Influence of temperature on diapausing forest tent caterpillar, Malacosoma disstria Hbn (Lepidoptera:Lasiocampidae)

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INFLUENCE OF TEMPERATURE ON DIAPAUSING
FOREST TENT CATERPILLAR, MALACOSOMA
DISSTRIA HBN. (LEPIDOPTERA: LASIOCAMPIDAE).

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Entomology

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INFLUENCE OF TEMPERATURE ON DIAPAUSING FOREST TENT
CATERPILLAR, MALACOSOMA DISSTRIA HBN.
(LEPIDOPTERA: LASIOCAMPIDAE)

by

Edwin Charles Masteller

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Zoology and Entomology

Approved:

In Charge of Major Work

Head of Major Department

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa
1967
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INTRODUCTION

Campbell (1963) reported that there was considerable variation in overwintering survival between egg masses of the forest tent caterpillar, *Malacosoma disstria* Hbn. After a six month overwintering treatment at 5°C followed by incubation at 22°C, a generally high hatch occurred in egg masses from new infestations that had been established by migratory females, whereas there was a poor hatch from egg masses from the center of an old infestation. Campbell (1964), after observing extreme developmental differences between colonies on the same host tree, suggested that the observed differences of hatch in the laboratory may have been the result of offspring of non-migratory females (i.e., those laying at the center of an infestation) requiring more than six months to complete diapause\(^1\) whereas the treatment was adequate for completion of diapause in offspring of migrators. Observed differences in seasonal development between the four types of colonies that comprised field populations of the species indicated considerable inter-colony variation in time of hatching and accordingly, intra-specific variation in the duration of diapause. Laboratory tests showed that the observed inter-colony variation in seasonal development was sufficient to produce complete

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\(^1\)Diapause is defined as a state of depressed development and/or activity that is independent of immediate environmental conditions.
temporal sexual-isolation between the two most divergent types of colonies and that genetic isolation was prevented only by the time of flight of the intermediate colonies.

Armstrong (1945) reported a comparable situation for codling moth, Carpocapsa pomonella (L.), in association with apple and pear orchards in the Niagara Peninsula of Ontario, Canada. Because young apples were susceptible to larval attack, whereas young pears were highly resistant, the progeny of early emerging adults (i.e., those having a relatively short prepupal diapause) could not become established on pears. Thus the progeny of only late emerging adults (i.e., those having a relatively long diapause) survived to reproduce in pear orchards. That the selection imposed by seasonal differences in the susceptibility of the two host species can result in inherent developmental differences between codling moth populations was evidenced by late seasonal moth emergence and one generation a year in pear orchards, compared to early moth emergence and two annual generations in apple orchards. From this it is evident that the species contained, within the same geographic region, considerable inherent variation in the duration of diapause, which permitted a wider primary host range than would otherwise have been possible.

Campbell (1962) found that by overwintering the diapausing, pre-feeding larvae of the third generation of a laboratory population of spruce budworm, Choristoneura fumiferana (Clem.),
for nine months at 6°C instead of at the usual 0°C, the sons of the large, highly fecund, small egg producing females were differentially selected against. As a result there was a four to five fold increase in overwintering survival in descendant generations and the frequency of small, lowly fecund, large egg producing females rose from 38 to 84 per cent. That the population responded to an abnormally high energy demand during diapause in one generation by decreasing the energy requirements and increasing the energy supplied through the egg for individuals of descendant generations suggested that the successful completion of diapause is a function of energy demand in relation to supply. The species apparently accommodates such periodic changes in this relation by shifts in relative frequency of physiologically divergent genotypes.

Hodson and Weinman (1945) studied the influence of various constant temperatures on duration of diapause, utilization of the yolk food reserves, and survival during diapause in *M. disstria*. Their results on minimal duration of diapause and the amount of yolk utilized, or the condition of the larvae, at the end of the minimum period for each of the treatment temperatures are shown in Figure 1. Whereas little or no yolk was utilized at treatment temperatures at 10°C and below, there was a rapid increase in the rate of yolk utilization at temperatures increasing above 10°C. However, the minimum duration of diapause prolonged with treatment temperatures both
Figure 1. The minimum time required at various treatment temperatures by diapausing pre-hatch larvae of *Malacosoma disstria* Hbn. to terminate diapause when incubated at 25°C, and the quantity of yolk remaining in the gut or the physiological condition of larvae at the end of these minimum treatment periods (After Hodson and Weinman, 1945)

Figure 2. A map of the Great Lakes Region of North America showing the geographic origin of the *Malacosoma disstria* Hbn. used in these studies and those of Hodson and Weinman (1945)
After Hodson & Weinmon, 1945.

minimum time to break diapause in weeks (in)

-5 0 5 10 15 20 25
treatment temperature C°

none
begin
partial
advanced
complete
dying
dead

yolk absorption and larval condition (mg)

-5 5 10 15 20

OulitI
Duluth
Chicago
Toronto

Map of the Great Lakes region.
Increasing and decreasing from 10°C. In the one instance diapause prolongs as the rate of yolk assimilation increases and, in the other, it prolongs after the rate of yolk assimilation has become minimal. Bucklin (1959) reported that, in *Melanoplus differentialis* (Thomas), there were "large amounts of lipid material in diapausing cells, stored as small droplets", and that excised whole embryos, as well as portions of embryos, even those isolated from endocrine structures, terminated diapause soon after removal from contact with the yolk substrate.

From these results Campbell (1966b) proposed a working hypothesis that diapause is the result of cellular hyper-assimilation of food (e.g. lipids) depressing respiration, possibly through interference with oxygen diffusion, and termination of diapause depends on the elimination of the surplus food from the cells. In the situation described by Bucklin (1959) cells of the embryos terminated diapause when they were able to deplete the surplus by respiration, after mechanical removal of the extra-cellular food source prevented replacement by assimilation. In the situation described by Hodson and Weinman (1945) (Figure 1), elimination of surplus intra-cellular food occurred most rapidly at 10°C, where the difference between the rate of yolk assimilation and respiration would be maximal because a further decrease in temperature caused no further depression in assimilation but would further
depress respiration, since the log rate of respiration decreases linearly with temperature (Richards, 1964). Accordingly, the duration of diapause prolongs at temperatures decreasing below 10°C because the rate of depletion of surplus intra-cellular reserves would diminish, since primarily only respiration would be further depressed. At temperatures increasing above 10°C, the rate of yolk assimilation rose rapidly so that the rate of replacement of intra-cellular food reserves utilized in respiration increased, and accordingly, the rate of depletion of intra-cellular reserves was reduced. Thus the diapause condition was prolonged. Moreover, if at a high temperature, assimilation rate becomes equivalent to the rate of utilization of intra-cellular food by respiration, starvation and termination of diapause will be coincident because no intra-cellular depletion can occur until the extra-cellular food reserves are exhausted. Hodson and Weinman found that at a constant 25°C M. disstria larvae rapidly depleted their yolk food reserves and died before hatching. Furthermore, it was found that when larvae were incubated at 25°C following treatment at the lower temperatures, they did not hatch until the yolk reserve was depleted, regardless of the quantity of yolk contained in the gut at the end of successful treatments. If the permeability of insect cell membranes increases exponentially with temperature, as found for ox-blood cells at temperatures from 0 to 50°C (Davson and
Danielli, 1943), then changes in the rate of yolk assimilation may be the result of depression in the permeability of cell membranes in response to decreasing temperatures. The relatively constant low level of yolk assimilation below 10°C conforms to the exponential nature of this relationship.

As mentioned previously, Hodson and Weinman (1945) found that the yolk food reserve was always depleted before hatching when incubation was at 25°C. It is somewhat doubtful if such starved larvae could survive up to two or three weeks without an external food source. However, such post-hatch survival periods have been reported by Riley (1871) and Hodson (1941), for example. But, if cell membrane permeability decreases with temperature, food deficits within the cells should accrue at lower incubation temperatures, so that a "normal" rate of respiration can be attained (i.e., diapause can be terminated) without depleting the extra-cellular food reserve prior to hatching. To test this possibility, the experiments of Hodson and Weinman (1945) on M. distria were repeated using post-treatment incubation temperatures of 18 and 20, as well as 25°C. Eggs from two populations were included to assess for possible intra-specific variation.
MATERIALS

Life History

Throughout its geographic range the forest tent caterpillar, _M. disstria_, has only one generation per year. In the main study area, which was located along the Georgian Bay of Lake Huron, adult flight and oviposition occur in early July. In the laboratory, mating and oviposition take place during the night, usually following the evening on which the adults emerge. Hodson (1941) reported that the moths mate shortly after eclosing, and oviposition takes place within three or four hours. Following copulation the females orientate outward on the small branches of the host tree. Hodson (1941) reported that trembling aspen twigs ranging in diameter from 0.064 to 0.168 inches were usually selected as oviposition sites.

A female lays the first row of eggs around the circumference of the twig and then backs proximally and deposits the next row of eggs behind and in contact with the first row. This process is repeated until her supply of eggs is exhausted. As each egg is deposited the female coats it with spumaline, a frothy secretion from her accessory glands. The number of eggs per mass observed in these experiments varied from 16 to 389 which closely agrees with the range of 15 to 327 reported by Hodson (1941) for Northern Minnesota. Craighead (1950) reported a range of 100 to 350 eggs per mass.
Field collected, newly laid eggs revealed germ band development after two weeks, and complete embryonic development after four weeks at 22 to 24°C in the laboratory. Hodson (1941) reported that embryonic development in the field required approximately three weeks. At the end of embryonic development, the pre-hatch larvae usually enter diapause. Occasionally some larvae, both in the field and laboratory, do not enter diapause and hatch immediately. However, these individuals do not survive naturally because the host foliage is unsuitable and the seasonal conditions are unsatisfactory for the completion of this potential second generation.

The diapausing larvae overwinter in the eggs and emerge the following spring. Riley (1871) stated that hatching of *Clisiocampa sylvatica* (Harr.) (*C. sylvatica = M. disstria*, J. HüeBner, 1922) in Missouri occurred from mid to late March and that the hatched larvae could survive up to three weeks without food during periods of inclement weather. Hodson (1941) found that in Northern Minnesota the date of hatching ranged from May 5 to 25. Blais et al. (1955) reported that 85 per cent of the eggs hatched between May 3 and 9 in 1953, at Cedar Lake in Northwestern Ontario, where the daily maximum temperature was 25.4°C. Craighead (1950) stated that the larvae hatch at the time that host leaf-buds are bursting. Campbell (1966a) suggested that variation in time of hatching between egg masses relative to inter-specific variation in the time of
foliation of potential host species determines different specific primary host associations both within and between climatic regions.

On emergence the larvae are gregarious and for a short period remain on the surface of the egg mass feeding on the spumaline. They then move outward as a colony to the tips of the branches and commence to feed on vegetative or floral tissue, if it is present. When food is available the larvae feed and then move inward where they spin a silken pad either on a branch or on the trunk, which serves as a communal resting and molting site. This may lead to the combining of larvae from more than one egg mass. If food is not present at the branch tip, they return to the egg mass temporarily before again moving to seek suitable host tissue.

There are usually five larval instars. Hodson (1941) observed that the first molt took place seven to nine days after hatching whereas Riley (1871) observed that the first instar lasted about two weeks. From our observations it seems probable that the length of the first instar, and indeed of all developmental stages, depends on the availability of food and the prevailing weather conditions. Whereas during the first three instars the larvae are gregarious and migrate as colonies, this behavioral tendency usually declines during the last two instars and the members of the colonies disperse.
At maturity the larvae spin cocoons within the foliage of the host trees when defoliation is not complete, or in the undergrowth and foliage of non-host species when the hosts are completely defoliated. Pupation occurs within approximately 24 hours of cocoon spinning.

In the study area the pupal stage lasted about two weeks and the adults emerged during early July. In emergence, the adults displayed a definite circadian rhythm. In the laboratory (22°C), all emergence occurred between 4:30 and 10:00 P.M. (CST) and, in an insectary, between 3:30 P.M. and 1:00 A.M. (CST), with the male emergence commencing about four hours before that of the females in both situations.

Investigation Area

Field populations of the forest tent caterpillar were observed in the Georgian Bay region in the province of Ontario, Canada, (45 to 47° North Latitude, Figure 2) from the initiation of hatching until adult flight and oviposition in 1964 and 1965, and during the hatching period in 1966. In the North trembling aspen, *Populus tremuloides* Michx., is the only major primary host species (i.e. one which facilitates survival of newly hatched larvae), whereas in the South large-tooth aspen, *P. grandidentata* Michx.; red oak, *Quercus rubra* L. (*Quercus borealis*) Michx.; and hard maple, *Acer saccharum* Marsh., are also major primary hosts. Campbell (1966a) reported that, in 1964, hard maple, for example, foliated about two weeks
earlier in the southern than in the northern limits of this region, whereas larvae were at the same stage of development at both ends of the region on June 9. This indicated that the insects had completed diapause and hatched at about the same time throughout the region, but variability in host flushing, both within and between species, resulted in different primary host associations.
For laboratory studies on the influence of temperature on diapause, newly laid egg masses were collected on July 13, 1965, at Azilda, which is 10 miles north of, and at Alban, which is 40 miles south of Sudbury, Ontario, Canada. At Azilda, where only trembling aspen was present, 672 egg masses were collected, and at Alban, where two species of aspen were represented, 469 masses were collected from trembling aspen and 343 from large-tooth aspen.

This material was brought to Ames, Iowa, on July 15, held at 24°C until July 31, and then transferred to 22°C. On September 15, the egg masses from each of the three samples were randomly divided into seven groups so that 96 egg masses from Azilda, and 116 from Alban (49 off large-tooth and 67 off trembling aspen) were placed at each of the treatment temperatures: -5, 0, 5, 10, 15, 18, and 20 ±0.5°C. Starting five weeks later, egg masses from each of the samples were transferred at four week intervals, for the next 24 weeks, to incubation temperatures of 18, 20 and 25 ±0.5°C. For all treatment and incubation a relative humidity from 50 to 75 per cent was maintained. At the end of each treatment period, as the eggs were being transferred to the incubators, two larvae from both the top and bottom of two egg masses, from each treatment, were excised and fixed in hot Bouin's solution (60°C) for future histological preparation. Newly hatched larvae from
each treatment and incubation condition were also fixed for histology. During incubation, egg masses were checked daily and the hatch recorded. After hatching was completed the eggs were counted, and sterility and parasitism assessed. Because sterility and parasitism were negligible, percentage hatch was calculated on the total number of eggs.

For histological studies the larvae that were fixed in Bouin's solution were transferred to 70 per cent ethyl alcohol after 24 hours. They were then infiltrated with and embedded in 56-57°C melting point Paraplast® (Biological Research, Inc.) following the schedule of Stairs (1960) shown in Table 1. Sections were cut at 8 microns, stained with alum haemotoxylin (regressive) and eosin, and mounted in Permount® (Fisher Scientific Co.).

Table 1. Schedule for embedding larvae of Malacosoma disstria after fixing in Bouin's solution and stored in 70 per cent ethyl alcohol

<table>
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<th>Step</th>
<th>1</th>
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<td>Time (Hours)</td>
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<td>2</td>
<td>2</td>
<td>8</td>
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<tr>
<td>Water</td>
<td>25</td>
<td>10</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>E</td>
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<tr>
<td>Ethyl Alcohol</td>
<td>50</td>
<td>40</td>
<td>24</td>
<td>5</td>
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<td>-</td>
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<td>M</td>
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<tr>
<td>N-Butyl Alcohol</td>
<td>25</td>
<td>50</td>
<td>75</td>
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<td>Paraplast® (56-57°C)</td>
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Following preliminary histological examination of larvae from the 1965 experiments, it was realized that interpretation would require the study of a detailed time series of the species during pre-, as well as, post-diapause development. For this newly laid egg masses were collected from trembling aspen on July 22, 1966, in the province of Alberta, Canada, at 53° North Latitude. These were brought to Ames, Iowa, on July 27 and held at 24°C until August 20 and then transferred to 22°C. On September 14 the egg masses were placed at 5 ±2°C for 13 weeks and then incubated at 20°C. Every two days from July 30 to the completion of hatching, embryos or larvae were prepared for histological study.

This material was fixed in the manner described previously. However, the infiltration and embedding method (Table 1) was modified by reducing all times in the schedule by one-half. By reducing the time in the embedding oven and alcohols, dehydration of the yolk was diminished, so that disruption of yolk in sectioning was greatly reduced. With the exception of early embryonic stages which were cut at 15 microns, this material was also cut at 8 microns. Alum haematoxylin and eosin were again used in staining.
RESULTS AND DISCUSSION

The influence of temperature on the fulfillment of diapause and possible intra-specific variation in the diapause phenomenon, in *M. distria*, were studied. Samples of egg masses from two populations, one of which was associated with two host species at Alban (Population A), and the other with a single host species at Azilda, Ontario (Populations B), were treated at several constant temperatures between -5 and 20°C for various periods and then incubated at 18, 20, and 25°C.

The results, as presented in Figure 3, show the proportion of hatch in egg masses from the two populations, after various combinations of treatment and incubation temperatures, for each of the treatment periods. Because the control of temperature in the 18°C incubator failed and killed the material from the 17 and 25 week treatments, valid data were not obtained. Consequently, these data are not included in Figure 3 and other presentations. A comparison of the percentage hatch for the two populations after 9 and 29 weeks of treatment indicates a higher heterogeneity in population A, which was associated with two host species, than in population B, which was associated with a single host. As would be expected, differences in heterogeneity were less evident after treatment periods of 17, 21, and 25 weeks, because both populations should be equally adapted to the intermediate situations. It is only
Figure 3. Percentage hatch of eggs from two populations of forest tent caterpillar, *Malacosoma disstria* Hbn., from Alban (Population A) and Azilda (Population B), Ontario, Canada, when incubated at 18, 20, or 25°C after treatment at -5, 0, 5, 10, 15, and 18°C for periods from 9 to 29 weeks.
when extreme conditions are applied that innate differences are revealed.

The situation described by Armstrong (1945) for intra-specific variation in diapause duration, relative to seasonal differences in the susceptibility of apples and pears to attack by *C. pomonella*, seems to be similar to that found here for *M. disstria*. Because large-tooth aspen in the Sudbury area foliated 1 to 3 weeks later than trembling aspen, it served mainly as a primary host for those *M. disstria* that had a late seasonal development. Accordingly, a fairly large proportion of the sample from Alban, where large-tooth aspen was present, survived up to 29 weeks under a fairly wide range of conditions, whereas survival for 29 weeks by insects from Azilda, where the late foliating host did not occur, was limited to the lowest treatment temperature.

That population A also terminated diapause more rapidly and under a wider range of conditions (i.e., 9 weeks, Figure 3), also suggests the selective influence of large-tooth aspen on the Alban population. Campbell (1966a) has shown that late colonies of *M. disstria* attained the adult stage and oviposited 1 to 2 weeks later than those from early colonies. Because all of the egg masses were placed at the treatment temperatures at the same time, the offspring of late adults had considerably less time to complete pre-diapause development. Later studies on pre-diapause development revealed that a pre-diapause
assimilation of yolk occurs for about one month prior to the complete onset of diapause. If, then, the duration of the assimilation period was reduced by 1 to 2 weeks for the offspring of the late adults (i.e., those associated with large-tooth aspen), the quantity of food assimilated by the cells of the latest of these individuals would be reduced and the duration of their diapause shortened.

Hodson and Weinman (1945) showed that M. disstria terminated diapause most rapidly at 10°C (Figure 1), whereas our results show the most rapid termination of diapause at 5°C (9 weeks, Figure 3). Such differences are not unexpected because in the first situation the test material was collected in Northern Minnesota during late August (i.e., near or at the end of pre-diapause development) and, in the second, from North Central Ontario during mid-July (i.e., prior to embryonic development). Therefore, this difference could have resulted from inherent variation between regional populations and (or) from pre-diapause development having occurred under oscillating temperatures in the first instance and under constant temperatures in the second. Regardless of this discrepancy, there is agreement that the duration of diapause prolongs with temperatures increasing and decreasing from the 5 to 10°C range. In both instances survival rapidly diminished with time at temperatures above this range but was maintained at a high level for relatively long periods at the lower
temperatures (21 weeks and 5 months, respectively, Figures 3 and 5).

In general (Figure 3) there was a gradual increase from the high to the low treatment temperatures in the percentage of insects terminating diapause with increasing storage periods up to 17 weeks. From 21 weeks on, superimposed on this increase, was a rapid decrease in survival at the higher temperatures. However, at 5°C, the treatment at which diapause was terminated the most rapidly, the insects survived for much shorter periods than at either 0 or 10°C. Relative to the working hypothesis this suggests that at 5°C the elimination of cellular food reserves occurs more rapidly than at either 0 or 10°C. A decrease from 5 to 0°C would be expected to depress mainly respiratory utilization of cellular reserves. On the other hand, an increase from 5 to 10°C would be expected to increase the rate of cellular assimilation of the extracellular yolk reserve relative to the rate of respiratory utilization. In both instances, the intra-cellular reserves would be maintained for longer periods at 0 and 10°C and the duration of diapause and survival prolonged accordingly.

To further interpret the relationship between temperature and time, relative to the working hypothesis on termination of diapause and survival, consider the results as presented in Figure 4. The treatment periods that resulted in maximal hatch at each of the treatment temperatures are indicated by
Figure 4. The number of weeks required by *Malacosoma disstria* Hbn. from Alban, Ontario, at each of the treatment temperatures to produce maximal hatch at a post-treatment incubation temperature of 20°C. Numerals on the graph are the mean number of days to hatch at 20°C after the various periods at each treatment. The time required for each treatment to give maximal hatch is indicated by an underlined mean.
the underlined numerals. For example, 21 weeks at \(-5^\circ C\) produced maximal hatch, whereas only 17 weeks at \(5^\circ C\) produced the same result. The broken lines join the treatment periods which gave maximal hatch at temperatures decreasing from 5 and increasing from \(10^\circ C\). Since at the two intermediate temperatures, 0 and \(15^\circ C\), a maximal hatch occurred after both 17 and 21 weeks, and 13 and 17 weeks, respectively, a solid line from 21 weeks at \(-5^\circ C\) to 17 weeks at \(5^\circ C\), as well as from 17 weeks at \(10^\circ C\) to 13 weeks at \(18^\circ C\), passes through the average period that resulted in maximal hatch at the intermediate temperatures. On this basis, there seems to be two different linear time-temperature relationships involved.

If the one relationship, decreasing from \(5^\circ C\), reflects mainly a depression of respiratory utilization of cellular reserves and the other, increasing from \(10^\circ C\), reflects a greater increase in the rate of assimilation of yolk than in the rate of respiratory utilization of intra-cellular reserves, the elimination of both intra- and extra-cellular food reserves should occur most rapidly at temperatures above \(10^\circ C\). Accordingly, the 4 week longer duration of diapause at the lower than at the higher temperature range is to be expected (Figure 4). Differences in the rate of yolk assimilation at the temperatures below 5 and above \(10^\circ C\) are evident from the relatively large amounts of yolk in the alimentary canal of larvae treated for 17 weeks at 0 or \(5^\circ C\) (Figures 17 and 20).
compared to the small quantities of yolk in larvae treated at 10 or 15°C for the same period (Figures 23 and 27).

Since the rate of yolk assimilation appears to be slow and relatively constant at temperatures below 5°C, prolongation of diapause at the lower temperatures is assumed to be mainly the result of decreasing respiratory utilization of intra-cellular reserves. If termination of diapause depends on the elimination of surplus intra-cellular food reserves, then the nutritional state of the cells at the end of the treatment will determine the period of incubation required to produce hatch. Therefore, the incubation period may be prolonged because elimination of this surplus, in addition to the common post-diapause pre-hatch processes, may have to be completed. This being the situation, the smaller the intra-cellular food deficit which is incurred by the cells during the treatment period, the greater the loss which must take place during incubation. Hence, a treatment that creates only a small cell deficit will be followed by a long incubation period prior to hatch, whereas one that produces a large deficit will be followed by a short incubation period. The mean number of days to hatch at 20°C, for the Alban population, following each period of treatment for each of the treatment temperatures is given on Figure 4. (These means and their standard errors are given in Figures 9, 10, 11, 12, and 13 for both populations at the three incubation temperatures.) The mean number of days to hatch (Figure
4) shows that, in accordance with the working hypothesis, the pre-hatch incubation period decreases with longer treatment periods and increasing temperature up to 5°C.

Since the fat-body cells, which are readily recognizable in histological preparations, have both a storage and metabolic function (Kilby, 1963), it seems reasonable that changes in their mass would reflect changes in the nutritional state of other somatic cells, and that they would thereby indicate the general intra-cellular food reserves at the end of each treatment. A comparison of the fat-body cells of larvae that had been stored at 0°C for 13, 17, and 25 weeks (Figures 16, 17, and 18) to those at 5°C for 9, 17, and 25 weeks (Figures 19, 20, and 21) shows a considerably greater depletion of cellular food reserves at the higher treatment temperature. Moreover, although the fat-body of larvae stored at 0°C from 13 to 25 weeks showed little change (Figures 16 and 18) (i.e., an extremely slow depletion of intra-cellular reserves at this low temperature), larvae at 5°C showed a rapid depletion of fat-body reserves (Figures 19, 20, and 21).

At temperatures increasing from 10°C both respiratory utilization of intra-cellular reserves and cellular assimilation of the yolk should increase, but the latter more rapidly than the former. Hence, at short treatment periods, when yolk is still abundant, rapid cellular assimilation of this extracellular source at the higher temperatures should tend to
maintain an initially high intra-cellular nutritional condition. Accordingly, after 9 and 13 weeks of treatment, longer incubation periods were required for hatching at 15 and 18°C than at 10°C (Figure 4) are to be expected. The more rapid termination of diapause at 10°C was further indicated by the presence of mitotic figures in larvae from the 10°C treatment after only 17 weeks (Figure 24). However, after longer treatment periods at the higher temperatures the yolk reserve should be greatly reduced so that the cells would not be able to assimilate extra-cellular reserves at a rate adequate to meet demands of cellular respiration. Initially, this would cause a rapid reduction in intra-cellular food so that at periods of intermediate duration, larvae from the higher temperatures would hatch as rapidly as those at 10°C (i.e., 17 and 21 weeks). Eventually, this situation, where respiratory demand exceeds the capacity of cells to assimilate, would lead to cellular starvation and death, even though extra-cellular reserves may not be depleted (i.e., 21 weeks at 18°C, and 25 weeks at 15 and 18°C).

The preceding results and interpretations are in accordance with histological observations. A comparison of the yolk content of larvae after 9, 17, and 25 weeks at 10°C to that of larvae after 9 weeks at 18°C, and 17 weeks at 15°C (Figures 22, 23, 25, 26, and 27 respectively) shows that yolk assimilation occurred more rapidly at the higher temperatures.
That both fat-body and yolk are more rapidly depleted at the higher temperatures is evident from the slight reduction in only the fat-body from 9 to 17 weeks at 10°C (Figures 22 and 23) in comparison to the drastic reduction in fat-body and yolk from 9 weeks at 18°C to 17 weeks at 15°C (Figures 26 and 27).

Observed differences in termination of diapause, when larvae from common treatments were incubated at different temperatures, also seem to fit the foregoing explanation. A higher percentage hatch occurred at 18°C than at 20 or 25°C after short treatments at the lowest treatment temperatures (i.e., 9 and 13 weeks at -5°C, and 9 weeks at 0°C, Figure 3). Following short treatments, depletion of most of the surplus food within the cells of larvae would occur during incubation because the depletion of intra-cellular reserves is extremely slow at the lowest temperatures (e.g., 13 weeks at 0°C, Figure 3). If at treatment temperatures increasing from 10°C both respiratory utilization of intra-cellular reserves and cellular assimilation of yolk increase, but the latter more rapidly than the former, a cellular food deficit could be incurred, without completely exhausting extra-cellular reserves, more readily at an 18°C than at higher incubation temperatures. The results of Hodson and Weinman (1945), which our experiments confirm, show that essentially no diapausing larvae survive at constant temperatures of 20°C and above. This suggests that the rate of assimilation of intra-cellular reserves becomes equal to
the rate of respiratory utilization at about 20°C. If at constant temperatures above 20°C starvation and termination of diapause are coincident, then, successful termination of diapause at such incubation temperatures will require a certain depletion of intra-cellular reserves (i.e., a partial reduction in the diapause condition), prior to incubation. The minimum depletion that must be incurred during a treatment for diapause to terminate upon incubation is reflected by decreased hatching success at temperatures increasing from 18 to 25°C following short treatments at the lowest temperatures (i.e., 9 and 13 weeks at -5°C, and 9 weeks at 0°C, Figure 3).

Assuming that the rate of assimilation exceeds that of respiratory utilization of intra-cellular food at 25°C, then successful incubation at this temperature will require almost complete elimination of the intra-cellular surplus and thereby the restoration of a normal, relatively high rate of respiration. If, prior to incubation at 25°C, insects have not increased their rate of respiration to a level equal to or in excess of the high rate of assimilation of extra-cellular reserves at this temperature, the original diapause condition will be restored. Therefore (Figure 3), maximal hatch occurred at 25°C, after a relatively narrow range of treatment conditions which were long enough to achieve elimination of the intra-cellular surplus but short enough to prevent exhaustion of the extra-cellular food reserve. Church (1955) reported
that post-diapause wheat-stem sawflies, *Cephus cinctus* Nort., reverted to diapause when placed at a high temperature (30-40°C). He observed that at this post-treatment temperature the thoracic gland returned to the inactive diapause state.

The wide range of treatment conditions that gave maximal hatch at the 20°C incubation (Figure 3) indicates that the insects do not need to acquire as precise a cellular nutritional state and respiratory condition at 20 as at 25°C to complete diapause. If in the initial diapause condition the rate of cellular assimilation is equivalent to respiratory utilization at 20°C, even relatively small depletions of intra-cellular reserves, incurred at lower treatment temperatures, will elevate respiratory utilization in excess of assimilation so that intra-cellular reserves will continue to be depleted at 20°C. That relatively small depletions of intra-cellular reserves, which would be incurred for short periods of treatment, are sufficient to induce continued depletion and eventual termination of diapause at 20, but not at 25°C, is evident from Figure 15. After only a 5 week treatment at temperatures from -5 to 15°C, some hatch occurred at 20°C but not at 25°C. Moreover, the reduction in total time to hatch (i.e., treatment plus incubation time, Figure 15) from 5 to 9 weeks, at 5, 10, and 15°C, indicates that the rate of depletion at 20°C is increased by incurring a greater depletion at the longer treatments. Essentially no change in total time is observed
with increasing periods of treatment up to 13 weeks, at -5 and 0°C, because the rate of cellular elimination of reserves through respiratory utilization would be extremely low at both of these temperatures.

The results (Figure 15) for 18°C incubation, a constant temperature at which some larvae terminate diapause, are similar to those for 20°C at all treatment temperatures. Because initially depletion can occur at a constant 18 but not at 20°C, the reduction in the duration of the incubation period from 5 to 9 weeks, at 5, 10, and 15°C, is less at the lower temperatures. As discussed previously, incubation at 18°C will increase the probability of hatch of individuals that had little opportunity to deplete intra-cellular reserves prior to incubation (i.e., 9 weeks at -5 and 0°C, and 13 weeks at -5°C, Figure 3).

To study the influence of various combinations of treatment and incubation temperatures on the nutritional condition at hatch, samples of larvae from within the low range (i.e., 5°C and below and from within the high range of treatment temperatures (i.e., -10°C and above) to each of the incubation temperatures, were prepared for histological examination. To maximize uniformity within the material to be compared, the samples were selected from temperature regimes that gave a uniformly high hatch at all three incubation temperatures (i.e., 10°C at 13 weeks and 0°C at 21 weeks, Figure 3). Comparing hatched material at the three incubation temperatures
from -5°C after 21 weeks to that from 10°C after only 13 weeks (Figures 31, 32, and 33 compared with 28, 29, and 30) generally shows a relatively larger amount of fat-body in those larvae from the lower temperature even though their treatment was 8 weeks longer. When hatching occurred at 18°C a temperature at which it is assumed that respiratory utilization exceeds cellular assimilation of intra-cellular reserves, those larvae from -5°C accordingly contained considerable yolk and fat-body whereas those from 10°C contained only a small amount of each (Figures 31 and 28). The smaller reserves in those from the 10°C treatment reflects the greater yolk utilization that occurred at this treatment. When incubation was at 20°C, the temperature at which cellular utilization and assimilation are assumed to be equal, larvae from both treatments that hatched at 20°C had completely utilized their yolk reserve (Figures 32 and 29) despite a large initial difference at the end of the two treatments. At 25°C incubation, where the potential rate of cellular assimilation is assumed to exceed the rate of utilization of intra-cellular reserves, will tend to be replaced if an adequate yolk reserve is available. Accordingly, the yolk reserve of larvae that had been incubated at 25°C was exhausted at hatching regardless of large differences in the yolk supply at the end of the two treatments, but those from the -5°C treatment contained considerably more fat (Figure 33) than those from 10°C (Figure 30).
If the termination of diapause requires the elimination of an excess intra-cellular food reserve, then it follows that the induction of diapause may be the result of hyper-assimilation of food by the cells. Fuzeau-Braesch (1966) reported that *Gryllus campestris* L. which were reared at 32°C grew rapidly, were large at maturity, and diapaused, but those reared at 20°C grew slowly, were small at maturity, and did not diapause. In other words, the lower temperature reduced the rate of assimilation and prevented diapause, whereas the higher temperature enhanced assimilation and induced diapause. As mentioned earlier, Church (1955) found that prepupae of *C. cinctus*, which had terminated diapause after a low temperature treatment, re-entered diapause during pupal development if they were kept at a high temperature (30-40°C) for more than two days. This could also be interpreted as the effect of hyper-assimilation by the cells at high temperatures restoring the diapause condition. That cellular assimilation does increase with temperature is supported by the results of Campbell and Sullivan (1963) on the influence of temperature on egg production in *Neodiprion sertifer* (Geoff.). They found that females held during oogenesis at 20°C, compared to those held at 10°C, produced full-sized eggs and completely utilized the food reserve from the fat-body, whereas those held at the lower temperature produced eggs that were 15 per cent lighter because they failed
diapause for approximately one month (Figure 41) also supports this conclusion.

Striking histological differences are evident between larvae that are entering, or are in diapause, and those which did not diapause but hatched immediately after completing embryonic development. Non-diapause larvae, which hatched about September 14, (Figure 40) contained no yolk and only a small amount of fat-body, whereas those either entering on September 1 or in diapause on October 16 (Figures 39 and 41) contained large amounts of both. The food reserve condition in these non-diapause larvae (Figure 40) was similar to those that had completed diapause after 13 weeks at 5°C and incubated at 20°C (Figures 42 and 43). The small fat-body reserve in these non-diapause larvae suggests that failure of the cells to assimilate large food reserves prevents the onset of the diapause condition. The reason that fat-body, in these non-diapause larvae, failed to assimilate from the yolk food reserve has not been determined. However, since none of the hatched non-diapause larvae contained yolk, possibly the total yolk reserve was depleted by the end of embryogenesis or maybe these larvae failed to devour that yolk which was not enveloped at dorsal closure.

The concept that temperature may modify the nutritional condition of cells by disproportionately influencing cell membrane permeability, and accordingly the rate of cellular
assimilation of food substrate relative to the rate of respiration, is supported by the studies of Richards (1957, 1958, and 1964). Richards (1964) reported that, over a broad range of insect orders, the log rate of respiration decreased linearly with temperature whereas the log rate of embryonic development decreased curvilinearly and diminished very rapidly at temperatures below 20°C. Such a differential reduction between respiration and development, in response to temperatures decreasing from 20°C, indicates that a decreasing proportion of the energy released through respiration is available for development at the lower temperatures. If, as the temperature decreases the permeability of the cell membrane is reduced (Davson and Danielli, 1943), the amount of energy that is required for transport of food across the membrane will increase and that remaining for developmental processes will decrease accordingly. As in situations where diapausing M. disstria, which were incubated at a temperature slightly below 20°C (i.e., 18°C), apparently reduced their intra-cellular reserves to a level at which diapause terminated without exhausting their yolk reserve, Richards (1957) observed that Oncopeltus fasciatus (Dallas) embryos died with yolk when incubated at a constant 14 or 15°C. Moreover, Richards (1957) found that at constant incubation temperatures only slightly below 20°C, both hatching success and post-hatch survival greatly diminished. However, O. fasciatus eggs were successfully incubated at 13°C,
when given short daily exposures at temperatures above 20°C. Apparently, short exposures at the high temperatures permitted the cells to assimilate food resources so that an intracellular energy source was maintained and developmental processes could proceed at the low temperature.

Schneiderman and Horwitz (1958) showed that mature larvae of *Mormoniella vitripennis* (Walker) require a minimum of 6 weeks at temperatures between 2 and 10°C to terminate diapause, and that the effect of low temperature is reversed (i.e., diapause prolonged) by intermittent exposures to 25°C. Under alternating periods at 5 and 25°C, the duration of diapause was greatly extended when periods of exposure to the two temperatures were equal (i.e., 2 and 2, or 7 and 7 days alternating), but was rapidly reduced as the time at the higher temperature was decreased (i.e., 7 hours at 5°C and either 4, 2, 1, or 0 hours at 25°C). Here again, longer exposures to a low temperature, which is expected to reduce the intra-cellular food reserves by depressing the rate of cellular assimilation below the rate of cellular utilization of food reserves, decreased the duration of diapause. Moreover, exposure of *M. vitripennis* larvae to 25°C under an oxygen free atmosphere, which would be expected to restrict assimilation of extra-cellular food sources by eliminating the energy for food transport that was provided by aerobic respiration, instead of reversing the effect at low temperatures enhanced the termination of diapause.
Kobayashi (1957) reported that larvae of *Bombyx mori* L. which were conditioned to produce non-diapause eggs, by incubating them during embryogenesis at 15°C, contained only half as many and smaller neurosecretory cells in the suboesophageal ganglion, than those that were destined to produce diapauing eggs by incubation at 25°C during embryogenesis. In this situation, embryogenesis at the lower temperature initiates a reduction in both the size and number of cells in this endocrine organ, by limiting the capacity of the cells of the anlage to achieve their innate potential for both growth and cell division. If at 15°C the permeability of these cells is diminished to a level at which the rate of cellular assimilation restricts the rate of cellular respiration, other metabolic processes involved in cellular growth and mitosis will be depressed accordingly. Since hormonal secretions of the suboesophageal ganglion are essential for the deposition of yolk (Van der Kloot, 1960), a reduction by one-half in the number of neurosecretory cells would be expected to drastically reduce the quantity of yolk supplied through the egg to individual offspring. Thus it may well be that the low temperature induced reduction in growth and replication of cells in a neurosecretory organ, during the embryonic stage of the mother, eventually causes a reduction in the production of hormones and that this restricts the nutrition of the offspring to a level at which diapause is prevented.
Figures 5, 6, 7, and 8 show the percentage of eggs of *M. disstria* that hatched after each treatment period at each treatment temperature, when incubated at 25°C (Figures 5 and 6), 20°C (Figure 7), and 18°C (Figure 8). From a comparison of these results it is evident that, besides variation between populations, there is considerable variation between the minimum duration of diapause and maximal duration of survival under the various temperature regimes. For example, a high percentage of larvae terminated diapause after only 9 weeks at 5 and 10°C when incubated at 20 and 18°C, respectively, whereas it required between 13 and 17 weeks at -5°C to terminate diapause at 25°C. In relation to duration of survival, more than 85 per cent of larvae hatched at 20°C after 29 weeks at 0 or -5°C, whereas almost all larvae stored at 5 and 15°C were dead by 25 weeks. There can be little doubt that temperature has a major effect on the duration and termination of diapause in *M. disstria*.

Considering the working hypothesis, that diapause is a state of reduced respiratory metabolism which may result from hyper-assimilation of food substrate under conditions of high cell membrane permeability at high temperatures, it is then possible that the prevailing temperature at several stages of development could have a profound influence on the induction and duration of diapause, and survival. Low temperature during egg development in the females would restrict the size of
individual eggs, whereas high temperature would increase the size of eggs, as reported for *N. sertifer* by Campbell and Sullivan (1963). If during embryogenesis high temperatures prevail, regardless of the temperature during oogenesis, the larvae should enter an intense diapause, but those in the large eggs will have a considerably greater yolk reserve and, except at temperatures below 5°C, a more prolonged diapause and survival period. However, if the temperature during embryogenesis is low, a less intense diapause should be induced, indeed possibly prevented, at temperatures slightly below 20°C. The larvae in eggs produced at high temperatures should, again however, have a longer diapause and survival period by having a larger yolk reserve. High temperatures during oogenesis and embryogenesis should enhance subsequent overwintering survival by increasing the food reserve and intensifying diapause (i.e., greatly depressing aerobic respiration). However, after the onset of diapause, low temperature conditions, which will further depress respiration and accordingly food utilization, will be more advantageous to survival.

The foregoing suppositions assume relatively constant temperature regimes and therefore may have to be modified when relating to specific natural situations where sizeable oscillations in temperature occur.
CONCLUSIONS

A comparison of the influence of temperature on diapausing *M. disstria* from two separate populations from the same geographic region, but having different host associations, showed a greater variability in duration of diapause for the population that was associated with two, rather than one, host species. This indicated that intra-specific seasonal variation between the two host species tended to maintain a higher heterogeneity within the insect population, with regard to duration of diapause, by selecting either early or late hatching individuals.

A working hypothesis, that diapause in *M. disstria* is a state of depressed respiration resulting from cellular hyper-assimilation of food substrate and that termination of diapause thus requires elimination of excess food substrate from the cells, was tested by subjecting diapausing pre-hatch larvae to various temperature regimes for increasing periods of time. Both hatching success and the nutritional condition of larvae were assessed at the end of the various treatments. On the basis of the supposition that the log rate of respiration increased linearly (Richards, 1957) whereas the log rate of cellular assimilation increased exponentially (Davson and Danielli, 1943) with temperature, the results of the experiment were consistent with the working hypothesis. At temperatures below 5°C, where respiration should decrease more rapidly than
assimilation, both the fat-body cells and the yolk in the gut remained unchanged for long periods, and diapause was prolonged. At 5°C where the respiratory utilization of intracellular reserves was assumed to be maximal relative to cellular assimilation of yolk, the fat-body but not the yolk was rapidly depleted and the duration of diapause was minimal. At 10°C and above, where cellular assimilation was expected to increase more rapidly than respiratory utilization, the rate of yolk depletion increased whereas the rate of fat-body depletion initially decreased with temperature, and diapause was completed more rapidly at 10°C. At longer treatment periods at the higher temperatures most of the larvae died after depletion of the fat-body but not of the yolk. Since some larvae hatched at a constant temperature of 18°C but not at 20°C, it was concluded that the rates of assimilation and utilization are approximately equal at the higher temperature. Accordingly, starvation and termination of diapause would be coincident at this temperature and above.
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Van der Kloot, W. G.  
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Figure 5. Percentage of *Malacosoma disstria* Hbn. eggs that hatched after each treatment period at each treatment temperature when incubated at 25°C (After Hodson and Weinman, 1945). (Circled numbers indicate months of treatment)

Figure 6. Percentage of *Malacosoma disstria* Hbn. eggs that hatched after each treatment period at each treatment temperature when incubated at 25°C: "A" from Alban and "B" from Azilda, Ontario, Canada. (Circled numbers indicate weeks of treatment)
incubation

20

population A

population B

at 25°C incubation

at 25°C incubation

percentage hatch

at 25°C incubation

3 weeks of treatment

population A

population B

treatment temperature °C

treatment temperature °C

Figure 7. Percentage of *Malacosoma disstria* Hbn. eggs that hatched after each treatment period at each treatment temperature when incubated at 20°C: "A" from Alban and "B" from Azilda, Ontario, Canada. (Circled numbers indicate weeks of treatment)

Figure 8. Percentage of *Malacosoma disstria* Hbn. eggs that hatched after each treatment period at each treatment temperature when incubated at 18°C: "A" from Alban and "B" from Azilda, Ontario, Canada. (Circled numbers indicate weeks of treatment)
Figure 9. The mean number of days (±2 standard errors) to hatch by eggs of *Malacosoma disstria* Hbn. at 18, 20, and 25°C after various periods at -5°C treatment.

Figure 10. The mean number of days (±2 standard errors) to hatch by eggs of *Malacosoma disstria* Hbn. at 18, 20, and 25°C after various periods at 0°C treatment.
Figure 11. The mean number of days (±2 standard errors) to hatch by eggs of *Malacosoma disstria* Hbn. at 18, 20, and 25°C after various periods at 5°C treatment.

Figure 12. The mean number of days (±2 standard errors) to hatch by eggs of *Malacosoma disstria* Hbn. at 18, 20, and 25°C after various periods at 10°C treatment.
Figure 13. The mean number of days (+2 standard errors) to hatch by eggs of *Malacosoma disstria* Hbn. at 18, 20, and 25°C after various periods at 15°C treatment.

Figure 14. The mean number of days to hatch, for the combined populations, by eggs of *Malacosoma disstria* Hbn. at 18, 20, or 25°C after various periods at -5, 0, 5, 10, or 15°C.
In a...
Figure 15. The average total cumulative time (i.e., treatment plus incubation period) by eggs of *Malacosoma disstria* Hbn. resulting from the various treatment periods in conjunction with each incubation temperature for treatments of -5, 0, 5, 10, and 15°C
Figure 16. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. that had been treated at 0°C for 13 weeks. (X80)

F - fat-body
Y - yolk

Figure 17. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. that had been treated at 0°C for 17 weeks. (X80)

F - fat-body
Y - yolk
Figure 18. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. that had been treated at 0°C for 25 weeks. (X80)

F - fat-body  
Y - yolk

Figure 19. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. that had been treated at 5°C for 9 weeks. (X80)

F - fat-body  
Y - yolk
Figure 20. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. that had been treated at 5°C for 17 weeks. (X80)

F - fat-body
Y - yolk

Figure 21. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. that had been treated at 5°C for 25 weeks. (X80)

Y - yolk
Figure 22. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. that had been treated at 10°C for 9 weeks. (X80)

F - fat-body
Y - yolk

Figure 23. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. that had been treated at 10°C for 17 weeks. (X80)

F - fat-body
Y - yolk
Figure 24. A longitudinal section through the gonad of an excised larva of *Malacosoma disstria* Hbn. after 17 weeks at 10°C showing a mitotic figure. (X1300)

Figure 25. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. that had been treated at 10°C for 25 weeks. (X80)

F - fat-body
Y - yolk
Figure 26. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. that had been treated at 18°C for 9 weeks. (X80)

F - fat-body  
Y - yolk

Figure 27. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. that had been treated at 15°C for 17 weeks. (X80)

Y - yolk
Figure 28. A longitudinal section through a larva of Malacosoma disstria Hbn. that hatched after treatment at 10°C for 13 weeks followed by incubation at 18°C. (X80)

F - fat-body

Figure 29. A longitudinal section through a larva of Malacosoma disstria Hbn. that hatched after treatment at 10°C for 13 weeks followed by incubation at 20°C. (X70)
Figure 30. A longitudinal section through a larva of *Malacosoma disstria* Hbn. that hatched after treatment at 10°C for 13 weeks followed by incubation at 25°C. (X80)

F - fat-body

Figure 31. A longitudinal section through a larva of *Malacosoma disstria* Hbn. that hatched after treatment at -5°C for 21 weeks followed by incubation at 18°C. (X70)

F - fat-body
Y - yolk
Figure 32. A longitudinal section through a larva of *Malacosoma disstria* Hbn. after treatment at -5°C for 21 weeks followed by incubation at 20°C. (X70)

Figure 33. A longitudinal section through a larva of *Malacosoma disstria* Hbn. that hatched after treatment at -5°C for 21 weeks followed by incubation at 25°C. (X80)

F - fat-body
Figure 34. A longitudinal section through an egg of *Malacosoma disstria* Hbn. showing germ band development at approximately 2 weeks after oviposition. (X125)

Y - yolk

Figure 35. A longitudinal section through an egg of *Malacosoma disstria* Hbn. two days older than the one in Figure 34. (X125)

Y - yolk
Figure 36. A cross-section through an egg of *Malacosoma disstria* Hbn. following dorsal closure of the embryo. (X200)

Y - yolk

Figure 37. A longitudinal section through a definitive larva of *Malacosoma disstria* Hbn. that was excised following dorsal closure. (X80)

Y - yolk
Figure 38. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. which had just become engorged with yolk. (X100)

Y - yolk

Figure 39. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. approximately 5 weeks after oviposition. (X100)

F - fat-body
Y - yolk
Figure 40. A longitudinal section through a larva of *Malacosoma disstria* Hbn. which hatched without diapausing 7 weeks after oviposition. (X100)

F - fat-body

Figure 41. A longitudinal section through a diapausing larva of *Malacosoma disstria* Hbn. approximately 11 weeks after oviposition. (X100)

F - fat-body

Y - yolk
Figure 42. A longitudinal section through an excised larva of *Malacosoma disstria* Hbn. at the time hatching was taking place from this particular egg mass, approximately 23 weeks after oviposition, after treatment for 13 weeks at 5°C followed by incubation at 20°C. (X100)

F - fat-body
Y - yolk

Figure 43. A longitudinal section through a hatched larva of *Malacosoma disstria* Hbn. approximately 23 weeks after oviposition and after treatment for 13 weeks at 5°C followed by incubation at 20°C. This individual hatched on the same day from the same egg mass as the individual in Figure 42. (X100)

F - fat-body
Y - yolk
Table 2. The effect of the duration of various treatment temperatures on diapausing pre-hatch larvae of *Malacosoma disstria* Hbn. from Azilda, Ontario, Canada, as it relates to percentage hatch and the mean number of days required to hatch at three post-treatment incubation temperatures

<table>
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<tr>
<th>Temp.</th>
<th>5 weeks</th>
<th>9 weeks</th>
<th>13 weeks</th>
<th>17 weeks</th>
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</thead>
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<td></td>
<td>No. eggs</td>
<td>% Hatch</td>
<td>No. eggs</td>
<td>% Hatch</td>
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<td>-5</td>
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<td>0.0</td>
<td>572</td>
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<td>20</td>
<td>719</td>
<td>0.0</td>
<td>634</td>
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<td></td>
<td>25</td>
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*b* Incubation temperature, °C.
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