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A. C. Hodson and C. J. Weinman[1]

Introduction

AN EXTENSIVE outbreak of the forest tent caterpillar, *Malacosoma disstria* Hbn., in northern Minnesota prompted a complete investigation of the environmental factors which might influence survival. In a report of field studies (Hodson, 7), evidence was presented showing that any one of several factors could account for high mortality, and particularly that overwintering eggs tolerated a wide variety of weather conditions. Eggs hatch successfully even though they remain on twigs of the host trees from July until the following May, the elements of weather having a considerable chance for action. These conditions, and the need for a standard procedure for rearing larvae and egg parasites, raised questions concerning the breaking of a diapause and optimum conditions for hatching. Initially the study was limited to variations of temperature in an attempt to ascertain their relations to the persistence of an embryonic diapause. Later the investigation was extended by the junior author to include an analysis of the effects of temperature and moisture on post-diapause development.

FACTORS AFFECTING RECOVERY FROM EMBRYONIC DIAPAUSE

In *Malacosoma disstria* and other related species, embryonic development is usually completed in about two to three weeks after the eggs are laid. The fully formed embryos then enter a state of dormancy which delays hatching until the following spring. Saunders *(13)* has stated that this phenomenon was first reported for the tent caterpillar by Mr. Gott, a nurseryman of Arkona, Ontario, in the fall of 1877. Until recently it was believed that eggs of the tent caterpillars had to experience temperatures

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below freezing before they would hatch. This opinion was not confirmed by a study of diapause in the eggs of the eastern tent caterpillar, *M. americana* Fabr., described by Flemion and Hartzell *(5)*. On the contrary, they found that eggs of *M. americana* hatched readily at room temperature after a pretreatment of 8 to 12 weeks at 1°, 5°, and 10° C. Twelve weeks at 15° C. induced light hatching, while only one larva appeared after 20 weeks at 20° C. Since preliminary tests with the eggs of *M. disstria* indicated a similar dependence upon moderately low temperatures for hatching, experiments were designed for a further study of the problem.

METHODS

Egg masses were collected in late August from several localities in northern Minnesota. Eight lots were placed in desiccators at temperatures of −5°, 2°, 5°, 10°, 15°, 20°, and 25° C. as well as under outdoor conditions in an open insectary. All experimental temperatures were controlled within ±0.5° C. and the relative humidity was maintained at 75 per cent with the proper mixtures of sulfuric acid and water. Twelve masses were removed from each conditioning temperature at intervals of one month, 10 of them being placed at 25° C. for hatching. Samples of larvae were taken from the two remaining egg masses and fixed in Bouin's solution for histological studies. Longitudinal sections of the preserved larvae were stained with iron hematoxylin.

At the end of each experiment all unhatched eggs were examined to determine the nature of their contents, a necessary precaution, because eggs which had not developed or had been parasitized were present in variable numbers in most of the masses. Although none of the undeveloped or parasitized eggs were included in the final calculations, all dead, fully formed embryos were considered to have failed to hatch as a result of the conditions of the experiment. The average number of eggs per mass was approximately 150, so with the usual variation there were between 1,000 and 2,000 "hatchable" eggs in each group of 10 masses.

EXPERIMENTAL RESULTS

When eggs of *Malacosoma disstria* were kept constantly at 25° C., there was no hatching observed in any of several tests, some of which were extended for as long as 10 months. In this respect the eggs of the forest tent caterpillar differ from those

of the nun moth, *Lymantria monacha* L., and the gypsy moth, *Porthetria dispar* L., two species studied by Tuleschkov *(16)*. He has shown that their eggs enter a state of diapause in which they remain for about three months after completion of the embryo. Under natural conditions hatching was delayed by low temperature in the fall and winter months, but in the laboratory hatching took place readily at room temperature after the three-month dormancy. Balch *(2)* also found that eggs of *Alsophila pometaria* would hatch without exposure to low temperatures but that low temperatures reduced both hatching time and mortality. Likewise, eggs of grasshoppers in a state of arrested development will resume normal growth after relatively long periods of time at 25° C. and above, as has been demonstrated by Parker *(10)*, Bodine *(3, 4)*, Andrewartha *(1)*, and others. In contrast, the eggs of *M. disstria* hatched only after they had been exposed to temperatures below 25° C. for a sufficient length of time. The results of temperature treatments are presented in table 1.

The data show that eggs hatch most quickly and in the greatest numbers after relatively short exposures to temperatures near 0° C. With longer treatments the range of temperature which will permit hatching is extended farther above and below freezing. Even at —5° C. there is a progressive change of the percentage of eggs hatching and the rate of hatching at 25° C. with additional time for conditioning. The changes in response to higher temperatures were similar for hatching rate but they differed with respect to survival. At both 10° and 15° C., an increase in the length of exposure time caused a progressive increase in the speed of hatching at 25° C., but this was accompanied by a marked decrease in the number hatching. Since other experiments will show that a good hatch can occur at both 10° and

Table 1. Per Cent Hatch (A) and Average Time in Days Required for Hatching (B) at 25° C. after Exposure for One to Six Months at Several Temperatures

Length of treatment	20° C.		15° C.		10° C.		5° C.		2° C.		—5° C.		Outside temperature	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
One month	0	0	0	0	0.1	24.0	0	0	0	0	0	0	0	0
Two months	0	0	0.3	39.5	2.9	25.7	24.3	22.0	31.1	20.6	0	0	0.1	56.0
Three months	0	0	9.2	17.8	90.3	11.4	86.9	11.5	92.9	11.7	6.3	12.8	1.8	33.8
Four months	1.4*	8.0	73.9*	8.4	80.4	5.6	91.3	8.4	93.7	10.2	24.5	15.7	76.6	26.5
Five months	0.1	7.0	12.5†	3.0	41.9	2.9	95.0	5.0	95.1	7.3	78.6	11.4	92.2	17.0
Six months	.....	.....	.....	.....	.....	.....	.....	.....	94.7	4.8	.....	.....	97.1	12.2

* One larva hatched after four months while eggs were being conditioned at 20° C.; 2.87 per cent of the larvae hatched after four months while at 15° C.

† While being conditioned at 15° C., 38.1 per cent of the larvae hatched during the fourth and fifth months.

15° C. after eggs have been exposed previously to 2° C. for three months, a continuous exposure to these temperatures must influence some preliminary development rather than the hatching process itself. In general, the eggs hatched much more uniformly under conditions of temperature and time which gave other evidence of being most favorable. The range of time in days from the first egg to hatch to the last in lots conditioned at 2° C. was 43, 31, 13, and 11 days after treatment for 2, 3, 4, and 5 months respectively. This trend toward less variation in the response of individual eggs was apparent at 5° C. and below freezing, but at higher temperatures the change was different. At 10° C., a temperature less efficient in breaking the diapause, the range in time required for hatching at 25° C. was 32, 27, 17, and 25 days, after exposures of 2, 3, 4, and 5 months respectively. In this case as well as in the experiments at 15° C., the variation in hatching time increased after reaching a minimum value at four months. As would be expected, there is a limit to the length of time during which eggs can remain at temperatures near O° C. and still be viable. No experiments were set up to determine the maximum duration of time, but 50 egg masses which were kept constantly at 5° C. for 13 months showed a hatch of 5.7 per cent after being brought up to room temperature.

The evidence presented shows definitely that continuous exposure to temperatures above 5° C. becomes increasingly less favorable for hatching. That 20° C. lies at the upper limit of the temperature range in which hatching is permitted is indicated by the fact that only 14 larvae out of about 2,000 hatched at 20° C. or subsequently at 25° C. A considerable number of them hatched at 15° C. after five months, but the total hatch after this treatment showed a gross loss when compared with the results after conditioning at the same temperature for only four months. There was no hatch while eggs were kept at 10° C.; however, hatching could be expected after a longer time because this temperature lies above the threshold for that process.

Because preliminary tests had suggested the probability that eggs of *Malacosoma disstria* would not hatch at temperatures as high as 25° C., an experiment was performed to test viability at this temperature. A large sample of eggs was placed at 25° C., and groups of 10 masses each were removed at intervals of one month to be given further treatment for three months at 2° C., after which the masses were returned to 25° C. to permit the hatching of viable eggs. The results of this study are shown in table 2.

Table 2. Response of Eggs to Exposures for Various Lengths of Time at 25° C. with Subsequent Treatment at 2° C. for Three Months before Being Returned to 25° C. for Hatching

Length of time at 25° C.	Number of eggs used	Per cent hatch when returned to 25° C.	Average hatching time in days
One month	1,939	73.5	25.7
Two months	1,558	51.8	18.7
Three months	1,652	1.6	21.0
Four months	1,832	0	.....
Five months	1,326	0	.....

In this experiment, fully formed embryos, which had been in the field for about six weeks before use, evidenced no hatch after being held longer than three months at 25° C. Further mention of the effect of continuous high temperatures will be included in a discussion of morphological changes in the embryo.

HISTOLOGICAL STUDIES

Bodine *(4)* has emphasized the unquestionable advantage of knowledge concerning the changes in insect morphology which may be correlated with the physiology of individuals in a state of diapause. In a study of grasshopper eggs, this is particularly true because the diapause occurs in a definite morphological stage shortly before blastokenesis, at a time when cell division and growth would be expected to be quite rapid (Slifer, *15*, and Andrewartha, *1*). Actually, Slifer *(14)* has shown by her histological and cytological studies that the grasshopper embryonic diapause is characterized by a complete absence of mitotic spindles or other evidence of continued growth. A similar condition would be expected in those eggs which become dormant at other times during the period of cell multiplication and differentiation, but there are several examples among the Coleoptera and Lepidoptera in which the diapause occurs only after embryonic development has been completed.

Although some of the characteristics of diapause in the mature embryos have been described (Flemion and Hartzell, *5*), apparently little attention has been given to the histological features. The most suggestive work has been reported by Tuleschkov *(16)*, who studied sections of the eggs of several species of Lepidoptera at intervals from the time of oviposition until hatching. The species used in his investigations were selected to represent two physiologically different groups: one, including *Bombyx mori, Stilpnotia salicis, Dasychira pudibunda,* and *Euproctis chrysor-*

rhoea, species producing eggs which hatch immediately upon completion of the embryo; the other, including *Porthetria dispar, Lymantria monacha,* and *Malacosoma neustria,* species laying eggs which enter a diapause as mature embryos. Tuleschkov found that in both groups there was a great abundance of yolk enclosed in the stomodeum and mesenteron of mature embryos, the significant difference being observed in the time of absorption of the yolk. In the first group the yolk was digested soon after embryonic growth was completed, and hatching took place as soon as all the yolk was exhausted. The species in the second group showed no change in the quantity of yolk for at least three months after growth ceased, but the yolk was digested rapidly just prior to hatching. Tuleschkov made no comment about other changes in the appearance of the embryos. He interpreted the yolk absorption merely as a necessary preliminary before hatching could be accomplished, and assumed that the caterpillars chewed their way out of the eggs in response to a hunger stimulus.

A similar study of sections made of the eggs of the forest tent caterpillar shows that a large amount of yolk remains in the digestive tract of the embryo from the time of maximum growth until just before hatching. Yolk is present in the stomodeum and mesenteron, usually being more abundant in the latter. As can be seen in figure 1, the yolk completely fills the lumen of the mid-gut of an embryo killed four weeks after the egg was laid. At this stage of the diapause the intestinal wall is much flattened

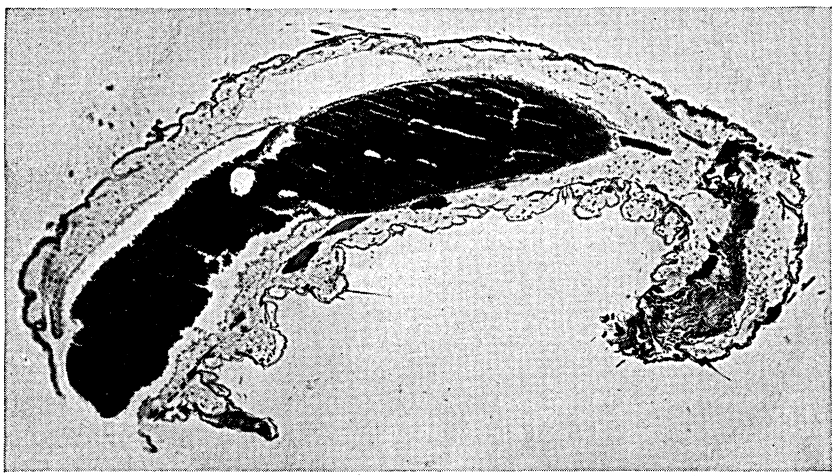

FIG. 1. Longitudinal section of egg made about one month after completion of embryo. Shows thin-walled mesenteron and compact Malpighian tubule (condition in diapause)

and the cells show no vacuoles or other evidence of activity. After six to seven weeks, the time at which most of the eggs were collected in the field, there was no reduction in the amount of yolk and only a slight change in the appearance of the cells of the mesenteron. When sections were made of samples of eggs taken from lots which had received the various temperature treatments described before, the general sequence of events associated with yolk absorption could be observed. At the time when the first structural modifications were in evidence, as illustrated in figure 2, three principal changes were noticed: a reduction in the amount of yolk in the fore-gut, a marked difference in the appearance of the mid-gut, and a change in the Malpighian tubules. The first sign of a reduction in the amount of yolk was indicated by ragged edges along the margin of the mass in the fore-gut. At this time some activity in the cells of the mesenteron was suggested by an increase in size and the presence of vacuoles. In the resting condition the lumen of the Malpighian tubules was barely discernible, and the dense nuclei were packed closely together to give the effect of two dark bands separated by a narrow light line (figure 1). As the mesenteron first became active the lumen of the Malpighian tubules enlarged somewhat, and the nuclei became more distinct.

As the process proceeded, the yolk in the fore-gut diminished in quantity, until only traces were left at the time of hatching.

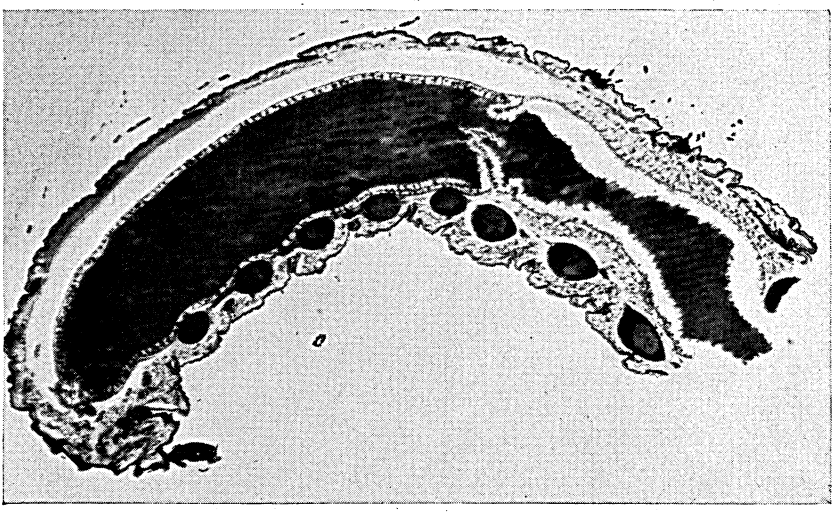

FIG. 2. Longitudinal section showing early change in amount of yolk and appearance of mesenteron wall

Likewise the yolk was absorbed slowly from the mid-gut until a few days before hatching, when all but small fragments were absorbed more rapidly. This active absorption of yolk apparently was accomplished by greatly increased activity of the cells of the mid-gut as shown in figure 4. As the yolk disappeared these cells became much enlarged and exhibited numerous vacuoles as well as goblet cells. In fact, just before hatching, the mid-gut cells nearly fill the lumen, in contrast to their inconspicuous nature at the beginning.

The Malpighian tubules also showed evidence of increasing activity as the preparation for hatching progressed. The lumen became more and more conspicuous, and the walls thinner in spite of an over-all increase in diameter. In the later stages (figure 4), the nuclei assumed the characteristic shape seen in larvae and adult insects.

The effects of temperature upon the histological changes described are of considerable importance when considered in connection with the study of survival and rate of hatching. When eggs were held at 20° and 25° C., there was only a slight change in their appearance after one month. At the end of two months (approximately three months after mature embryos were formed) there was a definite reduction in the amount of yolk, an increase in the size of the mid-gut wall, and there were signs of activity in the Malpighian tubules. Further exposure to these relatively high temperatures resulted in a nearly complete absorption of

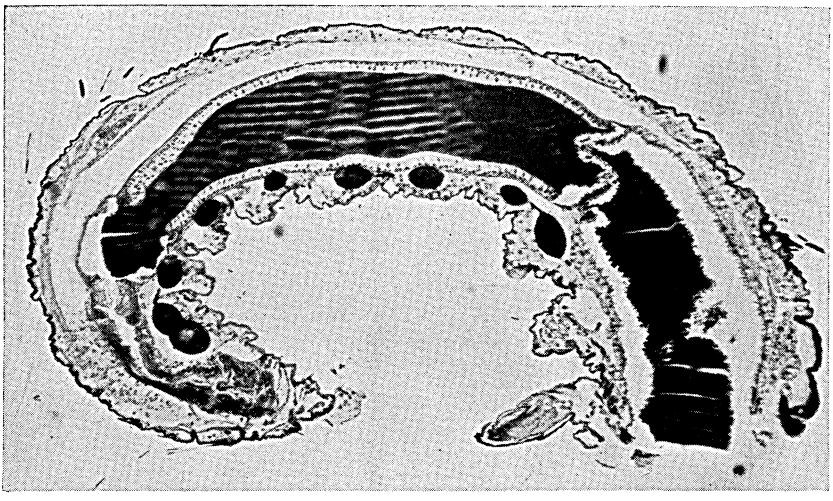

FIG. 3. Longitudinal section showing more advanced stage in process of yolk absorption and histological change

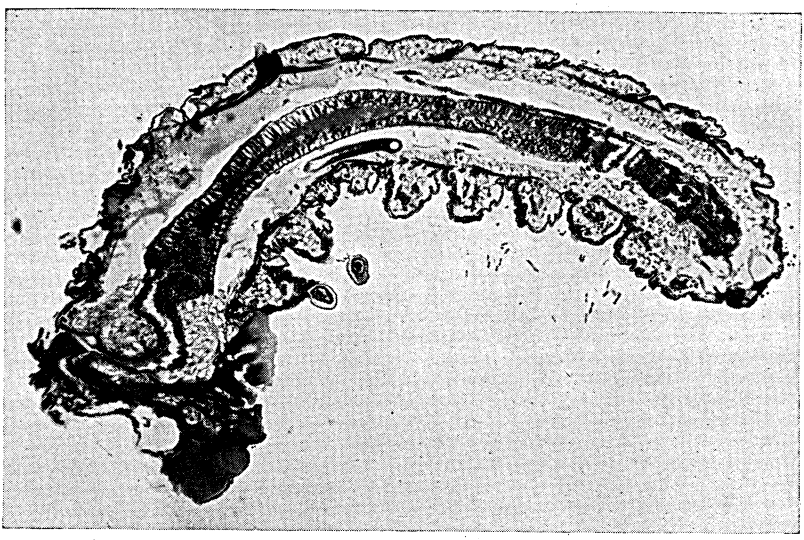

FIG. 4. Appearance of embryo a few days before hatching, showing great enlargement of mesenteron wall and Malpighian tubule, as well as a nearly complete exhaustion of the yolk

the yolk; however, most of the embryos were dead and had started to disintegrate by this time. After four or five months at 20° C. there were a few living embryos, although most of them appeared to be in about the same state of disintegration as those at 25° C. at the end of three months.

Sections of eggs conditioned at 15° C. showed a slight change in the mid-gut cells at the end of one month, and considerable difference could be seen in both these cells and the Malpighian tubules after two months. A treatment at 15° C. for three months produced an advanced stage of yolk absorption with the associated histological changes, while at the end of four months many of the sectioned eggs showed embryos with only fragments of yolk left. Reference to table 1 will show that some hatching took place after a three-month exposure to 15° C., and that some larvae hatched during the fourth month while the eggs were still at this temperature. A similar response to moderately low temperature was found for eggs conditioned at 10° C. The principal difference between the results at 15° and 10° C. was a slower rate of change during the first three months, so that eggs held at 10° C. were in about the same condition after four months as those at 15° C. were at the end of three months. No eggs hatched while at 10° C., although some of the embryos sectioned at the end of five months indicated that they were nearing the hatching condition, and

Table 3. Comparison of the Progress of Yolk Absorption and Histological Change in Embryos Conditioned at Several Temperatures

Length of treatment	Relative change at each temperature						
	25°	20°	15°	10°	5°	2°	—5°
One month	Beginning*	Beginning	Beginning	None	None	None	None
Two months	Advanced†	Advanced	Partial	Beginning	Beginning	Beginning	None
Three months	Complete‡	Complete	Advanced	Partial	Partial	Partial	None
Four months	Dead	Dying	Complete	Advanced	Partial	Partial	Partial
Five months	Dead	Dead	Complete	Complete	Advanced	Advanced	Partial

* Figure 2.
† Figure 3.
‡ Figure 4.

hatching took place in about three days when eggs similarly treated were placed at 25° C.

The responses after exposure to 5° and 2° C. were so nearly the same for the two temperatures that they can be considered together. Conditioning for one month caused no appreciable change, and after two and three months there was only a slight thickening of the wall of the mid-gut. At the end of four months the histological picture was much like that shown in figure 3. The cells of the mid-gut showed signs of activity, and the Malpighian tubules were enlarged. Sections made at the end of five months of conditioning showed some variation, from a condition of moderate yolk absorption to one approaching the hatching state. When —5° C. was used there was practically no change from the resting condition until the eggs had remained below freezing at this temperature for four months. Then there was a moderate development of the mid-gut and Malpighian tubules. Even after five months none of the sectional embryos showed an advanced stage of yolk absorption and cell activity. This survey of the morphological changes is summarized in table 3.

DISCUSSION

The purpose of this study was to determine the influence of temperature on the breaking of an embryonic diapause which prevents eggs of the forest tent caterpillar from hatching during the late summer. The figures representing percentage hatch indicate no response after one month of temperature treatment and only a partial breaking of dormancy after two months. Since the eggs used in this experiment were collected approximately six weeks after fully formed embryos were present in the field, the striking change in both percentage hatch and time required for hatching after a longer exposure suggests that the diapause persists for about three months under all temperature conditions.

When the ability to hatch is taken as the measure of success in breaking the diapause, the data lead to the conclusion that termination of the diapause is accomplished by a process which is not only initiated at moderately low temperatures, but which must be allowed to continue at these temperatures for a sufficient length of time. However, when the evidence is reconsidered in the light of information afforded by a study of sectioned embryos (see table 3), a different explanation of the effect of conditioning temperature is necessary. If the described histological changes and progressive yolk absorption are considered evidence of increased metabolic activity, then their inception marks the end of the true diapause and provides a means of evaluating the effects of temperature.

Reference to the histological study will show first that no significant change took place during the first month under any of the temperature conditions, thus supporting the belief that the diapause persisted for about three months after embryonic development was completed. This conclusion is confirmed by the fact that cell activity was observed after two months at all temperatures above —5° C. including 25° and 20° C. At this point the evidence provided by hatching records and morphological indications might seem to be conflicting, for in the case of the former there was no response at 25° C., while in the latter there were very conspicuous signs of cell activity and yolk absorption even at this temperature. Furthermore, preparation for hatching proceeded to the point where nearly all the yolk was digested at each of the highest temperatures even though there was no hatch at 25° C. and very little at 20° C. This means that low temperatures were not necessary to break the diapause but were essential, at least during the early stages of yolk absorption, before normal hatching would take place.

That the most critical period probably occurs during the early stages of yolk absorption is suggested in the results of experiments at 2° and 5° C. where little change in hatch is brought about by conditioning longer than three months, two months beyond the end of the diapause. Likewise the data in table 2, showing the results of placing eggs at 2° C. after various lengths of time at 25° C., suggest that there is a critical stage soon after the diapause is terminated, when exposure to high temperatures is unfavorable for hatching. Additional support for this conclusion is evident in the sections which show that after even a moderate change in the appearance of the mid-gut cells at low temperatures a good hatch resulted when the eggs were placed

at 25° C. Further enlargement of these cells, associated with an increased rate of yolk absorption, simply shortened the hatching time. This effect of temperature may be compared with its influence on growth, for the rate of hatching at 25° C. was accelerated most after treatment at 15° C. and slowed progressively as the conditioning temperature was reduced by degrees to —5° C.

The real cause of injury at high temperatures or, conversely, the benefits of exposure to moderately low temperatures as recovery from the diapause begins cannot be determined from the data available. It is possible that excessive dehydration may have been the determining factor, for Tuleschkov *(16)* has shown that a significant amount of water is lost during the diapause of similar species. Low temperatures may have had an effect on the yolk in making it more easily assimilated (Andrewartha, *1*), or the embryos may have been exhausted by the demands of a high rate of metabolism at 20° and 25° C. before the nutritional requirements could be satisfied by yolk absorption. Whatever the cause, these results can be interpreted to mean that although the diapause is of short duration, like that of the nun moth and gypsy moth, it is effective until cold weather comes along to slow down yolk absorption and prevent hatching until spring.

Effect of Temperature and Moisture on Hatching of the Eggs of *Malacosoma disstria*

After the diapause is terminated, as it is during November under Minnesota conditions, the eggs of the forest tent caterpillar are subjected to a great variety of weather conditions prior to the time of hatching. As temperature and moisture are the two most important factors that influence the percentage hatch and the rate of hatching, their action was investigated under controlled laboratory conditions. Experiments were designed to include the range of conditions common in the field and further to test the resistance of eggs to extreme desiccation and hydration.

METHODS

Dormancy was broken in all eggs used in the following experiments by storing them for about three months at 2° C. More than 700 egg masses were used in the various experiments, representing approximately 100,000 eggs. All experiments were carried on in constant temperature cabinets, ranging from 5° to 30° C. Five egg masses were selected for each temperature-humidity combination. Each egg mass was placed in a separate vial,

and the five vials were put into a desiccator made from a large-mouth pint jar. Sulfuric acid, in various concentrations, was used to maintain the desired relative humidity. In all experiments moisture conditions were selected and expressed in terms of saturation deficit as vapor pressure in millimeters of mercury. The original intent was to study the comparative advantage of the use of saturation deficit over relative humidity as a method of describing moisture conditions. This purpose was not followed through completely because of the difficulty in determining water loss from the eggs, which were left undisturbed on small sections of poplar twigs.

INFLUENCE OF TEMPERATURE

The data presented in table 4 and figure 5 show that under similar moisture conditions percentage hatch is not affected much by temperature until 30° C. is reached. This high temperature is well out of the range of the usual maximum experienced during the hatching period in the spring. The largest hatch took place in a saturated atmosphere at 15° C., although there is very little difference over the range from 10° to 25° C. No eggs hatched at 5° C., even after exposures for several months, so the threshold for hatching lies between 5° and 10° C.

INFLUENCE OF HUMIDITY

Whereas a rather wide range of temperature produced only the usual differences in rate of hatching and rather uniform results in percentage hatch, a variation of moisture conditions produced a different effect. An inspection of figure 5 will show that over the range of from 10° to 25° C., moisture conditions favorable for hatching were found between relative humidities of about 70 to 100 per cent. At 30° C., the lower limit of the optimum range was shifted slightly higher and the injurious effect of low humidities was more pronounced in general. In some respects these results differ from those obtained by Ludwig and Anderson *(8)*, who studied the eggs of four species of saturnid moths. Whereas they found the optimum relative humidity at about 76 per cent, with the exception of *Samia walkeri* at 15° and 20° C., the eggs of the forest tent caterpillar hatched best in a saturated atmosphere. This was true in spite of the development of mold at the two highest temperatures.

The data shown in table 4 indicate no striking effect of relative humidity or saturation deficiency on the time required for hatch-

Table 4. Effects of Temperature and Relative Humidity on the Hatching of Eggs of the Forest Tent Caterpillar

Temperature degrees C.	Relative humidity in per cent	Saturation deficit	Total larval hatch	Per cent larval hatch	Average time in days
10	100	0	181	90.5	37.2
10	78	2	262	80.9	37.9
10	56	4	262	66.3	38.3
10	35	6	100	20.8	35.5
10	13	8	0	0	0
15	100	0	394	95.1	20.0
15	69	4	348	89.0	22.3
15	53	6	168	69.4	23.0
15	37	8	83	18.1	26.0
15	21	10	0	0	.....
15	6	12	0	0	.....
20	100	0	392	90.1	14.4
20	77	4	343	86.2	11.6
20	66	6	294	89.3	11.7
20	43	10	80	24.3	12.8
20	31	12	26	10.8	14.4
20	20	14	0	0	.....
20	8	16	0	0	.....
25	100	0	319	90.6	8.8
25	75	6	365	90.8	8.3
25	58	10	300	74.3	8.2
25	49	12	157	35.7	7.4
25	32	16	2	2.1	9.0
25	15	20	0	0	.....
30	100	0	335	83.1	6.4
30	81	6	297	80.5	7.8
30	62	12	214	46.9	5.9
30	49	16	104	19.4	6.3
30	37	20	22	7.1	7.4
30	24	24	0	0	.....
30	11	28	0	0	.....

ing. When one considers the modification of the law of saturation deficit (Mellanby, *9*), which states that desiccation is proportional to the product of saturation deficit and time, then the effect of moisture on time of hatching becomes more complex. For example, in the results at 25° C., hatching required an average of 9.0, 8.78, and 8.26 days at saturation deficits (S.D.) of 16, 0, and 6 respectively. The eggs at S.D. of 0 hatched over a range of 19 days; the two at S.D. of 16 hatched 6 days apart, the first appearing only one day later than the first larva in S.D. of 0. Since the product, S.D. x time, increases at the rate of 16 units per day with a S.D. of 16 mm. of mercury, it would have reached 384 by the time the last larva in S.D. of 0 hatched. With so high a product, indicating a long exposure to dry air, it is little wonder that no larvae hatched during the later days in dry air while

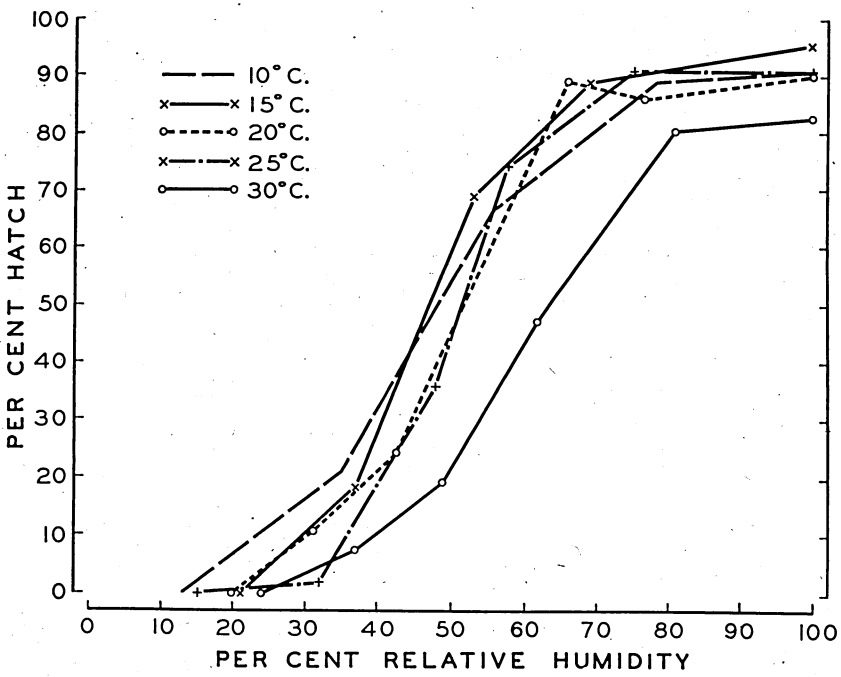

FIG. 5. Effect of different percentages of relative humidity over a range of 10° to 30° C. in the hatching of eggs of *Malacosoma disstria*

larva were still hatching in air saturated with moisture. In other words, the average time for hatching in S.D. of 16 was approximately equal to that in S.D. of 0 because the only larvae that hatched came out early. It is possible that the development of most of the eggs held at the highest saturation deficit was retarded by the dry air so long that, when they finally had reached the hatching stage, prolonged desiccation prevented normal emergence.

In this regard, the results of a subsequent experiment have a direct bearing on this question. The eggs which did not hatch at the highest saturation deficit after the experiment had run 14 days were moved from the dry atmospheres to containers with a saturation deficit of 0. Three egg masses from each group of five held at 15° to 30° C. were tested and two kept under the original conditions as checks. The results of this experiment are shown in table 5.

There was no hatch in the lots of two egg masses retained as checks under the original conditions of temperature and moisture, nor was there any hatch at 25° and 30° C., even in a saturated atmosphere. Those held at 15° C. hatched nearly normally, while

Table 5. Effect of a Saturated Atmosphere on the Hatching of Eggs Which Had Been Exposed to High Saturation Deficits over a Range of 15° to 30° C.

Temperature	Former saturation deficit	Former relative humidity	Total larval hatch
° C.			
30	28	11	0
30	24	24	0
25	20	15	0
25	16	32	0
20	16	8	9
20	14	20	0
15	12	6	208
15	10	21	190

only one other egg mass, that at 20° C. with a S.D. of 16, yielded any larval hatch. Since the relative humidity in each of the groups was approximately the same, the results show that either saturation deficit or the combined action of temperature and moisture is the controlling factor.

Eighty-eight days after this series of experiments was set up, two egg masses from each of the desiccators kept at 5° C. were moved to 25° C. and a saturation deficit of 6 (R.H. 75 per cent). All these egg masses, even those from S.D. 6 (R.H. 7.8 per cent), hatched in profusion. This temperature, 5° C., lies below the hatching threshold, and as a result the changes preceding hatching had not progressed far before the eggs were under conditions of favorable temperature and moisture.

Apparently there must be two reasons why forest tent caterpillar eggs do not hatch in atmospheres of low moisture content. The first is the physical alteration of the egg shell, or chorion. This evidently takes place before the larvae are ready to hatch, so that when they have reached the hatching point, the chorion is changed so much physically that they cannot break through it. This was the condition of the larvae held at 15° C., for they began to hatch within three days after they were moved to a saturated atmosphere. The second factor is physiological and involves the larvae directly. Where desiccation has been severe, the larvae are killed either through water loss, or through starvation and exhaustion; so that at the higher temperatures, few or no larvae hatched even after the chorion was restored to its normal physical condition. Ludwig and Anderson *(8)* found a similar condition when eggs of *Telea polyphemus* were incubated under a range of relative humidities. In this case more larvae developed than were able to hatch at low atmospheric moisture conditions, and it was assumed that these larvae were desiccated beyond the vital

limit before emergence could occur. Although the two sets of experiments are not entirely comparable, because the embryonic development of *Malacosoma disstria* was completed at the start of the experiment, the failure to hatch in a dry atmosphere is probably much the same in both instances. A change in the hardness of the chorion as well as desiccation of the embryos could be important as factors operating either alone or together to prevent normal hatching.

CHARACTERISTICS OF THE EGG COVERING

One of the familiar characteristics of egg masses of the forest tent caterpillar and related species is the frothlike substance covering the eggs. Various writers have referred to this material as "water-proof cement," "a brown sticky substance," "a light brown, frothy glue," or "a varnish-like covering." Most of these expressions are fairly descriptive of the appearance of the egg covering but are cumbersome to use and are even misleading. For the purpose of the following discussion this material will be called the *spumaline*. This term is a combination of the Latin words *spuma* meaning "froth," and *lino* meaning "to spread or smear over." It can be used to designate any kind of frothy, foamy, or cementlike material applied to the eggs by the female during oviposition.

When the results of the experiments were found to bear so close a relationship to relative humidity, it became apparent that some factor other than simple desiccation was importantly concerned. Although evaporation is proportional to saturation deficit under the conditions of these experiments, the amount of water which a hygroscopic substance will absorb is determined by the relative humidity. Accordingly an investigation into the hygroscopic properties of the spumaline was undertaken.

The spumaline was removed from about 80 egg masses, this number yielding almost 0.2 grams of the material. The whole amount was divided into two portions, a portion being put into each of two weighing vials previously brought to constant weight over concentrated sulfuric acid. When the spumaline had come to a constant weight, one portion of it was exposed in a desiccator maintaining a relative humidity of 7.8 per cent, and the other was exposed in a similar desiccator with a relative humidity of 84.5 per cent. If relative humidity alone is concerned, then the amount of moisture taken up by the material in either of the two vials should be approximately the same at any temperature. If saturation deficit is a factor, the material should absorb much

Table 6. Increase in Weight of Dry Spumaline Held over Weak and Strong Sulfuric Acid Solutions at 5° and 30° C.

Sample	Per cent sulfuric acid	Dry weight in grams	Increase in weight at 5° C.	Per cent increase in weight at 5° C.	Increase in weight at 30° C.	Per cent increase in weight at 30° C.
A	65.0	.0898	.0022	2.6	.0034	3.8
B	23.0	.0958	.0176	17.4	.0191	20.0

more water at a lower temperature. Sulfuric acid solutions, selected to produce a high and a low relative humidity, were used at 30° and 5° C. in a test of the hygroscopic properties of spumaline. The results of the experiment are given in table 6.

These data reveal that the spumaline does take up considerable moisture from atmospheres of high relative humidity. That there is a greater increase in weight at 30° C. than at 5° C. is explained by the fact that the vapor tension of a sulfuric acid solution varies slightly with temperature. In this experiment, the 23.0 per cent acid maintained a relative humidity of 84.5 per cent at 5° C., but with the same acid solution the relative humidity at 30° C. would be 91.5 per cent. Similarly, the stronger acid gave a relative humidity of 7.8 per cent at 5° C. and one of 10.5 per cent at 30° C. The difference in weight increase with the same acid solution used at different temperatures shows how closely the absorption of water is related to relative humidity, as can be seen by a calculation. The increase in weight of .0191 grams at 30° C. as compared with an increase of .0176 grams at 5° C. would indicate, by simple proportion, a relative humidity of 91.7 per cent. Actually, on the basis of vapor pressure, the 23.0 per cent acid should produce a relative humidity of 91.5 per cent at 30° C.

It is evident from the results of this experiment that relative humidity controls the amount of water taken up by the spumaline, a hygroscopic substance rather than a "water-proof cement." Eggs covered by such a substance lose water in a dry atmosphere, but the rate of loss would be affected, at least by the slowing down of the movement of water vapor away from the surface of the chorion. Also there might be a conservation of moisture after exposure to moist air or rain.

Host-Parasite Relationships

Other workers, for example Hefley *(6)* and Payne *(12)*, have found that temperature and moisture requirements of insects and their parasites may be distinctly different. Although too few parasites emerged in the experiments described here to merit a

final opinion, the parasites show some consistent differences. The most abundant parasite, *Telenomonus clisiocampae* Ashm., has an emergence threshold at about 15° C. while the host hatches readily at 10° C. A high humidity is most favorable for both host and parasite, but there is some evidence that the parasites tolerate a drier atmosphere than the eggs of the host. Further information on the resistance of parasites and host eggs to desiccation will be discussed in the section dealing with desiccation experiments.

SOAKING EXPERIMENTS

Since forest tent caterpillar egg masses are often subjected to thorough soaking from spring rains, it seemed desirable to determine their resistance to complete immersion at various temperatures. It may be objected that the effects of complete immersion are not comparable to the effects of rain; but the spumaline takes up enough water even from a light rain to become as soggy and thoroughly soaked as if the entire egg mass had been under water. It is significant here to recall again that this material has been referred to as "water-proof cement."

In the first experiment, egg masses were soaked in small jars of distilled water at temperatures of 5°, 10°, 15°, 20°, 25°, and 30° C. for periods of 2, 4, 8, 12, 24, and 32 days. At the end of each period, five masses were removed from each jar. These were dried superficially on paper towels for one hour and were then put into individual vials and incubated in a rearing chamber where the temperature varied from 23° to 26° C. and the relative humidity, from 68 to 74 per cent.

In a second experiment the eggs to be used were first divided into two groups, one of which consisted of egg masses well covered with spumaline. Masses in the other group were scraped free of their spumaline covering by means of a small, stiff brush. All the masses were put into jars of distilled water at 30° C. Three masses from each group were removed at the end of every hour from 1 to 24 hours, and after 26 and 28 hours. Two of the masses from each group were dried superficially as before and then incubated at 25° C. and a S.D. of 6. The third mass was opened and examined immediately under a binocular microscope.

The results of the first experiment, shown in table 7, make it clear that the temperature at which eggs are soaked is more important than the duration of soaking. A two-day soaking at 30° C. prevented hatch entirely, but eggs soaked at 5° C. for as long as 24 days permitted some hatch. Two possibilities are implied by

these results: first, that they show the effect of higher temperature on the respiration of the embryos; second, that they show the effect of temperature on the ability of water to penetrate the egg. To determine which of these was active in this first experiment was the purpose of the second. In this case the results are expressed (figure 6) as average hatch of the total larval emergence from eggs soaked for periods of four hours. The grouping of four hourly treatments eliminates some of the variation due to differences in egg mass size, or the number of "hatchable" eggs per mass, and emphasizes the general trend of the results. These data show a striking difference in response to soaking between masses from which the spumaline had been scraped and those

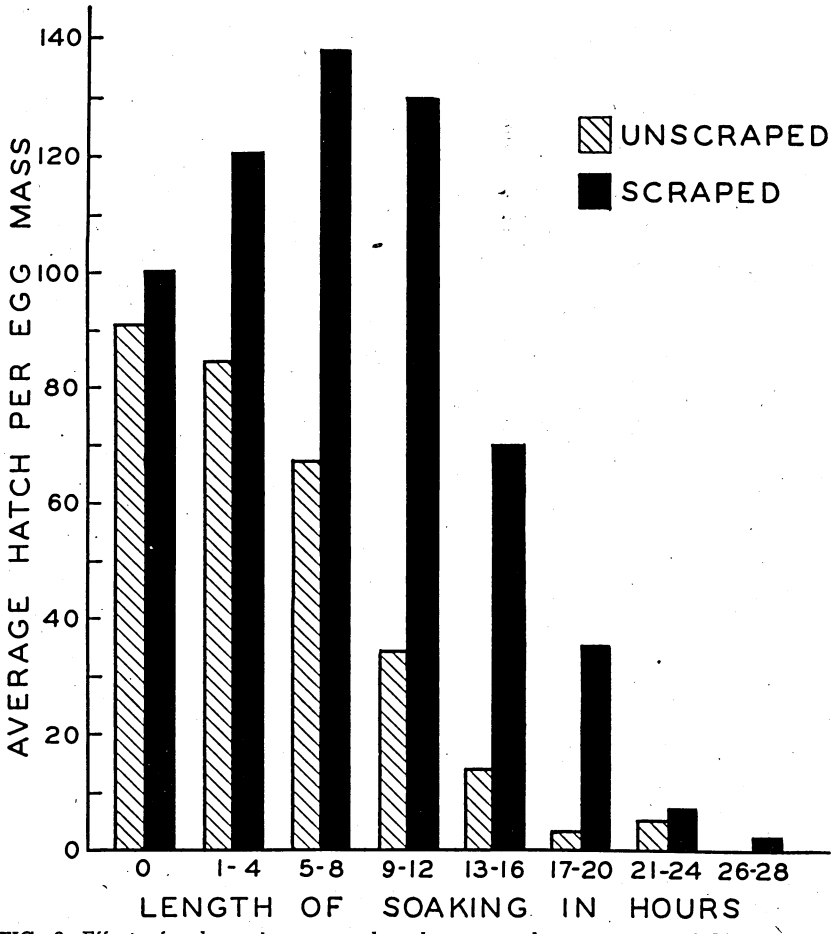

FIG. 6. Effect of submerging scraped and unscraped egg masses of *Malacosoma disstria* in water at 30° C.

which were left unscraped. There was a progressively smaller hatch from eggs which retained their covering as the length of soaking time increased, while the scraped eggs hatched better than the controls for about 12 to 14 hours and then began to show the injurious effects of submergence.

In addition to the hatching data, the results of direct examination of eggs after removal from the soaking chambers are of interest. An examination of samples of eggs revealed that water does penetrate the egg shell. After seven or eight hours, some of the eggs were found to have water in them, and at the end of the fourteenth hour most of the eggs contained water. No appreciable difference between the scraped and unscraped masses could be detected in this respect, but the decrease in hatch of unscraped eggs does bear a close relationship to the time when most of the eggs contained water.

It appears from these results that the penetration of water into the eggs must be considered as a factor responsible for some of the observed effects, but it does not explain fully the results of the first experiment. The fact that there was some hatch even after 28 hours of soaking leads one to the belief that at least a small amount of water within the eggs does not kill the larvae. In prolonged soaking at moderate temperatures and even in short periods of soaking at high temperatures, whatever oxygen was originally in the eggs would soon be exhausted, whether water entered the egg or not, unless the insects could lower their metabolic rate.

That the insects actually can adjust their metabolism—and therefore their rate of respiration—to the supply of available oxygen is a logical conclusion for reasons other than that given above. If respiration continued at the normal rate, some of the larvae in the first experiment would have attempted to hatch while the eggs were still under water. There was no evidence that this had happened. Moreover, the length of time required for hatching after the eggs were removed from the water was not appreciably affected either by the temperature at which they were soaked or by the length of time that they were soaked. This can mean only that no appreciable extent of development took place while the eggs were immersed, and this, in turn, means that the rate of metabolism was lowered to a point at which life could just be maintained. The ability of the insects to lower their metabolism to this point must bear an inverse relation to the temperature. For those held at 5° C., only slight adjustment was necessary, since no hatching can take place at this low tempera-

ture. The higher the temperature at which the larvae were soaked, the greater this adjustment had to be, and the more difficult it became. At such a high temperature as 30° C., it was impossible for the larvae to reduce their metabolic rate sufficiently to survive even one or two days of soaking. By similar reasoning, the hatch or lack of hatch throughout the other conditions of the experiment can be explained.

There remains to be explained the great difference in amount of hatch that obtained between the scraped and the unscraped masses (figure 6), and this involves the manner in which water gets into the eggs. Here again two possibilities are apparent: the water may enter through the micropyle, or it may pass directly through the chorion. Although the first of these may be correct, the second certainly is. After the eggs have been soaked only a few hours at 30° C., the physical properties of the egg shell are altered. When dry, the chorion is brittle; but a little water soon softens it considerably, and more water renders it almost spongy. Even before water in droplets can be detected within the egg, the inside of the shell appears to be damp. The chorion seems even to have some affinity for water, for it will absorb enough from an atmosphere of high humidity to cause it to lose its brittleness. If water passes into the egg through the chorion, it is safe to assume that it passes out in the same way. But water cannot be evaporated from the spumaline-covered eggs until the spumaline itself has become sufficiently dry. The effect of the spumaline then is to prolong the period of soaking, even though the eggs are no longer under water; and this is the reason that in almost every instance more larvae hatched from the scraped eggs than from the unscraped.

Host-Parasite Relationships

A glance at table 7 reveals that under several conditions parasites emerged when no larvae hatched. This must be interpreted to mean that the parasites are better able to adjust themselves to a diminished oxygen supply than are the larvae. It does not indicate that the oxygen requirement of the parasites is less than that of the larvae, for the length of time for emergence after incubation does not vary appreciably with the condition to which the eggs were subjected. As was true of the larvae, the parasites showed no evidence of having undergone development during the time that the eggs were soaking.

The figures for percentage hatch of parasites are significant only in that they do not show a gradual reduction with length

Table 7. Percentage of Larval Hatch and Parasite Emergence from Eggs Soaked from 2 to 32 Days at Various Temperatures

Temperature of soaking in degrees C.	Length of soaking in days	Total larval hatch	Per cent larval hatch	Total parasite emergence	Per cent parasite emergence
5	2	99	86.8	13	92.9
5	4	169	71.0	20	62.5
5	8	18	43.9	17	51.5
5	12	10	12.8	24	70.6
5	18	16	19.1	5	100.0
5	24	98	36.8	14	60.9
5	32	0	0.0	4	57.1
10	2	278	73.0	19	100.0
10	4	206	71.7	8	100.0
10	8	49	44.0	24	85.7
10	12	11	18.3	9	60.0
10	18	0	0.0	2	100.0
10	24	0	0.0	1	50.0
10	32	0	0.0	3	75.0
15	2	109	94.0	13	76.5
15	4	152	86.0	13	92.9
15	8	0	0.0	12	80.0
15	12	0	0.0	2	40.0
15	18	0	0.0	0	0.0
No hatch from eggs soaked longer than 12 days at 15° C.					
20	2	151	74.8	42	76.5
20	4	0	0.0	14	70.0
20	8	0	0.0	1	100.0
20	12	0	0.0	0	0.0
No hatch from eggs soaked longer than 8 days at 20° C.					
25	2	2	12.5	8	100.0
25	4	0	0.0	0	0.0
No hatch from eggs soaked longer than 2 days at 25° C.					
No hatch from any eggs soaked at 30° C.					

of time of soaking. There are variations in percentages, of course, but these show no general tendency. Whenever any parasites emerged, the percentage of emergence was high. In some instances, the percentage had to be based on the emergence of a very few individuals, but this could not be avoided.

DESICCATION EXPERIMENTS

In Minnesota the eggs of *Malacosoma disstria* are subjected to desiccation as often as to prolonged soaking during the season of development preliminary to hatching. This series of experiments was designed to demonstrate the effects of desiccation under controlled conditions in the laboratory. All the egg masses were put into desiccators over calcium chloride maintained in the rearing chamber, where the temperature varied from 23° to 26° C. and the relative humidity, from 68 to 74 per cent. Fifteen

egg masses were removed after each period of 1, 2, 4, 8, 12, 16, 21, and 32 days of desiccation. Five of these masses were incubated immediately under the conditions of the rearing chamber mentioned above; five were incubated in desiccators over distilled water; and the remaining five were soaked in distilled water for 24 hours before being incubated under the conditions of the chamber. Thirty egg masses were used as checks, 10 masses being subjected without desiccation to each of the conditions described above.

Unfortunately, it was impossible to examine all of the eggs after the experiment had run its course to determine the actual percentage of emerged larvae and parasites, so the results given in table 8 are based on average larval hatch per egg mass and total parasite emergence. Quite apparently the eggs of the forest tent caterpillar are not very resistant to desiccation, being quite different in this respect from those of the tussock moth, *Hemerocampa leucostigma* Smith and Abbott, which Payne *(11)* states can be kept over calcium chloride for two years without losing their ability to hatch. Moreover, the effects of the desiccation are irreversible, as shown by the fact that after 12 days of drying, the larval hatch was greatly reduced even in those eggs which afterward were soaked or exposed to a saturated atmosphere. The effects of soaking and a high relative humidity show an advantage from this experience as the desiccation period is prolonged, but have little corrective value much after the time when normal hatching would have occurred.

The influence of desiccation on the rate of development elucidates the nature of the unfavorable effects of drying. Eggs incubated at the conditions in the chamber show a steady decrease in average time for hatch up to 8 days of desiccation. This means that development is taking place during the desiccation and at about the same rate as in those eggs which were not desiccated. The average time for hatch in the check eggs of this group was 8.0 days. After one day of desiccation, the time was 7.7 days; after 2 days of drying, 6.7 days; and after 4 days of drying, 5.3 days. If now we add to the average time for hatch after incubation the time the eggs were in the desiccators, the values for check, 1, 2, 4, and 8 days of desiccation become 8, 8.7, 8.7, 9.3, and 11.9 days, respectively. Except for the last, these figures do not vary beyond the normal, and it can be seen that development does take place at about the same rate during the early days, whether the eggs are subjected to desiccation or not. This means also that the larvae within the eggs are ready to hatch at about

Table 8. Average Hatch of Larvae and Total Parasite Emergence from Eggs Desiccated at 23°-26° C. from 1 to 32 Days

Duration of desiccation in days	Condition of incubation	Total larval hatch	Average larval hatch per hatched egg mass	Average time of larval hatch after incubation in days	Total parasite emergence	Average time of *Telenomus* emergence after incubation in days	Average time of *Telenomus* emergence plus time of desiccation
Check	68-74 per cent r.h.	504	56.0	8.0	43	38.2	.....
Check	100 per cent r.h.	467	46.7	8.5	64	34.8	.....
Check	soaked 24 hrs.	331	47.3	9.6	54	38.4	.....
	(Ten egg masses used in checks; five in all the others)						
1	68-74 per cent r.h.	263	65.7	7.7	20	40.4	41.4
1	100 per cent r.h.	199	49.8	9.0	1	.....	.....
1	soaked 24 hrs.	96	32.0	8.0	36	39.5	40.5
2	68-74 per cent r.h.	334	111.3	6.7	46	36.1	38.1
2	100 per cent r.h.	163	40.8	5.8	4	37.8	39.8
2	soaked 24 hrs.	20	20.0	10.7	22	40.1	42.1
4	68-74 per cent r.h.	156	52.0	5.3	21	32.6	36.6
4	100 per cent r.h.	337	94.3	4.6	22	32.6	36.6
4	soaked 24 hrs.	71	23.7	10.6	31	36.0	40.0
8	68-74 per cent r.h.	95	23.3	3.9	30	32.7	40.7
8	100 per cent r.h.	217	54.3	2.7	22	26.5	34.5
8	soaked 24 hrs.	73	36.5	2.6	21	34.4	42.4
12	68-74 per cent r.h.	2	2.0	5.5	37	30.1	42.1
12	100 per cent r.h.	57	14.3	3.2	37	25.8	37.8
12	soaked 24 hrs.	68	22.7	8.3	24	30.6	42.6
16	68-74 per cent r.h.	1	1.0	3.0	14	25.8	41.8
16	100 per cent r.h.	16	5.2	5.8	44	22.7	38.7
16	soaked 24 hrs.	54	13.5	4.4	18	29.4	45.4
21	68-74 per cent r.h.	0	0.0	.....	13	24.8	45.8
21	100 per cent r.h.	1	1.0	2.0	45	21.0	42.0
21	soaked 24 hrs.	3	3.0	2.0	38	27.7	48.7
32	68-74 per cent r.h.	0	0.0	.....	3	17.7	49.7
32	100 per cent r.h.	0	0.0	.....	16	12.1	44.1
32	soaked 24 hrs.	0	0.0	.....	11	16.7	48.7

the same time in desiccated as in undesiccated eggs. That they do not actually hatch at the same time is again the function of the properties of the chorion and also of the spumaline. The larvae, even though they have reached the hatching point, cannot bite through the brittle, dry chorion—or if they get through the chorion, the spumaline is so hard that they cannot penetrate it. Eggs were often observed with holes in them large enough for the larvae or parasites to get out but with the insects still in them.

As was pointed out in the discussion on the temperature and humidity experiments, desiccation beyond the hatching point results in a decreased hatch of the larvae. One or both of the same two possible conditions obtain in this experiment: either the larvae die from starvation and exhaustion in attempting to escape, or else they die from too great a loss of water. It seems likely that both these factors are operative.

Host-Parasite Relationships

The parasites again exhibit a reduction in rate of development under conditions of desiccation. The figures in table 8 show that the average time for emergence began to increase gradually but definitely after 12 days of desiccation. However, the parasites on the whole are much less seriously affected by prolonged desiccation than are the larvae, as can be seen from the table. The reason for this lies in the fact that the developmental period is much longer for the parasites than for the larvae. Most of the larvae in the check eggs reached the hatching point in from 6 to 9 days. The time for development of the earliest parasites to emerge from these eggs was 32 days. Since 32 days was the longest period of desiccation to which the eggs in these experiments were subjected, the parasites found conditions for emerging quite favorable within a few days at most after they had developed to the point of emergence. From this information it can be inferred that, at least so far as the parasites are concerned, the factor of loss of water was rather unimportant; and this suggests that perhaps the factors of starvation and exhaustion and not loss of water were primarily responsible for preventing the hatching of those larvae desiccated considerably beyond the time of their development to the hatching condition.

Summary and Conclusions

A study of the effect of temperature on the termination of an embryonic diapause of the forest tent caterpillar, *Malacosoma disstria*, has shown that the diapause lasts for about three months after embryonic development is completed. The hatching response after exposures to temperatures ranging from 25° to —5° C. suggested that the diapause could be broken only after conditioning at temperatures below 20° to 25° C. for a sufficient length of time and that optimum conditions prevailed near freezing. A histological study of embryos undergoing similar treatment showed first that the fore- and mid-gut were packed with yolk during the diapause and, what is more significant, that nearly complete absorption of the yolk was necessary before hatching took place. When the histological changes associated with yolk absorption were compared with hatching data it became evident that temperature had little effect on the diapause, and, instead, temperature influenced the course of developments after the diapause was broken. The differential hatching after exposure to both high and moderately low temperatures is believed to have been caused either by some injury from high temperatures or desiccation, or by a beneficial effect of low temperature during a critical period occurring when the early stages of yolk absorption were in progress.

A further study of postdiapause development showed that temperatures covering a range of 10° to 25° C. were nearly equally favorable for hatching, the optimum conditions being found in a saturated atmosphere at 15° C. A significant decrease in percentage hatch occurred at 30° C., and no larvae emerged even after several months of incubation at 5° C. Over the range of favorable temperatures, the best moisture conditions were found to lie between relative humidities of 70 and 100 per cent. Failure of the eggs to hatch in a dry atmosphere was the result of a change in the physical properties of the chorion as well as direct water loss through desiccation of the embryos.

The "spumaline," a term proposed to designate the colleterial secretion with which the eggs were covered, was found to be hygroscopic and absorbs considerable moisture from atmospheres of high relative humidities. Its principal function seems to be to prevent a rapid desiccation of the eggs in dry air and to conserve moisture which has been absorbed.

In experiments on the effects of soaking, the temperature at which eggs were soaked was found to be more important than

the length of time of submergence. The parasites were affected less adversely by soaking than were the embryos. Both embryos and parasites apparently are able to adjust themselves to a reduction in oxygen supply or to the accumulation of carbon dioxide. The spumaline on soaked eggs prolongs the effect of immersion after eggs have been removed from the water.

The eggs of the forest tent caterpillar are not very resistant to desiccation, for hatch was reduced considerably after eight days of drying over calcium chloride at 23° to 26° C., and no hatch took place after 32 days of desiccation. The egg parasites were considerably more resistant to desiccation. This experiment offered further evidence that the degree of hardness of the chorion is a determining factor in the escape of larvae from the egg.

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