboratory and the field will enable workers to give early and accurate estimates of egg fertility.

Although this is primarily a study of the embryo, no attempt is made to study the early development or organogeny of the embryo. The incubation observations are based on the identification of various morphological characteristics in the order of their appearance. A detailed cytological study of embryological development has not been attempted.

Some effects of temperature on the rate of development of the embryo have been studied. From these an indication of egg reaction in the field might be determined.

REVIEW OF LITERATURE

This review will not attempt to outline all the work that has been done on insect embryology. Only those publications which help to give a sound basis for a study of the morphological development of an insect embryo, together with some of those which discuss the effects of temperature and humidity on the rate of that development, will be mentioned. The review will therefore be divided into two categories, one dealing with insect embryology,

and the other with temperature and humidity effects on rate of development. Exceptional types of egg development will not be discussed.

INSECT EMBRYOLOGY

Embryology has been defined as the study of the embryo, its formation and development. The structural unit of the animal or plant embryo is the cell (23). It is with the cell, ie. the ovum, that most papers on insect embryology begin. The fertilization of the ovum nucleus by the nucleus of the spermatozoon gives rise to the zygote which in turn becomes the embryo. In Prodenia eridania Cram., Gross and Howland (17) found that fertilization occurred within the yolk just beneath the micropyle.

This act of fertilization usually occurs in insects just prior to the deposition of the egg (23). Rempel (28) states that in Mamestra configurata (Walker) fertilization is accomplished within the second half-hour following oviposition. Gambrell (12) found that the nucleus of some eggs of Simulium pictipes Hagen was at the stage of polar body formation at the time of deposition, while in others early cleavage had begun.

Cleavage and blastoderm formation now occurs. Auten (1) observed that in Phormia regina (Meig.) cleavage was under way within two hours of oviposition. Jeffrey (21) was able to identify the blastoderm in the embryo of Botys hyalinialis Hb. (Microstega hyalinialis (Hb.)) as an extremely fine marginal line eight hours after the egg had been laid. Woodsworth (35) noted that the blastoderm of the embryo of Eu Vanessa antiopa L. (Nymphalis antiopa (L.)) was complete 24 hours after oviposition. He mentions no developmental temperature.

The germ band develops next, followed by the embryonic envelopes, the amnion and serosa. These processes were found to occur between the sixteenth and twenty-eighth hours after oviposition in Diacrisia virginica Fabr. by Johannsen (22). However, Butt (3) showed that in Sciara coprophila Lint. the embryo is entirely covered over and protected by the two embryonic envelopes at the fifteenth hour following oviposition.

As the embryonic envelopes are forming, gastrulation of the germ band takes place. Butt (4) observed that the gastrular groove in Brachyrhinus ligustici L. was complete by the third day. After the lateral and anterior amniotic folds have formed blastokinesis takes place. Miller (25) says that in Pteronarcys proteus Newman a shortening accompanies

consolidation of the body regions, and in order that further growth may occur, blastokinesis must take place. Rempel (28) showed that blastokinesis occurred in the embryo of Mamestra configurata (Walker) at the seventieth hour of development at room temperature, ie., 68°-72° F.

During blastokinesis segmentation and appendage formation takes place. The abdomen of some primitive insects such as Lepisma spp. may consist of 12 segments (23). However, most insects normally have eleven segments. Johannsen (22) states that segmentation of Diacrisia virginica Fabr. begins soon after the fourtieth hour. Indication of legs budding on some segments of Botys hyalinialis Hb. was observed by Jeffrey (21) on the fifth day of development. Eastham (9) says that the appendages develop as paired outgrowths of the body wall on all segments of the body of the embryo of Pieris rapae L.

After differentiation of the mesoderm, ie., segmentation, coelomic sac formation, etc., the development of the alimentary canal begins. Invaginations at the anterior and posterior ends of the embryo indicate the beginnings of the stomodaeum and proctodaeum. In the embryo of Sciara coprophila Lint. Butt (3) noted that the stomodaeum appeared as a shallow depression in the anterior region of the embryo at the twenty-fifth hour. However, he was not able to discern the proctodaeum until the fiftieth hour.

When closure of the dorsal wall is complete, which Johannsen (22) says occurs at about 95 hours in Diacrisia virginica Fabr., formation of the heart begins. Jeffrey (21) noted the first pulsations of the dorsal vessel in Botys hyalinalis Hb. on the ninth day of embryonic development.

The nervous system has its origin in the neural groove according to Johannsen and Butt (23). Butt (3), in his investigations of Sciara coprophila Lint., found that the neural groove begins forming at the twenty-fifth hour. Working with Simulium pictipes Hagen, Gambrell (12) showed that the neural groove was developing soon after the twenty-fourth hour following oviposition.

Shortly after the outer neural ridges have become segmented the stigmata of the tracheal system appear. Eastham (9), in his paper on the embryology of Pieris rapae L., says that the respiratory system develops from spiracular invaginations.

With the formation and disappearance of the dorsal organ (Snodgrass 32), and the advent of dorsal closure, the body of the embryo has become an elongate sac with continuous walls. Gambrell (12) says the dorsal wall closes early on the fourth day of development in Simulium pictipes Hagen.

Salt (29) states that in several Melanoplus spp. dorsal closure is complete by the nineteenth day when development is at 25° C. According to Rempel (28), the embryo of Mamestra configurata (Walker) is about 130 hours old when it sets itself free by rupturing the serosa, vitelline membrane, and the egg chorion.

TEMPERATURE AND HUMIDITY

Wigglesworth (34) says that the most important factor controlling the rate of embryological development is temperature, within the vital limits of each species. Generally speaking, development is accelerated by high temperatures and retarded by low.

Evans' (11) data on the eggs of Lucilia sericata Meig. indicate that 100 per cent hatch will occur when the eggs develop anywhere between 14° C. and 35° C. Beyond these limits he observed that mortality increases rapidly with a comparatively small increase or decrease in temperature. He found that the limiting temperatures for the development of eggs of Lucilia sericata Meig. were about 10° C. and 40° C.

Bodine (2) concluded that it is possible to calculate the time of hatching of insect eggs if previous temperature history is known.

Temperature is an important factor in ensuring the fertility of deposited insect eggs. Wigglesworth (34) says that although the temperature limits for oviposition may be wide, the limits between which mating may occur are often much narrower. Wigglesworth also says that males seem to be more sensitive to abnormal temperatures than females. Hanna (18) showed that when the chalcid, Euchalcidia was exposed to 16° C. for 10 days, females still laid normal complements of eggs, but 70 per cent of the males were sterile.

Actually very few quantitative data on the effect of temperature on the duration of the incubation period are available. Of the many embryological papers which give very concise information on time of embryo development, very few mention a specific temperature at which the egg was incubated. Several mention room temperatures, others speak of normal seasonal temperatures, while some, either make no mention of temperature, or they give a probable range of temperatures that may have occurred during the incubation period. Melvin (24) showed from his observations on muscoid

flies that high as well as low temperatures prolong the developmental period of the eggs.

Buxton (5), working with eggs of Melanoplus atlanis Sauss. (Melanoplus mexicanus mexicanus (Sauss.)) found that over a wide range of temperatures, mortality was governed by humidity. Evans (11) says that the important factor governing mortality is loss of water and not temperature directly. He observed that eggs of Lucilia sericata Meig. are killed by relatively low saturation deficiencies at high or low temperatures, but at median temperatures, higher saturation deficiencies are tolerated.

According to Evans (11), Holdaway (19) showed that humidity had very little effect on the duration of the egg stage in Tribolium confusum Duval when incubated at 27° C. On the other hand Evans says that Janisch (20) found, when studying the egg of Prodenia littoralis Gn., that the duration of development, percentage of eggs completing development, and mortality were profoundly affected by the humidity of the surrounding air. Schipper (30) noted that retardation of development in eggs of Melanoplus spp. occurred when the eggs became dehydrated, development continued nevertheless, as long as the eggs contained enough water to sustain growth.

The stage of development of the embryo is often a determining factor in its ability to resist desiccation.

Buxton (6) says that an egg ready to hatch contains an embryo that is tracheate and covered with chitin and that such an egg is capable of resisting conditions that would have been fatal earlier in development. Buxton also says that Zwolfer (36) was able to show that eggs of Panolis flammea Neum. (Apantesis flammea (Neum.)) tolerate a wide range of humidities but total mortality occurs in saturated air. Conversely, Janisch (20) found that eggs of Prodenia littoralis Gn. require a very high degree of humidity and are not harmed by the air being saturated. However, Buxton (6) concluded that in almost every example, a larger number of eggs hatched in air which was at or near saturation, than in air which was dry.

LIFE HISTORY OF THE PALE WESTERN CUTWORM

Gibson (16) contributed the first paper on the life history of the pale western cutworm. This report included field observations made by Strickland in 1913. A more detailed report on the life cycle was published by Parker, Strand, and Seamans (27) in 1921. In 1930 Cook (8) enlarged on this latter paper.

The female adult deposits her eggs in the soil in the fall of the year, usually between August 15 and September 15. According to Seamans (31), these eggs appear to have completed the period of incubation before freeze-up. The winter is usually passed in this state although there have been reports of fall hatching by Parker, Strand, and Seamans (27), and by Cook (7).

The larvae normally emerge from the egg early the following May. Hatching, or eclosion from the egg, is accomplished by the larva chewing a hole through the chorion with its mandibles (32).

The larval stage, at which time the cutworm does its crop damage, is usually completed by the end of June. Temperature and moisture are two of the factors which cause variation in the length of the feeding period.

The pupal stage is also spent in the soil. The insect remains in this state for a period of three to six weeks. Normally, the adult moths emerge from the soil shortly after the middle of August and mating and oviposition begin soon afterwards.

DESCRIPTION OF THE EGG

According to Seamans (31), pale western cutworm eggs are larger than those of most cutworms. They are about one mm. in diameter.

Newly laid eggs are milky white, but as they develop their colour becomes bluish with a pearly lustre. Fully developed eggs are dark gray-purple. Since the eggs are laid in the soil and when fresh the chorion is sticky, a layer of soil particles usually adheres to them (Fig. 1).

The egg is sub-spherical in form with the micropylar area, and its opposite pole, somewhat flattened. The chorion shows a series of reticulations radiating from the micropyle (Fig. 2).

The eggs are usually laid in masses in the soil, each egg cemented to its neighbor by the sticky substance covering the chorion. A cluster of eggs may vary from a few to well over one hundred.

MATERIALS AND METHODS

The female moth emerges from the pupal state almost sexually mature. Eidmann (10) says that most Lepidoptera on

emergence from the pupa have nearly always a great number of undeveloped eggs in their ovaries, and that such eggs are developed post-metabolically, so becoming suitable for deposition.

Dissection of female moths indicated that this does apply to Agrotis orthogonia Morr., but the fact that the female usually begins ovipositing two or three days after emergence from the pupa shows that many of her eggs are very close to being mature.

In the laboratory moths were kept in large glass jars containing two or three inches of fine, dry soil. A honey and water solution was provided. Four or five pairs of moths were usually kept in each jar although fertile eggs were recovered from single pairs of males and females.

High percentages of fertile eggs resulted when the moths were kept in an environment which included light and a temperature of 30° C. for eight hours, and 5° C. in darkness for a sixteen hour period. Mating and some oviposition occurred during the 5° C. period. Copulation was observed on one occasion.

When a female had begun to deposit fertile eggs she could be removed from possible contact with male moths and continue to lay fertile eggs for almost three weeks.

At the end of each sixteen hour, 5° C. period, the soil was removed from the jars and sifted through a fine wire mesh screen which retained the deposited eggs. These eggs had been at a temperature of 5° C. for the few hours that they may have been laid, so that a minimum of development could have occurred. They could then be utilized in the various incubation trials. Eggs from both field-collected and laboratory-reared moths were obtained in this manner.

The tests were carried out in controlled temperature rooms. The temperature in each room varied within $\pm 0.25^{\circ}$ C. Freshly deposited eggs were incubated at the various temperatures. After it had been established that 30° C. was the optimum temperature for egg development, it was used as a check temperature for all tests. Fifty eggs of every collection were allowed to develop at 30° C. in order to ensure that the majority were fertile. Fifty eggs were incubated at each temperature in each replicate. Small petri dishes were used to contain the eggs.

When eggs were removed for examination they were gently washed, using a moist camel hair brush to remove excess dirt, before being dissected. A binocular dissecting microscope was used and the egg chorion was ruptured with a pair of dissecting needles. The egg contents flowed or were squeezed

on to a glass slide where a drop of glycerol was added to prevent desiccation of the embryo before it could be examined. Some dissections were made under water.

Most egg dissections were made using a magnification of 160X but some examinations required 250X or 400X. Daily dissections were made of eggs from each trial.

When observations indicated that the embryo had absorbed the surrounding yolk, moist blotting paper was added to the egg container. This moisture kept the chorion soft enough that the fully developed embryo had no difficulty in escaping from the egg.

Tests on the effect of varying relative humidity were conducted at 35° C. and 40° C. The test eggs were put into desiccators in which the desired relative humidity was maintained by solutions of sulphuric acid as described by Solomon (33).

When eggs were removed from the desiccators in the relative humidity tests, the lid of the desiccator was removed and replaced as quickly as possible in order that the equilibrium would be disturbed as little as possible. Equilibrium would probably be established within one hour of being disturbed so that the incubating eggs were subject to the desired relative humidity a minimum of 23 hours out of every 24.

Fig. 1. Pale western cutworm egg with a layer of soil particles adhering to it.

Fig. 2. A washed egg showing reticulations of the chorion.

RESULTS

THE ORDER OF MORPHOLOGICAL DEVELOPMENT IN THE EMBRYO OF AGROTIS ORTHOGONIA MORR.

When freshly deposited eggs are dissected they are found to contain a homogeneous, creamy fluid. The first indication that embryological development is underway is when a few small droplets of clear matter are noted in the yolk fluid. As development progresses these masses become larger.

A cellular, colourless membrane, presumably the serosa, soon completes its formation. This membrane is underneath the chorion and completely envelopes the egg yolk and embryo. The egg shell may be dissected away leaving this membrane and its contents intact if extreme care is taken. This membrane usually, but not always, develops a few cream or rust coloured strands or masses. These strands are incorporated into the membrane itself and are not included in the egg yolk or embryo. If the chorion of an egg is cleaned of soil particles at this stage, the rust coloured matter is easily seen through it.

One of the clear masses in the yolk becomes larger than the others and this can soon be identified as the developing embryo. It is a rather shapeless mass of clear matter before

assuming its definite, elongate form. The fluid surrounding the embryo remains a creamy-gray colour.

The embryo can soon be seen to be a definite, segmented, form approximately 2 mms. long and 0.5 mms. in width.

The pigmentation of the larval eyes is the next notable step in development of the embryo. They appear at first as two very small, light tan coloured spots. They serve to identify the anterior end of the embryo. As they grow they become darker brown in colour. It can now be seen that there are three pairs of eyes on each side of the head. They become a dark red-brown and finally black as the embryo develops. When they have acquired the dark brown colour they are clearly visible through the washed chorion of a complete egg.

At the same time as the larval eyes are acquiring their first pigmentation, the outlines of the mandibles may be observed but they are completely lacking in pigment. If the embryo is dissected in water the rudiments of the thoracic legs, also unpigmented, can be seen.

As the eyespots darken, a faint amber area appears in the embryo just behind the head. It is best viewed dorsally. As this amber area darkens to brown, it will be seen that a smaller amber area is present in most of the abdominal

segments. These areas are the ganglia of the embryonic nervous system.

Shortly after the first of these amber areas appears mandibular pigmentation begins. An embryo dissected under water at this stage shows movement of the unpigmented thoracic legs. Also present are short unpigmented setae on the thorax and abdomen of the embryo. Spiracles lacking in pigment may also be seen.

The pigmentation of the mandibles begins with the biting edges. As this region darkens from a yellow to a red-brown colour the remainder of the mandibles becomes lightly pigmented. During this period the other mouthparts begin to acquire pigment. Occasionally at this stage gas bubbles may be present in the egg fluid.

When the mandibles have reached a state of moderate red-brown pigmentation, the head capsule and the dorsal thoracic plate both begin to show a slight gray colouration. By this time the spiracles, setae, and thoracic legs are usually darkly pigmented. The gray pigmentation of the head capsule gradually darkens until it finally becomes a shiny, dark brown colour. When the mandibles and head capsule have acquired partial pigmentation they are visible through the washed chorion of the complete egg.

As the head capsule becomes pigmented the chorion of the complete egg begins to show a bluish-purple tinge. This tinge deepens until by the time the head capsule (Fig. 3) is fully pigmented, the chorion is a deep blue-purple. This is the colour often associated with fully developed pale western cutworm eggs.

When the head capsule is still a dark gray colour the embryo ingests the yolk. Dissections of the embryo's digestive tract were made prior to, and immediately after, the ingestion of the yolk. These dissections show definitely that the yolk is ingested by the embryo (Figs. 4, 5).

Shortly before the yolk is ingested the embryo becomes much more resistant to desiccation in air. This would imply that the epicuticle of the embryo was functioning normally.

Even though the head capsule is now fully pigmented a dissected embryo shows the abdomen to be a pearly-white colour. The abdomen slowly becomes pigmented until it is a definite tan or buff colour. This is the state of the larva when it finally emerges from the egg.

Although the embryo may now be fully developed morphologically, it does not hatch immediately even at optimum developmental temperatures. Embryos incubated at 25° C. or 30° C. require a period of two or three days before they will hatch naturally, even though contact moisture is provided.

Although the embryo is morphologically complete, a further period of physiological development may be required.

Fig. 3. A fully incubated
egg showing the mature
embryo through the chorion.

Fig. 4. The stomodaeum and head capsule of an embryo before absorption of the yolk.

Fig. 5. The stomodaeum and head capsule of an embryo after absorption of the yolk.

A KEY TO THE EMBRYOLOGICAL DEVELOPMENT OF
AGROTIS ORTHOGONIA MORR. AT 30° C.

The more outstanding aspects of morphological development have been described in a key that relates them to the duration of incubation at 30° C. This key is intended as a convenient practical means of reference to embryonic development, so the time is given in days. The relationship between development and time, as given in the key, should be interpreted as not being too rigid, but rather as an optimum. Occasional embryos fell slightly behind or were slightly ahead of the described character. Only those characters observable under a binocular dissecting microscope have been described.

THE 30° C. KEY

<u>DAY</u>	<u>DESIGNATION</u>	<u>STAGE OF DEVELOPMENT</u>
1	A	Two or three small, clear masses present in the cream coloured fluid of the egg.
2	B	Many small, clear masses present in yolk fluid.
3	C	Two or three large, clear masses in yolk.
4	D	Embryo can be identified as a clear mass, segmented, with larval eyes observable as two faint tan coloured spots.

5	E	Eyespots are dark red-brown colour.
Slight buff coloured patch on dorsal
thorax behind head capsule.
6	F	Buff coloured patch easily seen.
Mandibles show slight pigmentation.
7	G	Mandibles well pigmented. Head
capsule is lightly pigmented gray.
Fluid still surrounds embryo.
8	H	Head capsule dark brown or dark gray.
Fluid has been absorbed by embryo.
Head capsule easily seen through
egg chorion.
9	I	Head capsule black. Abdomen is still
pearly-white. Embryo may be fairly
active when dissected out of egg.
10	J	Egg chorion shows blue-purple colour.
Embryonic abdomen is a slight tan colour.
11	K	Embryonic abdomen is dark tan colour and
larvae begin hatching.

THE EFFECT OF TEMPERATURES WITHIN THE DEVELOPMENTAL RANGE

ADDITIONAL INCUBATION TESTS AT 30° C.

The trials at 30° C. were conducted in a constant temperature room in which the relative humidity was maintained at 45[±]2 per cent. When the eggs had become fully developed moist blotting paper was added to the petri dish to ensure that the chorion was soft enough for the larva to escape from the egg.

Three series of 50 eggs each were incubated at this temperature. When the eggs had completed development no direct moisture was added to the eggs as in the other 30° C. tests. In all three series larvae still managed to escape from the egg although no contact moisture was provided. When the eggs are moistened directly larval emergence begins on the eleventh day and is usually complete about the thirteenth. In the three test groups two series began to hatch on the thirteenth day and completed it on the nineteenth. The third group commenced hatching on the fourteenth day and concluded on the nineteenth.

The three check groups averaged a hatch of 84 per cent. The three test series averaged 39 per cent hatch. The unhatched eggs were dissected on the twenty-first day and the majority of them contained fully developed but dead, desiccating embryos.

A single test series of 50 eggs was incubated at 30° C. and a relative humidity of 10 per cent. The check eggs were incubated as usual at 30° C. and a relative humidity of 45 per cent. Ninety per cent of the check series hatched normally. The 50 eggs in the test group developed normally. Contact moisture was added on the ninth day and the larvae began emerging on the eleventh. Seventy-eight per cent of these eggs hatched.

Several clutches of eggs from field reared moths in captivity were obtained in the fall of 1951. Three groups of 50 field eggs each were incubated at 30° C. Daily dissections showed that the embryos developed at the same rate as those in the eggs from the laboratory reared material. Larval emergence began on the eleventh day in two groups and on the twelfth day in the third. These results corresponded very closely with those of the laboratory reared eggs.

Several moths were observed ovipositing in the field during the fall of 1951 and several egg masses were recovered from the soil. A cluster of 40 of these eggs was incubated at 30° C. Their rate of development coincided with that of the laboratory reared material. In view of these results it was not considered necessary to repeat the developmental tests at other temperatures with eggs from field reared moths.

The results show that when pale western cutworm eggs are incubated at 30° C. and a relative humidity of approximately 45 per cent, they will hatch without the addition of contact moisture. However, when moisture is added directly to fully developed eggs they will begin hatching sooner and a greater percentage of larval emergence will result.

If moisture is added to fully developed eggs which have been incubated at 30° C. with a relative humidity of 10 per cent they will hatch normally and in the usual time.

Eggs from field reared pale western cutworm moths develop at the same rate as eggs from laboratory reared material.

THE EFFECT OF MEDIAN TEMPERATURES ON THE RATE OF DEVELOPMENT

The developmental rate of pale western cutworm eggs at 30° C. has already been recorded in a key. The rates have also been determined using temperatures of 10° C., 15° C., 20° C., and 25° C. The development of the embryo at these temperatures was traced using the same criteria of development as in the 30° C. tests.

Three replicates were completed at each temperature and the results graphed. Each replicate was plotted on a graph (Fig. 6) but in several instances the results at particular stages agreed, so that these points overlap on the graph. The mean of the three replicates has been shown by a straight line graph for each test temperature.

The criteria of development which were used in preparing the 30° C. key have been used in graphing the incubation rates at the other temperatures. These criteria may be identified on the vertical axis of the graph by letters which correspond with the various stages of the 30° C. key.

Fig. 7 shows the result of plotting temperature against the developmental time in days. This graph illustrates that there is an increase in rate of development when the developmental temperature is increased, ie., there is a positive correlation between temperature and rate of development, at least within the limits 10° C. and 30° C.

Also shown on Fig. 7 is a graph of linear regression derived by plotting the temperature in degrees centigrade against the reciprocal of the incubation time in days.

The regression line indicates that the theoretical threshold of development is approximately 8° C. This agrees with the incubation results. Eggs would not complete development at 5° C. but they incubated successfully at 10° C. This graph also shows that with an increase in temperature there is an increase in rate of development.

Using these data it is possible to calculate the approximate time of hatching of eggs of the pale western cutworm, provided the previous temperature history of the eggs is known. It has also been shown that freshly deposited eggs will successfully complete development and hatch normally at temperatures within a range of 10° C. and 30° C.

Fig. 6. Embryological development of eggs of the pale western cutworm, Agrotis orthogonia (Morr.).

Fig. 7. The effect of temperature on the developmental period of eggs of Agrotis orthogonia (Morr.)

THE EFFECT OF ABNORMAL TEMPERATURES

TESTS CONDUCTED AT 35° C. and 40° C.

When 50 eggs were incubated at a relative humidity of 10 per cent and 35° C. development at first seemed to proceed quite normally. The larval eyespots were first observed on the fourth day of incubation. This agreed with the developmental rate for 30° C. as shown in the key.

As development continued it became evident that it was abnormal in some respects. The amber coloured areas of each segment, which were the ganglia of the nervous system, had become a much darker brown than was usual at the median temperatures. The integument of the embryo was a yellow colour rather than the pearly-white observed in the lower temperatures.

By the ninth day most of the embryos had black head capsules but the yolk fluid had not been absorbed. Many of these embryos showed slight movements of the thoracic legs.

At this time moist blotting paper was added to the egg container. No larvae had hatched by the fourteenth day so several embryos were dissected out of the eggs. Very few of these embryos showed any movement. It was noted that most of the head capsules were becoming distinctly wrinkled. No larval

emergence had occurred by the twentieth day so more embryos were dissected out of the eggs. All eggs contained dead embryos.

Although embryos would not complete normal development at 35° C. and a relative humidity of 10 per cent it was thought that there might be a humidity level at which development would proceed normally to completion at this temperature. A test was carried out at 35° C. which provided the undeveloped groups of eggs with various levels of relative humidity, ie., 25 per cent, 50 per cent, and 90 per cent. Another group of freshly deposited eggs had contact moisture added throughout the test period.

As in the 10 per cent relative humidity trial, development seemed to progress normally in all tests until about the eighth day. At this time the excessive yellowing of the abdomen and browning of the nerve ganglia was observed at all levels of relative humidity. No larvae hatched naturally in any of the tests. It was noted that in only a few instances was the egg yolk absorbed by the embryo. Soon after the head capsules had become fully pigmented they began to wrinkle. All dissected embryos were dead by the fifteenth day. Even in the test where direct moisture was provided, or where a relative humidity of 90 per cent was maintained, no larval hatch occurred.

Attempts were now made to determine whether incubating eggs could withstand a period of exposure to 35° C. One hundred and fifty unincubated eggs were exposed at 35° C. Blotting paper in the egg container was kept moist. Three groups of 50 eggs each were transferred to a temperature of 30° C. after three and one-half, seven and 14 days respectively.

Larvae of the three and one-half day group began hatching on the eleventh day of incubation. After four days 82 per cent of the eggs had hatched. The check series showed a hatch of 88 per cent.

No natural hatch took place in either the seven or 14 day groups. After 21 days these eggs were dissected. They contained dead, desiccated embryos.

Two groups of 50 undeveloped eggs were allowed to incubate at a temperature of 40° C. One group was kept at a relative humidity of 10 per cent and the other at 90 per cent.

After four days the eggs at 10 per cent relative humidity were completely desiccated. The eggs incubating at 90 per cent relative humidity were not desiccated at this time but the embryos were distorted. On the sixth day, even though the yolk was fluid, the embryos were all dead. None of them had acquired any pigmentation.

Twenty-five unincubated eggs were put into 30° C. until examination of the embryos showed that the eyespots were

lightly pigmented. The remaining eggs were then transferred to 40° C. where they were placed on constantly moist blotting paper.

After a period of three days in this environment the embryonic masses had become quite fluid. They were soon completely desiccated.

The results indicate that freshly deposited pale western cutworm eggs will not complete incubation and hatch at 35° C. regardless of the level of relative humidity. The non-absorption of the egg yolk indicates that most of the embryos were probably dead prior to stage H of the 30° C. key. The fact that the embryonic head capsules showed wrinkling at this same time also indicated that the death of the embryo had taken place.

The results of the succeeding test show that eggs will hatch normally at 30° C. after a period of three and one-half days at 35° C. However, embryos which have been exposed to this temperature for a period of seven days or more are not likely to hatch normally.

As expected eggs develop at 35° C. at a rate very close to that for 30° C. up to the point where the embryo begins to deteriorate. The embryo completes morphological development at 35° C. but there seems to be some interference with a

physiological process. This interference must occur sometime between three and one-half and seven days. It is doubtful if the embryo deteriorated as a result of desiccation because the yolk was still in a fluid state at the time of death.

Eggs will not develop at 40° C., nor will partially developed embryos complete development at this temperature.

SOME EFFECTS OF INCUBATING EGGS AT 2° C. AND 5° C.

Three different tests were conducted at both 2° C. and 5° C. with freshly deposited pale western cutworm eggs. These tests were carried out at temperatures of 2[±]1° C. and 5[±]0.25° C. Check groups of eggs for both tests incubated normally at 30° C. and showed an average hatch of 86 per cent.

In the first test three groups of 50 eggs each were exposed to 2° C. and a similar three groups were stored at 5° C. This test was an attempt to determine whether undeveloped eggs would partially or completely develop at these temperatures. Periodic egg dissections were made. The observations are recorded in Table I.

Table I. Embryological development of undeveloped pale western cutworm eggs at 20° C. and 50° C.

Period of time	Stage of development at 20° C.	Stage of development at 50° C.	Remarks
30 days	Gray fluid only	Two or three small clear droplets in gray fluid	-
60 days	Two or three small clear masses in gray fluid	Several droplets present in gray fluid and a new membrane, possibly the serosa, is entire	-
90 days	Many small clear droplets in gray fluid and a new membrane, possibly the serosa, is entire.	One clear mass much larger than the others and is probably the embryo	-
120 days	No change	No change	-
150 days	No change	No change	-
180 days	No change	No change	-
210 days	No change	Granular material in the egg fluid indicated the beginning of deterioration.	It is now assumed that development has become static. When 25 eggs from each test were transferred to 30° C. they all deteriorated.

An attempt was made to determine if there was any period of time that newly laid eggs could be exposed to either 2° C. or 5° C. and still complete development after being transferred to a more favourable temperature.

Five groups of 50 freshly deposited eggs were exposed to 2° C. for periods of one, two, four, eight, and 16 days respectively. Five similar groups were subjected to 5° C. for the same periods of time. At the end of the exposure period each group was transferred to 30° C. where observations were taken and recorded as in Table II. Control groups of eggs developed normally at 30° C.

Table II. The effect of 2° C. and 5° C. on undeveloped pale western cutworm eggs.

Initial incubation temp.	Period at initial temp.	Final incubation temp.	% Hatch	% distorted embryos	% deteriorated eggs	% infertile eggs	% failed to hatch. Reason unknown.
2° C.	1 day	30° C.	92	0	0	4	4
2° C.	2 days	30° C.	84	0	0	6	10
2° C.	4 days	30° C.	90	0	0	2	8
2° C.	8 days	30° C.	8	84	0	2	6
2° C.	16 days	30° C.	0	4	84	4	8
5° C.	1 day	30° C.	84	0	0	10	6
5° C.	2 days	30° C.	84	0	2	8	6
5° C.	4 days	30° C.	64	0	28	4	4
5° C.	8 days	30° C.	72	0	20	6	2
5° C.	16 days	30° C.	12	22	58	6	2

The third group of tests at 2° C. and 5° C. utilized partially developed eggs. Two series of 200 eggs each were incubated at 30° C. for five days. The embryos then showed slight pigmentation of the mandibles but no colouration of the head capsule. One group of 200 eggs was now moved to 2° C. and the other to 5° C. Observations were made on both series to determine whether partially developed eggs will complete development at 2° C. or at 5° C. The observations made from periodic egg dissections are recorded in Table III.

Table III. Embryological development at 2° C. and 5° C. in eggs previously developed to the five day, 30° C. stage.

Period of time	Stage of development at 2° C.	Stage of development at 5° C.	Remarks
40 days	Embryos developed to the 7-day, 30° C. stage	Embryos fully incubated and first larva hatched in 5° C.	-
80 days	Embryos fully developed and first larva hatched in 2° C.	15 per cent of the 200 eggs hatched	-
120 days	6 per cent of the 200 eggs hatched	50 per cent hatched	50 eggs of 5° C. test transferred to 25° C.
140 days	18 per cent hatched	-	-
160 days	50 per cent hatched	-	50 eggs of 2° C. test transferred to 25° C.

When the 50 per cent hatch level had been reached in each test series, 50 of the remaining eggs in each test were moved to 25° C. Of the eggs which had completed development at 2° C., 76 per cent hatched. The 5° C. test eggs showed a hatching percentage of 88 per cent after removal to 25° C. Contact moisture was provided the eggs on being transferred to the higher temperature. The test eggs had previously been stored without the addition of contact moisture.

Although the data shows that freshly deposited pale western cutworm eggs will not completely develop at either 2° C. or 5° C. the indications are that embryological development will progress further at 5° C. than it will at the lower temperature. Once development has reached its limit in either temperature, the eggs will not complete development at temperatures which normally favour incubation. The eggs deteriorate quickly after being transferred to the higher temperature.

Eggs which have developed to the five-day stage at 30° C. will complete development, and at least 50 per cent of them will hatch, at a temperature of either 2° C. or 5° C. These eggs require a longer period to complete development at 2° C. than they do at 5° C.

Of the unhatched eggs in 2° C. and 5° C. when the 50 per cent larval emergence point had been reached, very high

percentages hatched when the eggs were removed to 25° C.

The tests show that fully developed pale western cutworm eggs will hatch without the addition of contact moisture at 2° C. or 5° C.

Indications are that undeveloped eggs may be stored at both 2° C. and 5° C. for periods of one, two or four days and still successfully complete development when removed to 30° C. After an eight-day period at 2° C. the percentage of eggs that develop normally in the higher temperature is very low. No eggs were able to withstand a 16-day exposure to 2° C. and develop normally.

However, the eggs which were exposed to 5° C. for eight days showed no unusual effect when transferred to 30° C. A small percentage of eggs withstood a 16-day exposure at 5° C. and developed normally at the higher temperature.

THE EFFECT OF EXPOSING EGGS TO 0° C.

Groups of 50 freshly oviposited pale western cutworm eggs were stored at 0° C. for one, four, eight, 12, and 63 days. At the end of these periods the various groups were removed to 30° C. where they were allowed to develop in a relative humidity of 45⁺-2 per cent. Embryological development was traced by

making egg dissections every second day. The results of the observations are recorded in Table IV. They indicate that undeveloped eggs are unaffected by periods of exposure to 0° C. up to 63 days prior to removal to a favourable developmental temperature.

Table IV. The result of exposing undeveloped pale western cutworm eggs to 0° C. for various periods prior to transfer to 30° C.

Period at 0° C.	% Hatch	% distorted embryos	% infertile eggs	% failed to hatch. Reason un- known.
1 day	84	0	6	10
4 days	80	0	10	10
8 days	80	0	8	12
12 days	72	0	8	20
63 days	78	0	10	12

SOME EFFECTS OF BELOW FREEZING TEMPERATURES ON DEVELOPMENT

A single series of groups of undeveloped eggs was exposed to temperatures of -5° C., -10° C., -15° C., and -20° C. for periods of one, two, four, eight and 16 days. The various groups were then moved to the 30° C. room. Check eggs were incubated at 30° C. and showed an average hatch of 84 per cent.

The results are recorded in Table V.

Freshly deposited pale western cutworm eggs may be stored for a period of at least two days at -5° C. and successfully complete development after being removed to 30° C. When eggs remain at -5° C. for a period of four days prior to incubation at 30° C., the percentage of eggs which hatch normally is reduced by approximately 50 per cent. Most of the eggs which fail to hatch deteriorate soon after being transferred to 30° C. At the same time a few of these eggs showed embryos which became distorted from the normal as the developmental period progressed. The proportion of distorted embryos is much larger in eggs which were exposed to -5° C. for an eight-day period. This period also showed a greatly decreased percentage of hatch.

When eggs are stored at -10° C. a low percentage hatches even if the storage period is only one day. After four days

or more at -10° C. the number of eggs developing successfully at 30° C. approaches zero. Any period between one and eight days at -10° C. results in a large number of distorted embryos. However, the majority of these eggs deteriorate before an identifiable embryo has developed.

Exposure to -15° C. for one day prior to incubation at 30° C. results in a very low percentage hatch and a high proportion of distorted embryos. When this storage period is increased to two or more days both the percentage of hatch and the number of distorted embryos decreases. After an eight-day exposure to -15° C. the majority of the eggs deteriorate at 30° C.

No eggs completed development after exposure to -20° C. for one or more days. A few distorted embryos resulted when the period at -20° C. was one day, but when the exposure period was increased to two or more days, only deteriorated eggs were observed.

Table V. The result of exposing undeveloped pale western cutworm eggs to below zero temperatures prior to incubation at 30° C.

Ini- tial temp.	Period at ini- tial temp.	% hatch	% distort- ed embryos	% deteri- orated eggs	% infer- tile eggs	% failed to hatch. Reason un- known.
-50° C.	1 day	80	0	0	10	10
-50° C.	2 days	88	0	0	4	8
-50° C.	4 days	40	2	44	10	4
-50° C.	8 days	8	22	42	10	18
-50° C.	16 days	18	24	36	10	12
-10° C.	1 day	16	24	48	12	0
-10° C.	2 days	24	28	42	4	2
-10° C.	4 days	6	30	50	4	10
-10° C.	8 days	0	14	74	6	6
-10° C.	16 days	0	10	82	2	6
-15° C.	1 day	16	32	48	0	4
-15° C.	2 days	2	24	70	2	2
-15° C.	4 days	0	12	84	2	2
-15° C.	8 days	0	0	92	8	0
-15° C.	16 days	0	0	88	8	4
-20° C.	1 day	0	14	80	6	0
-20° C.	2 days	0	0	88	8	4
-20° C.	4 days	0	2	92	6	0
-20° C.	8 days	0	0	90	4	6
-20° C.	16 days	0	0	92	6	2

DISCUSSION

DISTORTED EMBRYOS

Fig. 8 shows two of the distorted embryos caused by exposing undeveloped eggs to 2° C. for eight days before moving them to 30° C. A normal fully developed embryo is also shown. A large variety of distortions have been observed in these embryos. Mouth parts, larval eyes, head capsule, thoracic legs and abdomen are all subject to malformation.

Although most of these embryos are dead when dissected out of the egg the individual structures have usually completed development, even though they may have been subject to distortion. The mandibles may be located well apart but they will usually be fully pigmented. Malformed abdomens are always pigmented and possessed of setae and spiracles. Many of these embryos are completely lacking in one or more anatomical characters.

It is possible that certain essential enzymes or hormones are inhibited wholly or partially by the influence of 2° C. and other low temperatures. These compounds may be responsible for the cell differentiation which, if not carried out correctly

Fig. 8. (1) A normal fully incubated embryo.
(2) and (3) are distorted embryos caused by temporary exposure to temperatures just above the freezing point.

in the early embryo, results in the monsters and distortions observed in the tests.

Possibly the cause of embryo distortion at 2° C. and 5° C. differs from that of embryos at sub-zero temperatures. The monsters caused by exposure to the above freezing temperatures may be caused by differential development, ie., certain cells in the early embryo are able to develop in the usual manner while others fail to function normally at these low temperatures. This differential development probably occurs during the exposure period and then continues when the eggs are transferred to the higher temperature.

The distorted embryos caused by exposure to below freezing temperatures may be caused by a different influence. Although eggs may not freeze in a particular below zero temperature, there would be a chilling effect on the embryo. The lower the temperature the eggs were exposed to, or the longer the eggs were exposed at a specific temperature, the greater the influence of the chilling effect on the embryo. It is unlikely that any embryological development took place in the below freezing temperature but the chilling might be sufficient to cause distortion of the embryo after it is removed to the warmer temperature and has begun to develop. If the undeveloped eggs suffered sufficient chilling, either because of a

prolonged spell in a mildly freezing temperature, or a short spell in a very cold environment, the embryo would no doubt be killed and deteriorated eggs would result.

Eggs which were exposed to 0° C. for a prolonged period still produced a high percentage of normal larvae after being transferred to 30° C. The reason for this normal development may be that the 0° C. temperature is too low for any differential development to take place but at the same time it is too high for the chilling effect to be sufficient to influence the embryo, at least over a period of 63 days. This may be the reason why high percentage hatch and no distorted embryos resulted from exposure of eggs to this temperature.

INFERTILE EGGS

Some female moths deposited large numbers of eggs which were 100 per cent infertile even though males had had access to the females. These moths had been kept at the same environmental temperatures 24 hours a day. As previously described, an eight hour period at 30° C. followed by a 16 hour period at 5° C. was sufficient to stimulate copulation. This resulted in almost 90 per cent fertile eggs being deposited.

In the field it is very unlikely that females would lay large percentages of infertile eggs because of a similar lack of copulation stimulation. There are bound to be sufficient fluctuations in temperature to ensure the deposition of fertile eggs.

THE CRITERION OF FULLY DEVELOPED EGGS

The usually accepted criterion of fully developed pale western cutworm eggs has been the deep blue-purple colour which the eggs acquire during the period of development. Eggs in this condition are generally thought to be in condition to hatch. In the course of the experiments it was found that even at 30° C. blue-purple eggs would not hatch for a day or two. As the developmental temperature is lowered this period between the acquiring of the colour and the time of hatching lengthens. When eggs were incubated at 20° C. this period was found to be eight or nine days, and at 15° C. the interval became approximately 20 days. Therefore it would seem that blue-purple eggs may or may not be ready to hatch.

FUTURE INVESTIGATIONS

Several tests, especially those dealing with embryological development at the below freezing and slightly above freezing temperatures, will be repeated when additional eggs become available. Some work on the effect of these low temperatures on morphologically developed eggs is under way at the present time.

Additional work will be undertaken to determine the reasons for distortion of embryos by temporary egg storage at low temperatures. This will entail a detailed cytological study of the embryo. It may be possible to establish the exact periods at various temperatures which will affect cell differentiation at the time organogeny is taking place.

A single combination of temperatures within a 24 hour period which is favourable to the deposition of fertile eggs in the laboratory has been established. Other combinations will be investigated. The effect of the various temperature combinations on fecundity will also be studied.

Arbitrary criteria of embryological development have been established. Attempts will be made to apply these data in field studies. The failure of forecast outbreaks to materialize may be partially explained by the fact that undeveloped eggs will not complete development after fairly short periods of exposure to 2° C. or below freezing temperatures.

SUMMARY AND CONCLUSIONS

The late development of the embryo of the pale western cutworm, Agrotis orthogonia Morr., as viewed through a binocular dissecting microscope is described. The effect of median temperatures on the rate of development is reported. A descriptive key to the rate of development at 30° C. has been proposed. This temperature is shown to be the optimum for incubation of pale western cutworm eggs. The various effects of temperature may prove useful in determining the reasons why forecast outbreaks have sometimes failed to materialize.

The effect of temporary egg storage at temperatures from 5° C. to -20° C. on future development are shown. Several of these temperatures have proven unsuitable for egg storage.

Eggs from both laboratory reared and field collected moths are used in the experiments. It is shown that eggs from both sources will develop at the same rates. It may be concluded from these tests that no period of diapause is required by embryos of the pale western cutworm.

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