Initiation of post-diapause development and reinstatement of diapause in Cephus Cinctus Nort.
by Norman Stanley Church

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Entomology
Montana State University
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Abstract:
Sawfly larvae normally absorb contact water during the latter part of diapause and early prepupal development. Moisture thus absorbed is unnecessary for diapause development but is sometimes required for the initiation of post-diapause morphogenesis. Desiccation at 25° C. or higher had little effect on diapause reinstatement in undeveloped postdiapause larvae in their stubs. Moreover, previous dehydration at 0° C. did not enhance the diapause-reinstating activity of heat. Prolonged desiccation at 0° C. often prevented the initiation of post-diapause morphogenesis in larvae subsequently incubated moist; in other larvae it only delayed morphogenesis.

Ligation showed post-diapause morphogenesis to depend upon the secretion of a differentiation hormone from the prothorax. Its secretion, in turn, is prompted by a stimulatory substance from the head. Blood transfusion showed diapause to be primarily the result of a lack of differentiation hormone. It is proposed that the original stimulus releasing the endocrine mechanism is exerted by the nervous system, and that diapause development is a conditioning of the insect preparatory to the nervous system's becoming suitably activated.

In larvae just out of diapause, heat at first stimulates the endocrine mechanism, and thereby the initiation of morphogenesis. Longer heat exposure reinstates diapause, presumably by destroying the activated endocrine mechanism. Diapause can not be reinstated after the differentiation hormone from the prothorax has been released. That the developmental block induced by heat is a true diapause is confirmed by the fact that it can be eliminated by chilling. Chilling, however, was unsuccessful in eliminating the developmental block introduced by dryness. Because of this and other differences between the effects- of dryness and those of heat on the initiation of post-diapause morphogenesis, presumably the results of their influence on the endocrine mechanism, it is suggested that the developmental block caused by dryness is not a true diapause.

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TABLE OF CONTENTS

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INTRODUCTION

Farstad in 1938 observed the failure of post-dia-
pause larvae of wheat stem sawfly (Cephus cinctus Nort.) to
develop during a hot dry spring. Salt (1947) confirmed that
diapause, though once broken, could be reintroduced by the
action of heat. Exposure to 35° C. for 25 days during the
larva's transition from diapause development to post-diapause
morphogenesis caused all larvae to revert to diapause. On
the Great Plains heat and drought are usually closely associ-
ated, so the possibility presented itself that dryness, too,
might influence the reinstatement of diapause.

Only one other indication of a possible similar
reinstatement of diapause was found in the literature.
Prebble (1941b) observed that a dry spring prevented the
development of Diprion, which undergoes a prepupal diapause.
In a bivoltine race development commenced if water was
supplied later during the summer, but members of a univoltine
race did not so respond. Possibly the latter had been
returned to diapause.

Such a unique event in the life history of an insect
as a reinstatement of diapause warranted further investi-
gation. The possible influence of dryness in returning

_C_. _cinctus_ to diapause was of particular importance and was chosen as the subject of this work.

REVIEW OF LITERATURE

A discussion of literature pertaining to diapause in general is presented in the first part of this section. It is followed by more detailed accounts of literature dealing with relationships between moisture and diapause, the "hormone failure" theory of diapause, and diapause in C. cinctus, each of which directly concerns the original work to be presented in this thesis.

Much has been learned about insect ecology and physiology since the phenomenon of diapause was recorded by Duclaux in 1869. Many diverse records on diapause have been reported, especially in relation to ecology. However, the real nature of diapause remains unknown.

Wheeler (1893) invented the term "diapause" to describe a stationary period during blastokinesis in Xiphidium. Henneguy (1904) extended the term to include an arrest of development at any stage of an insect's life. The word has frequently been used very loosely since then, even to include development temporarily retarded directly by cold, starvation, or drought. It is now most commonly and most satisfactorily restricted, as it was by Shelford (1929), to more or less spontaneously arrested development where further activity can not immediately be induced by providing the

kind of environment normally suitable. Duclaux's (1869, 1876) work on Bombyx eggs first pointed out this distinction. He found that their arrested development was brought to a conclusion by refrigeration but not by continuous rearing at room temperature.

This distinction between diapause and otherwise arrested development is an important one, but the division is arbitrary. Insects in which development is arrested directly by a sub-optimum external condition must adjust themselves physiologically to this state of inactivity. Though their development is resumed when the unfavorable condition is removed, this response sometimes becomes apparent only after considerable delay, during which they readjust themselves to activity. This behavior is intermediate between temporarily arrested development and a typical diapause.

General Characteristics of Diapause

Numerous workers have reported that diapause insects have a very low rate of respiration combined with a lowered respiratory quotient. The work of Bodine, Williams, and their associates proved that diapause respiration involved more than a mere quantitative change. Respiration of diapause Melanoplus eggs was unaffected by carbon monoxide, cyanide, and sodium azide, all of which affect the cytochrome-

cytochrome oxidase system. All but about one-fifth of the respiration of non-diapause eggs was affected by these agents (Bodine and Boell, 1934; Bodine, 1950). Williams (1947b) and Sanborn and Williams (1950), working with Platysamia, found that cytochrome respiration was broken down during diapause, except in the abdominal muscles, and life was maintained by a system of respiration involving flavoproteins.

The diapause state is also generally characterized by a lowered moisture content, increased food reserves in the form of an enlarged fat body, physical inactivity, and a pronounced decrease in mitotic activity as evidenced in grasshopper embryos (Carothers, 1924; Slifer, 1931). These characteristics have been noticed by many authors, most of whose papers on these and other aspects of diapause have been reviewed competently by Prebble (1941a), Simmonds (1948), and Lees (1950, 1952) and especially thoroughly by Andrewartha (1952).

Manifestations of diapause are not necessarily exhibited in all body functions. A few or many tissues or organs may be relatively unaffected by diapause. Muscular activity in diapause C. cinctus is relatively uninhibited (Salt, 1947). This is true also of the abdominal muscles of Platysamia (Williams, 1951). Imaginal diapause in many species affects only gonad development directly. On the

other hand, extensive cellular activity of the gonads continues in C. cinctus larvae kept at 10° C. (Mackay and Church, unpublished). Birch (1942) and Andrewartha (1943) observed visible growth and differentiation during diapause in Austroicetes embryos. It is wrong, then, to consider diapause simply as a state approaching "suspended animation". Although it is a useful term, "arrested development" can be and has been at times misleading.

Causes of Diapause

Sajo (1896) and Baumberger (1917) suggested that diapause was the result of "developmental fatigue" in which there was an accumulation of metabolic waste materials that inhibited development. Roubaud (1922, 1928, 1929) enlarged upon the hypothesis, concluding that excretion of these wastes had failed to keep pace with other physiological processes; a period of rest at a low temperature or low humidity was required in order that it catch up. When postulated, Roubaud's theory was readily compatible with the known developmental histories of the species of Diptera that he experimented upon, but more recent work has discredited the theory as an overall explanation of diapause.

Bodine (1932) suggested as the cause of the embryonic diapause in Melanoplus a similarly inhibitory "x-factor"

that could be eliminated best at low temperatures. Its

existence has never received experimental support.

Although Roubaud's and Bodine's hypotheses do not

completely explain diapause, inhibitory substances possibly

do contribute to it. They may be the agents whereby exter-

nal conditions can influence diapause. Generally unfavor-

able environmental conditions that commonly cause the rate of

development to be reduced usually strengthen the tendency

towards diapause in species having a facultative diapause,

i.e., in species in which every individual of each generation

is not obliged to enter diapause. The extensive literature

on this subject was critically reviewed by Andrewartha (1952).

Deficient or excessive moisture, food quality and its dryness,

low or unusually high temperatures, and over-crowding and

isolation have all been implicated. Unfortunately, in some

of the literature in which the influence of these factors is

described, no distinction is made between diapause and develop-

ment arrested only temporarily.

There are other diapause-inducing factors that

ordinarily are not associated with an unfavorable environ-

ment. Among them are declining, but not necessarily low,

temperatures (Dawson, 1931). In many species of Lepidoptera,

photoperiods in which "day" and "night" are roughly equal

tend to increase diapause within a population. Wide

departures in either direction, but especially in the direction of a longer light period, tend to prevent diapause (Dickson and Sanders, 1945; Dickson, 1949; Way and Hopkins, 1950; Lees, 1952). In one species, Antheraea, the situation is reversed (Lees, 1952).

The effect of declining temperature and photoperiod show that diapause is not solely an undesirable delay in the life cycle arising from an unhealthy condition, as it is commonly considered to be (e.g., Simmonds, 1948). Diapause is an adaptation naturally selected for many species because of its value in winter survival. The ability of an insect to overwinter is often dependent on its being in a state resistant to cold and desiccation that is most easily attained during dormancy. Some of the previously mentioned environmental factors that tend to induce diapause, e.g., low or declining temperature, dryness or richness of food, and short photoperiod, are associated with the end of the growing season. They serve as signals to synchronize the onset of diapause with the seasons.

The distinction between obligatory and facultative diapause is arbitrary. According to definition, an insect that undergoes an obligatory diapause must enter it regardless of environmental influence, whereas entry into a facultative diapause depends largely on external conditions.

In the latter case the insect may possess either a strong or weak tendency towards diapause. In some species where diapause at first appeared to be obligatory, upon investigation it has been found to be conditioned by environment (Cousin, 1932). Some insects may respond only to extremes not possible in nature (e.g., Danilyevsky, 1948).

The causative influence exerted by environment on the occurrence of diapause is through long and devious physiological processes. This is well demonstrated by the time lapse between cause and observed effect (Andrewartha, 1952). It is sometimes the next generation that is affected. The work of Kogure (1933) on Bombyx is particularly interesting. He found that light and temperature acting on incubating eggs partly determine whether or not eggs of the next generation will enter diapause.

Diapause Elimination

As previously implied, if the onset of diapause has been furthered by a certain environmental factor, the removal of or compensation for that factor does not break diapause. Low temperature is a common cause of diapause. However, almost invariably the best temperature for diapause elimination is 10° to 25° C. lower than the morphogenetic optimum.

Diapause-breaking is a progressive process. The rate-temperature curves for diapause-breaking and for normal non-diapause development are similar, except that the entire curve for diapause-breaking is displaced towards the lower end of the temperature scale. In both normal development and diapause-breaking, the rate of the process gradually diminishes as the temperature falls below optimum, and the rate falls off more abruptly if the temperature is raised. The similarity suggests that diapause-breaking is also a form of development (cf. Andrewartha, 1952). This "diapause develop=ment" must be completed before the more rapid post-diapause development and morphogenesis can begin.

Sometimes the rate-temperature curves for the two processes overlap extensively; sometimes not. In C. cinctus the optimum for morphogenesis is about 25° C. and for diapause development, 10° C. (Salt, 1947). An extreme overlap is evident where Matthée (1951) found that diapause in Locustana was eliminated at 35° C. Another is evident in Dickson's (1949) work on Grapholitha which showed diapause development to be more rapid at 26° C. than at lower temperatures.

There are numerous papers reporting the efficacy of various "shock" treatments such as pricking, singeing, oviposition by a parasite, and the action of acids and other chemicals in breaking diapause. But, as Andrewartha (1952)

has suggested, apparently these have been found effective only in insects with a "weak" diapause or in those nearing the end of a "stronger" one. In similar circumstances, sudden temperature changes have been found to have the same effect in Diprion by Gobeil (1941) and Melanoplus by Bodine and Robbie (1940). Browning (1952) demonstrated that Gryllulus eggs required shorter low-temperature exposures to break diapause if they were incubated at much higher temperatures afterwards. Some species require a final stimulus at the end of diapause development. In Locusta, Le Berre (1951) notices that even in eggs in which diapause had been broken at a constant temperature well above the threshold of development (17°, 21°, and 25° C.) a slight increase in temperature was necessary to stimulate post-diapause development.

Dryness and Diapause

Dryness of either the habitat or food has often been reported as inducing diapause. This has been best substantiated in Pectinophora (Squire, 1937, 1940; Fife, 1949) and Loxostege (Strelnikov, 1936). Less moisture in diapause than non-diapause forms has been recorded in Leptinotarsa (Fink, 1925), Lucilia (Mellanby, 1938), and Carpocapsa (Ushatinskaya, 1949).

It has been demonstrated in Leptinotarsa (Fink, 1925), Pyrausta (Babcock, 1927), Epiblema (Rice, 1937), Lucilia (Mellanby, 1938), Diprion (Prebble, 1941b), Melanoplus (Slifer, 1946), Carpocapsa (Ushatinskaya, 1949), and Locustana (Matthée, 1951) that, the water lacking during diapause must be replaced before post-diapause development can proceed. Squire (1937) found that the addition of contact water to the habitat of Pectinophora expedited its emergence from dormancy or facilitated its subsequent development, but that it was not essential. From such information has arisen the idea, often casually repeated, that an external moisture supply is necessary for diapause development. But as Andrewartha (1952) emphasized, data presented by most of the authors just mentioned have indicated that the provision of water is only essential at the end of or after hibernation. None showed water to be necessary during diapause development. The water deficit must be remedied before post-diapause development can take place, but an external water supply has not been proven to have much influence on diapause development itself. Readio (1931) found that Reduvius completed diapause even more successfully at low humidities. Similarly, Matthée (1951) specified that in Locustana diapause development is best promoted by a dry environment, though moisture is required at its completion.

An apparent exception to the generalization that contact moisture is unnecessary for diapause development is found in the work of Slifer (1946), who showed that diapause in Melanoplus differentialis eggs could be broken by treating them with zylol and other fat solvents. These presumably dissolved a waxy coat and permitted the entry of water into the egg, thereby breaking diapause. However, Andrewartha (1952) suggested that xylol may have eliminated diapause by way of other, direct effects on the egg contents. Andrewartha's suggestion is supported by the fact that xylol can break diapause in Myrmus eggs, which contain enough moisture when laid to carry them through to hatching (Woodward, 1952). Pepper (1937), too, found xylol and other chemicals effective in breaking diapause in Loxostege prepupae where impermeability to water is not likely a factor.

The Hormone Failure Theory of Diapause

Wiggleworth (1934), nearly twenty years ago, proposed that diapause may be primarily the result of a hormone failure. This viewpoint is gaining increasing support. It has been proved that molting, including that which produces a pupa or adult, depends for its initiation on a hormone secreted by a pair (usually) of diffuse organs, the thoracic glands. These have variously been called the prothoracic

glands, ventral glands, corpora incerta, suboesophageal
glands, and hypostigmatic glands (Sellier, 1951). They are
generally situated in the prothorax, but frequently extend
into the head and/or mesothorax. These glands or their
homologues have been seen in Lepidoptera, Hymenoptera,
Orthoptera, Hemiptera, and Odonata. They exist in Diptera as
giant lateral cells of the ring gland. Work on this subject
has been reviewed by Williams (1948, 1949). Pflugfelder
(1947) reported the presence of ventral or thoracic glands
in a number of other, heterometabolous, orders. By ligation,
gland extirpation and implantation, and blood transfusion
experiments the control over molting exerted by the thoracic
glands has been demonstrated in Bombyx (Fukuda, 1944) and
Platysamia (Williams, 1947) among the Lepidoptera, in Diptera,
Odonata, and Phasmida, and finally in the Hemipteran
Rhodnius (Wigglesworth, 1952). Most of this work was reviewed
by Wigglesworth (1951).

Kopec (1922) discovered in Lymantria that the brain
controlled the initiation of molting, apparently by way of
glandular action. Dependence of molting upon the brain has
been revealed in other Lepidoptera, Hemiptera, Orthoptera,
Phasmida, Coleoptera, and Diptera (reviewed by Wigglesworth,
1948, 1951), and in Hymenoptera (Schmieder, 1942). In some
species it has been determined that the brain is the source

of the stimulation that activates the thoracic glands. A small number of neurosecretory cells in the protocerebrum secrete a hormone which, when it reaches the thoracic glands, causes them to begin secreting. This series of reactions has been traced in Rhodnius and Platysamia by the classic experiments of Wigglesworth (1934, 1940, 1951, 1952a) and of Williams (1946, 1947a, 1952), and in Calliphora by Possompès (1950) and in Bombyx by Bounhiol (1952a, 1952b) and Fukuda (1944). The sole function of the neurosecretory cells at this stage appears to be the stimulation of the thoracic glands. Once this has been done, the secretory activity of the thoracic glands and the molting process are no longer dependent upon the brain.

Protocerebral neurosecretory cells, since first found by Weyer (1935) in Apis, have been disclosed histologically in other Hymenoptera, Lepidoptera, Coleoptera, Diptera, Trichoptera, Orthoptera, Hemiptera, and Neuroptera (reviewed by Day, 1940, and Scharrer and Scharrer, 1945). Their homologues have been found in the Apterygota (Hanstrom, 1953). According to E. Scharrer (1952) neurosecretory cells are undoubtedly links of communication between the two physiological control systems, the nervous and the endocrine.

Platysamia experiences a pupal diapause before the endocrine mechanism controlling its imaginal molt and meta-

morphosis is set in motion. Williams (1946) demonstrated that during this diapause the implantation of brains from chilled, post-diapause pupae was enough to activate the thoracic glands and elicit normal metamorphosis. Active brain implants also proved to be effective in breaking diapause in Gryllus (Sellier, 1949).

Diapause seems to result from failure of the molting hormone mechanism to operate. In Andrewartha's (1952) opinion, since diapause (at least larval, nymphal and pupal diapause) occurs near the end of a stadium, the hormone failure theory of diapause is probably generally applicable. He believes the brain does not release the hormone mechanism because it is inhibited by the accumulation of reserve food in a form thatiis not immediately available to the tissues, and that diapause development is a process of food mobilization or processes prerequisite to it. The abundance of reports of marked differences in fat body and other tissues between diapause and non-diapause insects gives circumstantial support to this theory.

Diapause in Cephus cinctus

Ainslie (1929) noticed the inability of wheat stem sawfly larvae enclosed in their wheat stubs to develop indoors. It is now known that soon after the mature S-shaped

larva cuts off the wheat stem above the stub in which it over-
winters, it enters an obligatory diapause. By spring diapause
development is completed and post-diapause development can
begin. Salt (1947) found that diapause development proceeds
most rapidly at 10° C., and much more slowly at 15° and 5° C.
He also found that, in the field in 1946, diapause was
broken in every larva by January. It is invariably broken
by spring, although, as already related, diapause can then be
reintroduced.

Ainslie (1929) also observed that larvae in the
field sometimes failed to develop in the spring though they
were obviously viable. He attributed this to insufficient
moisture. There seems to be little doubt that the larvae had
been returned to diapause early in the spring. In extensive
areas of Alberta and Saskatchewan a severe early drought in
1937 killed the sawfly's host plants and nearly annihilated
the brood of offspring produced that year. In spite of this,
there was a sawfly outbreak in 1938. Large numbers of
larvae of the previous brood must have returned to diapause
in the spring of 1937, and emerged in the spring of 1938 to
cause the unexpected outbreak (Farstad, unpublished). In
1944 a similar carry-over occurred. In one infested field
in eastern Alberta, 86% of the stubs still contained
undeveloped S-larvae after the adult flight was over. Many

of the larvae survived, and in the spring of 1945 they

emerged as normal adults about the same time as others of the

next generation. Another observation of this kind was

reported by Mills, Callenbach, and Reinhardt (1945) who

wrote that undeveloped S-larvae were found in 50% of a stand

of infested stubs on July 18, 1944. This "spring diapause"

occurs frequently in small portions of a population whose

microhabitats are especially hot and dry (Farstad, unpublished).

EXPERIMENTAL OBJECTIVES

This study was undertaken to establish and explain the influence of dryness on diapause reinstatement in C. cinctus. This objective may be subdivided into the following: 1) to determine the role of contact water at the end of the proceding "fall" diapause; 2) to measure the over-all effect of moisture lack in reinstating diapause and preventing post-diapause development; 3) to investigate the application of the hormone failure theory of diapause to C. cinctus; and 4) to find the relationship to diapause reinstatement of the hormone mechanism controlling post-diapause development.

In order to obtain a general idea of the importance of water to diapause development the role of contact water during a normal "fall" diapause was first investigated. The more important water is to diapause development, the more effect it should have on diapause reinstatement.

A series of experiments were then done on the influence of dryness and desiccation on diapause reinstatement. In some species of insects, dryness has been reported to induce the original entry into diapause. Possibly it could also re-induce diapause in C. cinctus. In this species high temperature is known to cause diapause reinstatement. Experiments were performed at a variety of temperatures

because the influence of dryness could not be studied without taking heat into consideration. In the course of the experiments it became apparent that the relations between dryness, temperature, and diapause reinstatement were more complex than at first anticipated. Dryness did not simply add to or subtract from the effect produced by heat.

The next steps were to find out whether post-diapause morphogenesis was initiated by an endocrine mechanism and whether a hormone failure was the immediate cause of diapause in C. cinctus. It was anticipated that the relationship of the hormone mechanism to diapause would suggest some explanation for the effects of heat and dryness on diapause reinstatement.

EXPERIMENTAL PROCEDURES

Because of the variety of experiments to be discussed, only the experimental procedures generally applicable to a number of them are outlined in this section. Procedures specifically relating to individual experiments are described in the sections on experimental results.

Source of Material

The mature sawfly larvae used in this work were collected from wheat fields near Lethbridge, Alberta. Roots and debris were removed from the infested wheat stubs, which were then packed loosely in moist garden soil in closed pint jars for storage.

Conditioning and Storage of Larvae

Nearly all the experiments reported here required larvae that were ready to begin post-diapause development but had not yet begun it. In a few experiments spring-collected post-diapause material was used, but generally it was not reliably uniform and undeveloped. Material for most experiments was gathered in the fall while still in diapause. Diapause was broken in the laboratory under strictly controlled conditions in order to obtain the desired material. Since

diapause development progresses most rapidly at 10° C., this temperature was used for the first part of their conditioning. Post-diapause development also will take place at 10° C. Therefore the stubs were removed from 10° to 0° C. as soon as the first few larvae were out of diapause. Several months storage at 0° C. broke diapause in the remainder, and permitted them to finish most of the development transitory between diapause and post-diapause morphogenesis. None of them could begin post-diapause morphogenesis at 0° C. In this way a stock was prepared of fairly uniform post-diapause larvae, ready to recommence morphogenesis as soon as they were incubated. This method of conditioning was essential to experiments concerning initiation of post-diapause development and diapause reinstatement. The ligation experiments reported in a later section would have been impossible without it.

A supply of diapause larvae for use in a few experiments was maintained by storing fall-collected stubs in slightly moistened soil at 25° C., a temperature too high to permit any diapause-breaking. This temperature and the 10° and 0° C. temperatures mentioned above were maintained in constant temperature rooms with an accuracy of $\pm 0.5^\circ$ C.

Techniques Used in Moisture Relationship Experiments

One experiment dealt with moisture absorption during and after diapause development. Others concerned the effects of dryness and heat on diapause reinstatement. In all of these experiments, larvae were treated and incubated while intact in their stubs because the initiation of development after diapause is very easily inhibited if they are removed. After a post-diapause S-larva has begun to develop and has transformed into a prepupa, or even a day or so before this has happened, it will continue to develop under most conditions until it either emerges as an adult or dies. At moderate temperatures, and if excessive drying is prevented, it develops normally and at about the same rate as usual. Removal from the stub at this time has little effect. Just before this, during the stage from the end of diapause until shortly before the larva is ready to become prepupal, removal from its stub usually inhibits further development, even when temperature, moisture, and light conditions are apparently favorable. The insect remains active and healthy for months, but will not develop. This inhibition affects a large and variable portion of any sample of naked larvae in this stage. Moreover, the only criterion available for determining the presence or absence of diapause in <u>C</u>. <u>cinctus</u> is whether or not it will develop

when incubated. Any attempt to measure the influence of dry-
ness on diapause reinstatement using naked larvae is futile;
they must be kept in their stubs.

In experiments where the humidity was controlled,
it was done over sulphuric acid solutions or dry calcium
chloride in glass desiccators. High temperature exposures
(over 25° C.) were made in cabinets in which the temperature
fluctuations were less than ±1° C.

Whenever it was desired to determine the number of
larvae capable of development, or conversely, the number still
in or returned to diapause, samples of stubs were covered with
moist soil in closed pint or half-pint jars and incubated at
25° C. The minimum incubation period was three weeks, which
was long enough to permit any larva that began development
promptly to become adult. Wherever a treatment tended to
delay the initiation of morphogenesis incubation was extended
as much as three weeks longer.

The moist soil in which larvae were treated or in-
cubated was sandy loam containing about 15% moisture. The dry
soil used in some experiments was similar soil, air-dried at
room temperature.

Moisture contents were determined on individual
larvae. They were removed from the stub, weighed on a
spring-torsion balance (150/0.1 mg.), oven-dried, and re-

weighed. Drying for one day at 95-100° C. was sufficient
to bring the specimens to constant weight. Standard errors
for the average ratios of water to dry material of the
samples represented in Table 1 were small. Since other
samples were similar in size and range, standard error
calculations for them were omitted.

Techniques Used in Hormone Relationship Experiments

Ligation experiments were performed to resolve
whether an endocrine mechanism controlled post-diapause
development in C. cinctus, and, if a mechanism existed, to
determine the source and timing of its hormone secretions.
Larvae were removed from the stub and ligatures of fine silk
thread, dipped in molten paraffin and beeswax, were tied
tightly around them in the various positions to be described
later. A single knot was sufficient because the wax held it
firmly. The ligatures prevented seepage of fluids from one
section to another. In ligated insects most organs thought
to possess an endocrine function, e.g., the supra- and sub-
oesophageal ganglia and associated structures, the corpora
allata, and the corpora cardiaca, were contained in the head
section. An isolated prothorax included the organs believed
to be the thoracic glands, and an abdominal section con-
tained the gonads.

In parabiotic experiments intended to investigate the effect of post-diapause larval blood on larvae still in diapause, larvae were joined in pairs, tail to tail. The tips of the abdomens of two larvae in different stages of development were severed and the ends of a glass microtube thrust into the wounds. Ligatures were applied just in front of the wounds, binding the larvae to the tube. The blood from one larva was continuous through the tube with that of the other. Finally, the operated parts were covered with a mixture of paraffin and beeswax.

Ligated larvae and parabiotic pairs were incubated at 25° C. in wax cells lined with blotting paper. Slot-shaped cells were melted into blocks of commercial paraffin wax, each quarter-pound block holding 25 slots. Each cell was 5 mm. deep and just wide and long enough to accommodate a sawfly or pair of joined sawflies. It was lined with a small folded rectangle of blotter. After an experimental larva was inserted, one end of the blotter was folded over and pressed in around the insect. The blotter was kept slightly moistened. This rearing technique did not completely solve the problem of inducing post-diapause larvae to develop when extracted from their stubs. However, enough did develop when reared this way so that useful data could be obtained.

EXPERIMENTAL RESULTS

Normal Moisture Absorption at End of Diapause

The water present in a sawfly larva's tissues during diapause is normally supplemented by absorption of soil moisture before pupation. This was shown by the following experiment. Diapause larvae obtained from a most field on September 19, 1952, were kept in moist soil at 10° C. to continue their diapause development. After 0, 50, 75, and 100 days at 10° C. samples of more than 70 of these larvae were tested for water content, and other samples, each containing more than 55 larvae, were incubated at 25° C. to determine the number in which diapause had been eliminated. Young prepupae developing from larvae given 110 days at 10° C. plus 9 days incubation were also tested for water content. The results are shown in Table 1. Some water was taken up during the latter part of diapause development. Some more was absorbed during the transition period between diapause development and active post-diapause development. In addition, moisture was probably absorbed during prepupal morphogenesis.

The transition between diapause and post-diapause development can be successfully accomplished at either a moderate (25° C.) or low (10° C.) temperature. The

TABLE 1

Water absorption as represented by ratio of water
to dry matter in C. cinctus larvae during diapause,
transition, and prepupal development

Number of days at 10° C.	Percentage of larvae out of diapause	Average ratio of water to dry matter
0	0	1.22 ($\pm$ 0.02)
50	84	1.43 ($\pm$ 0.02)
75	99	1.41 ($\pm$ 0.03)
100	100	1.52 ($\pm$ 0.02)
Prepupae	-	1.64 ($\pm$ 0.03)

physiological processes occurring during this interval are unlike those occurring before or after. They do not depend upon low temperature as does diapause development, but neither is their activity as greatly increased by higher temperatures as is that of hormone secretion and morphogenesis. The transition period ends when hormone activity begins, less than a week before the visible change of the S-larva to a prepupa. This subject will be discussed more fully in a later section.

Additional sawflies from the same source as those mentioned above were enclosed in jars of dry soil for 50, 75, and 100 days at 10° C., after which they were incubated in moist soil. Each group comprised about 90 larvae. The percentage of each sample that developed upon incubation, and therefore in which diapause must have been eliminated, is compared with that of larvae chilled in most soil (Table II). It is evident that the differences are small. In the dry series there was no possibility of water absorption; probably a little dehydration occurred. Evidently diapause development is not dependent upon absorption of external moisture.

Other larvae were kept in dry soil at 10° C. for 90 and 110 days, and then incubated in dry soil, thereby receiving no moisture at any time. Only 37% and 19%,

TABLE II

Diapause elimination in C. cinctus
at 10° C., with and without access to moisture

Number of days at 10° C.	Percentage developing when incubated moist after chilling at 10° C. in	
	dry soil	moist soil
0	0	0
50	83	84
75	97	99
100	92	100

respectively, developed. In the insects in which development

ultimately did occur its beginning was delayed. Water

absorbed near the end of diapause must be necessary for some

of the insects, if they are to undergo post-diapause morpho-

genesis. But a moisture supply during diapause development

is not a requisite. All of the 100-day dry series in Table

II no doubt would have developed if the desiccation that

accompanied chilling had not interfered with post-diapause

development. That diapause development in the sawfly is

independent of an external moisture supply offers additional

support of Andrewartha's (1952) thesis that this relationship

is general among insects.

Effect of Dryness on Diapause Reinstatement and Initiation of Morphogenesis

Although contact water is unnecessary for the

elimination of diapause, it is still possible that dryness

can cause diapause to return to C. cinctus. It has already

been pointed out that dryness has been found responsible

for the original initiation of diapause in many species of

insects. However, the two situations are not identical. In

C. cinctus, we are concerned with the action of dryness after

a long period of relative inactivity, and long after feeding

has ceased. In the other species, the influence of dryness

is exerted during active growing and feeding stages.

If it is assumed that dryness helps to return post-
diapause larvae to diapause, it could exert an effect under a
variety of conditions: (1) an effect could be produced
independently of the influence of temperature extremes, i.e.,
during incubation at 25° C.; (2) dry surroundings might also
be expected to augment the action of heat in reinstating dia-
pause if heat and dryness were combined in one treatment;
(3) a decreased water content, previously produced by dehy-
dration at a sub-developmental temperature, could induce a
larva's return to diapause upon incubation; or (4) it could
facilitate the action of high temperature in returning the
larva to diapause. These four possibilities will be con-
sidered in turn.

(1) <u>Influence of Dryness Applied During Incubation</u>

The first possibility was easily eliminated. It was
soon realized that in post-diapause larvae incubated at 25° C.,
lack of moisture was not an intensive enough factor to prevent
morphogenesis. Larvae incubated from the beginning of their
transition period in a current of dry air developed past the
critical period for diapause reinstatement before dryness
could exert its supposed influence. Even when 1952 material,
which had a long transition period lasting about 2 weeks at
25° C., was subjected to this treatment practically all
larvae developed. As a group the larvae lost only 9.6% of

their original water before the critical period was past.
The average ratio of body water to dry matter was reduced
from 1.36 to 1.26.

    (2) <u>Influence of Dryness when Combined with Heat</u>

        Sawfly larvae were subjected to drying and heat
simultaneously. Post-diapause larvae were exposed for a
number of days to 35, 40, or 45° C. in either dry or moist
soil. They were then incubated, and developing forms and
undeveloped S-larvae were counted. Each sample of 60 stubs
contained about 50 living larvae. The data in Table III
showing the percentages of living insects in which diapause
was apparently reinstated suggested at first that reinstate-
ment was more successful in the dry series. However, that
is probably not true. Any S-larvae in which diapause had
been reinstated, being somewhat more resistant to desic-
cation (Salt, 1946), would have a better chance of surviving
in the dry soil than would developing insects. In the dry
series, mortality tended to be greater among developing
forms. This left a misleadingly high percentage of S-larvae
among the survivors. Note that there is less consistent
difference between the two series in the numbers of larvae
that survived but did not develop than there is in the
percentages. After allowance has been made for differential
survival of S-larvae and developing insects in the two

TABLE III

Diapause reinstatement in post-diapause larvae
by exposure to heat in moist and dry soil

Temperature, degrees C.	Time, days	Treated in moist soil			Treated in dry soil		
		Number surviving	Number not developing	Percentage of survivors not developing	Number surviving	Number not developing	Percentage of survivors not developing
35	5	25	25	100	40	36	90
	10	42	42	100	41	41	100
40	2	52	6	12	38	10	26
	5	47	21	45	27	22	81
	10	8	8	100	1	1	100
45	1	19	18	95	13	13	100
	2	4	4	100	1	1	100
Control, no treatment		....			46	3	7

series, this experiment offers no support to the hypothesis that dryness promotes return to diapause.

In the first of two other similar experiments, groups of 100 stubs each were given treatments shorter than those of the last experiment. They received exposures of six hours at 0 and 80% relative humidity and various temperatures between 37.5 and 50° C. They were then incubated in most soil at 25° C. Over 80% of the stubs contained larvae, most of which were in the critical period for diapause reinstatement when treated. The results of incubation are given in Table IV.

In the second experiment, similar critical period larvae were given six and eighteen hours at saturation deficits of 4 and 42 mm., and 35, 40, and 47.5° C., whereupon they were incubated as above. About 65 larvae were used in each sample. The results are shown in Table V. Moisture content samples of an equivalent series showed that the higher saturation deficit produced severe desiccation of the larvae, completely desiccating them in 18 hours at 47.5° C. At high temperatures, the lower saturation deficit caused slight desiccation.

The data of Tables IV and V show that, despite the brevity of the treatments, some larvae were successfully prevented from resuming morphogenesis. Humidity had no con-