

THE EFFECT OF LOW TEMPERATURES ON BRUCHUS OBTECTUS SAY, AN INSECT AFFECTING SEED¹

By WALTER CARTER,
Assistant in Animal Biology, University of Minnesota

INTRODUCTION

The common bean weevil, *Bruchus obtectus* Say, has been the subject of considerable study. This is probably due to its economic importance and the fact that it is a fairly simple insect with which to work. It can be readily propagated in large numbers, ordinary laboratory conditions constitute a normal habitat, and there is freedom from complications due to periodicity in its life cycle.

The investigation presented here covers two phases. The first is concerned with the general phenomenon of supercooling and freezing, as found in *Bruchus obtectus*. Working along the lines suggested by Bachmetjew (3)², attention has been given to the individual freezing of larvae, pupae, and adults. The second phase deals with the control of this insect by means of low temperatures. It is generally known that temperatures such as obtain in midwinter in the Northern States are fatal to *Bruchus obtectus*. The upper limits of these temperatures have not previously been worked out, although data on the effect of continued cold storage on the bean weevil have been recently published (10).

REVIEW OF LITERATURE

Much of the earlier work on the freezing of living tissues was done by botanists. An admirable résumé and bibliography of that work, together with a summary of the latest work on freezing of plant tissues, is given by Rosa (14). The outstanding worker on the subject of vital temperature in insects is Bachmetjew (1, 2). His first volume contains a review of the theories contributed up to his time, and then extensive data on supercooling and freezing considered from the standpoint of the following influences: Rate at which the insect was cooled, sex and development, hunger, repeating the act of cooling the lymph, lymph coefficient, and season. In an appendix at the end of his first volume is a figure of the apparatus used. His second volume was published later (1907) and gives but little attention to the subject of vital temperature.

Pirsch (13) reviews some of the earlier work on individual freezing of insect larvae, particularly with reference to bees. His citation includes references to the earliest users of the electrothermal method of taking insect temperatures.

¹ Received for publication July 18, 1924; issued September, 1925. Published with the approval of the director as paper No. 483 of the Journal series of the Minnesota Agricultural Experiment Station. The work on which this paper is based was done in the laboratories of the Division of Entomology, University of Minnesota, where all the apparatus mentioned herein is to be found. The writer gratefully acknowledges his indebtedness to the members of the staff. To Dr. R. N. Chapman, under whose immediate supervision the work was done, the writer is particularly indebted for constant guidance and much helpful suggestion.

² Reference is made by number (*italic*) to "Literature cited," p. 181.

Hine (7) reports observations on the effect of freezing insect larvae. Those of *Bellura obliqua* were subjected to temperatures below 0° F. and revived. He records that larvae were frozen solid and could be snapped in two, but the pieces were alive when thawed out. He also records observations on tomato hawk moth larvae. Those that had pupated before the first frosts came carried through normally; immature forms were frozen as the plant was frozen, those on the tips being killed with the first touch of frost while those on the lower parts of the plant were not killed until frost struck that part of the plant. Sir John Ross's account of his Arctic expedition is quoted by Hine. Ross records that *Larria rossi* withstood successive freezings of -40° F. Further references along the same lines are found in Houlbert (8, p. 255). This writer, quoting Justi, a worker in 1753, reports that larvae could be frozen so hard that they could be snapped in two like ice. If the frozen larvae were permitted to return to normal temperatures, then development proceeded normally.

Duval and Portier (4) found the temperature limits of *Cossus cossus* to be -22° C. They report larvae so hard as to be easily broken in two without water appearing at the point of section. They incline to accept Bachmetjew's conclusion that only the intercellular water freezes and the rest supercools.

The latest paper available to the writer is that of Knight (9) on the nature of the color pattern of *Perillus bioculatus* Fabr., which contains a table of data on the supercooling of that insect considered from the standpoints of season (involving the colloidal state of the lymph) and repetitions of freezing.

EXPERIMENTAL WORK ON SUPERCOOLING AND FREEZING

METHOD

The electrothermal method of taking individual temperatures was used. This method is described by Taylor (17), Harvey (6), and White (18). A portion of the apparatus is figured by Pirsch (13), but in the present study a mirror galvanometer was substituted. This provided an admirable indicator of high sensitivity. A modification of the method for taking individual temperatures will be described later.

In each case of individual freezing tabulated in Table I, a finely pointed thermocouple of constantan and copper was thrust into the insect at approximately the ventral middle. The temperature readings were taken at intervals while the insect was being cooled.

It was found best to avoid using thermocouples made of heavy wire, the best and most uniform results being obtained by the use of No. 36 wire. The only trouble encountered with this wire was short-circuiting due to the rapid wearing of the silk insulation. Erratic jumping of the galvanometer soon gave notice of this trouble. When heavy wire was used, even though it could be tapered to a very fine point by means of acid, considerable difference was found to persist between the temperature registered by the instrument and a control thermometer.

All the specimens used for individual freezing were taken from exactly the same environmental conditions, so that variables due to changes in environment were eliminated. The variables due to

stage (and therefore also probable differences in the colloidal character of the lymph) have been considered, as well as the influence of the rate at which the insect was cooled.

GENERAL DISCUSSION

The typical graph of an insect's reaction to temperature, both high and low, is given in great detail in Bachmetjew's second volume (2). Knight (10) gives a simplified graph, which includes the essential features with which the writer is concerned.

There is first a gradual lowering of the insect's temperature to a point designated by Bachmetjew as the "critical point." It is presumed that the freezing of the body fluids causes the typical rebound to a point which the same author calls the "freezing point of the lymph." Bachmetjew concluded that it was necessary to again cool the insect to its supercooling temperature, or "critical point," after the rebound had taken place, before death ensued. It has been the experience of the writer that one rebound was sufficient to cause death. This was also the experience of Pirsch (13) and Knight (9). Careful examination of the data on which Bachmetjew based his conclusions shows considerable discrepancy. If the freezing was hurried or incomplete it is possible that muscular contractions after the insect thawed out again might have given the impression that life still remained. The data presented in this paper show that there is also a distinct ante-mortem zone of muscular activity. From the purely physical standpoint it seems reasonable to conclude that crystallization results in a disruption of cell tissue which, while not necessarily fatal to a plant, is fatal to an insect. There is a possibility, however, that when a fairly large insect is frozen the disruption of cell tissue may be only local and not sufficient to cause death. This would be most likely in the case of an insect which has been impaled on a thermocouple point.

FREEZING EXPERIMENTS WITH BRUCHUS OBTECTUS

Table I gives the results obtained by freezing 25 individuals in each stage—adults, pupae, and larvae. The adults were taken out of the bean just previous to emergence. This stage was the most satisfactory from the experimental standpoint, since variables due to the more active metabolism, histolysis, and histogenesis, encountered in the more immature stages, were absent. The criterion of color was used to determine approximately the age of the pupae; the age of the larvae was determined by size.

TABLE I.—Data on freezing of *Bruchus obtectus*

UNEMERGED ADULTS

Temperature at start	Time taken to reach 0° C.	Time taken to reach super-cooling point	Rate at which insect was cooled	Super-cooling point	Re-bound point	Remarks
° C.	Seconds	Seconds	Degrees per minute	° C.	° C.	
2	3	18	0.63	-9.50	-7.75	
18	19	35	.55	-8.75	-5.80	
15	19	35	.56	-9.00	-7.20	
5	8	29	.40	-8.40	-5.85	
7.5	14	36	.45	-10.00	-6.50	
5	7	32	.36	-9.00	-6.75	
0	-----	23	.41	-9.50	-5.00	
17	26	50	.46	-11.00	-7.25	
14	20	43	.35	-8.00	-4.25	
15	28	47	.39	-7.50	-3.75	
8	13	34	.37	-7.75	-4.30	
3	15	40	.32	-8.00	-4.75	
10	14	35	.36	-7.50	-3.75	
15	14	40	.44	-11.50	-8.50	
13	15	36	.41	-8.60	-4.80	
9	7	25	.47	-8.50	-3.75	
12	-----	38	-----	-7.75	-5.30	
8	6	19	.58	-7.60	-4.50	
10	13	38	.29	-7.75	-4.25	
1	2	6	1.81	-7.25	-6.50	
13	18	43	.38	-9.50	-5.75	
6	14	33	.38	-7.25	-4.00	
10	12	28	.45	-7.25	-3.50	
12	-----	16	-----	-8.75	-7.25	
9.5	9	32	.50	-11.50	-7.75	
Average	-----	-----	-----	-8.68	-5.55	
PUPAE						
15	24	61	0.26	-9.50	-5.00	Cream-colored.
10	12	45	.29	-9.80	-5.25	Light brown; wings grayed.
8	11	33	.43	-9.50	-4.50	Do.
9	11	29	.46	-8.25	-3.75	Cream-colored.
15	15	40	.44	-11.00	-5.75	Do.
8	9	40	.38	-11.75	-6.00	Do.
10	14	36	.36	-8.00	-3.75	Cream-colored; wings grayed.
8	13	35	.36	-8.00	-4.00	Cream-colored.
10	15	37	.43	-9.50	-4.25	Do.
5	3	25	.44	-9.75	-5.00	Do.
0	-----	55	.23	-12.50	-6.25	Do.
-6	-----	23	.14	-9.25	-5.00	Red-brown; wings black.
12	17	45	.34	-9.60	-5.80	Cream-colored.
5	6	21	.52	-7.75	-4.80	Red-brown; wings grayed.
0	-----	28	.35	-9.70	-5.20	Cream-colored.
14	12	40	.32	-9.00	-5.75	Do.
8	18	40	.29	-6.50	-4.25	Red-brown; wings grayed.
10	25	57	.23	-7.25	-4.50	Cream-colored.
16	18	40	.39	-8.50	-5.25	Thorax black; wings black.
11	15	45	.33	-9.75	-6.75	Red-brown; wings gray.
16	20	40	.34	-6.75	-3.80	Cream-colored.
9	15	35	.35	-7.00	-4.50	Do.
-5	-----	15	.43	-11.50	-5.25	Do.
5	9	26	.53	-9.00	-6.75	Do.
0	-----	23	.50	-11.50	-7.00	Do.
Average	-----	-----	-----	-9.22	-5.12	

TABLE I.—Data on freezing of *Bruchus obtectus*—Continued

LARVAE

Temperature at start	Time taken to reach 0° C.	Time taken to reach super-cooling point	Rate at which insect was cooled	Super-cooling point	Re-bound point	Remarks
° C.	Seconds	Seconds	Degrees per minute	° C.	° C.	
15	19	48	0.35	-10.20	-7.20	Less than quarter grown.
15	20	48	.34	-9.50	-5.50	Full grown.
-2.5	-----	24	.36	-8.75	-5.50	Half grown.
3	8	39	.28	-8.80	-5.25	Full grown.
1	2	27	.35	-8.75	-6.60	Do.
15	30	62	.25	-8.00	-4.00	Two-thirds grown.
-3	-----	33	.28	-9.20	-5.10	Half grown.
9	11	31	.42	-8.40	-4.40	Full grown.
12	24	54	.28	-8.30	-6.75	Quarter grown.
10	14	60	.29	-13.25	-9.75	One-third grown.
7	10	52	.29	-12.50	-11.00	Quarter grown.
11	12	32	.50	-10.00	-7.50	Two-thirds grown.
-3	-----	14	.66	-9.30	-5.25	Do.
15	23	50	.32	-8.75	-6.10	One-third grown.
7	11	31	.45	-9.00	-6.50	Full grown.
14	15	48	.30	-10.00	-7.25	Do.
19	39	70	.40	-12.50	-8.80	Do.
3	2	8	1.96	-11.75	-8.60	Quarter grown.
16	20	70	.24	-12.00	-6.10	Full grown.
19	9	26	.66	-11.25	-7.75	Do.
10	4	9	2.00	-10.00	-5.00	Two-thirds grown.
-----	3	5	1.92	-9.60	-4.50	Half grown.
18	5	10	2.05	-10.25	-7.75	Do.
19	5	10	1.80	-9.00	-6.00	Do.
-----	3	7	2.31	-9.25	-4.75	One-third grown.
Average	-----	-----	-----	-9.53	-6.52	

The averages in Table I are brought together here for convenience of reference:

	Adults	Pupae	Larvae
Supercooling point.....	° C -8.68	° C -9.22	° C -9.53
Rebound point.....	-5.55	-5.12	-6.52

This shows a progressive lowering of the supercooling point from adults to larvae. The differences between these are slight, and may find their explanation in the metamorphic differences existing between these three stages. It is interesting to note that the supercooling point is depressed in the purely vegetative larvae and is highest in the adult. It should be noted, however, that in the case of the unemerged adults much greater uniformity of development is found than in any of the more immature stages. These first named were all obtainable at a point just previous to emergence. The pupae on the other hand, presented variations due to differences in the progress of histolysis and histogenesis, while the larvae were in all stages of growth, from very small specimens just big enough to contain the thermocouple point to full-grown larvae full of fat body.

Reference to Figure 1, B, will show that while the general trend of the curve is similar to those of Figure 2, A and C, the points are more widely separated. This suggests that individual differences among pupae are due to differences in *kind* while those of the larvae are dif-

ferences in *degree*. Stated in another way, this means that the larval period is one in which accumulation of material of the same kind and with the same properties goes on from the first establishing of the larva within the bean to its final maturity as a larva, while with the pupa histolysis and histogenesis produce changes in the chemical and physical constituents of the body contents, these changes varying with the age of the pupa. With the advent of the adult these changes are completed and the short period of quiescence before emergence simply permits of a hardening process taking place whereby the chitin becomes hard and brittle.

EFFECT OF INJURY ON THE INSECT'S RESISTANCE

Early in the course of the work the question as to the effect of injury on the resistance of these insects was recognized. Practically all the work of previous authors has been done with the thermocouple method which involves piercing the insect. Is the vital temperature so recorded any index of the capacity of the insect to resist low temperature? In order to test this, quiescent adults, pupae, and larvae

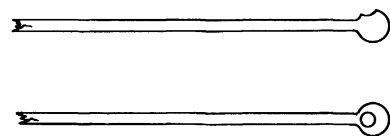


FIG. 1.—Diagram of glass tube used in freezing insects without injury due to piercing by thermocouple

were removed from the beans and put into a gelatin capsule through which the thermocouple point was thrust. The specimens were then uninjured and were lying around the couple point. The method was then modified in order to secure more rigidity by taking a piece of glass tubing, blowing a bulb at the end and then making a small hole in the bulb. The thermocouple was then thrust down until it was almost to the bottom of the bulb and the insects placed down in the bulb and close to the couple point. When several insects are so placed it so happens that one of them will "rebound," but the heat given off will be dispersed and only a mere indication of the rebound will be recorded by the instrument. Rebound under these circumstances then is simply an indication that inoculation has taken place and the amount is not significant (Table II).

TABLE II.—Experiments to determine the effect of injury on an insect's resistance

Super-cooling point	Rebound point	Stage	Number used	Result
° C.	° C.			
-13.60	-10.70	Pupal...	14	All died.
-17.00	None.	...do....	15	Lived.
-20.00	None.	...do....	3	Do.
-20.00	None.	...do....	3	Do.
-20.00	None.	...do....	3	Do.
-13.25	None.	Larval...	1	Do.
-16.50	None.	...do....	1	Do.
-16.00	None.	...do....	1	Do.
-20.00	None.	...do....	24	Do.
-17.00	Slight.	...do....	4	Three lived.*
-15.50	None.	...do....	1	Lived.
-17.00	None.	Adult ^b ...	10	Seven lived.
-17.00	None.	...do. ^b ...	15	Six lived.
-22.00	None.	...do. ^b ...	10	One lived.

* Of these four larvae one was slightly injured putting it into the tube and a thin film of its body fluid held it to the glass. The couple was not touching it. Only that larva died; the other three lived.

^b Unemerged adults.

Comparing these figures (Table II) with the averages for individual freezings quoted above, it becomes apparent that all these stages are capable of resisting lower temperatures when they are not injured. This may be due to the cuticula preventing the rebound or, in other words, preventing "inoculation." It may be that the presence of free water in the vicinity of the couple point may hurry inoculation in the case of the injured specimens. That an uninjured organism can better withstand extreme conditions than an injured one is a point that has been overlooked in much previous work.

When an insect is impaled on a thermocouple point and cooled, the temperature recorded is that of the lymph and broken-down tissue in the immediate vicinity of the point. It may be of some physiological significance that the freezing point of the impaled insect's lymph is not the same as that of the insect as a living uninjured organism.

The experiments described in the second part of this paper, when bean weevils in all stages were able to withstand temperatures as low as those shown to be the limit for injured specimens and for a greater length of time, seems to establish this point still further.

RELATION BETWEEN SUPERCOOLING AND REBOUND

Figure 2, A, B, and C, are dot charts wherein the supercooling temperatures have been plotted against the rebound points for each set of data on individual freezing. The charts suggest that there is a relationship between the two factors, so the Pearsonian method of arriving at the coefficient of correlation was used (12).

The correlations that exist between supercooling and rebound are thus found to be:

	Correlation	Probable error
Adults.....	+0.779	0.053
Pupae.....	+ .614	.084
Larvae.....	+ .890	.028

These high correlations indicate very definitely that the supercooling point bears a distinct relationship to the rebound point. Just what this relationship is, however, is another matter. According to chemical law (11, pp. 172-180) the rebound point should be the same no matter how low supercooling goes. If this law applies, then the explanation of this correlation must be sought elsewhere.

Knight (9) showed that when freezing *P. bioculatus* Fabr. a thermocouple placed near the insect registered a rise in temperature when the insect rebounded, showing that heat was given off to the surrounding air. This means, of course, that the thermocouple on which the insect is impaled does not register the total amount of heat of crystallization given off by the insect but that some is lost by radiation. These insects (*B. obtectus*) are very small, not more than 3 or 4 mm. long, so that their surface is very great in proportion to their mass. It follows then, that radiation to the air of the chamber must consume a considerable portion of the heat of crystallization.

Because radiation varies as the difference in temperature between the source of the heat and the surrounding medium, it is reasonable

to assume that the lower the temperature of the cooling chamber the more heat will be absorbed by radiation when the insect rebounds. The lower the insect supercools the lower will be the point to which the heat of crystallization available to the thermocouple will enable the temperature to rise, or, in other words, the lower will be the point of rebound. If this is true the correlation between supercooling and rebound points is simply due to the amounts of radiation being related to the temperature of the cooling chamber. Whatever the explanation may be, the fact of the existence of this correlation seems to have been demonstrated.

RELATION BETWEEN SUPERCOOLING AND THE RATE AT WHICH THE INSECT WAS COOLED

Bachmetjew expressed the belief that possibly there may be a relationship between the rate at which the insect is cooled and the supercooling point. His "abkuhlungsgewindigkeit" is described as a "shadow in the background."

Figure 3, A, B, and C, are dot charts wherein the supercooling points have been plotted against the rate at which the insect was cooled. In computing these rates the time taken to bring the insect from 0° C. to the supercooling point was used in order that the rates could all be calculated from a common starting point, as the initial temperature of the bath varied. The coefficients of correlation and the probable errors for the three stages are as follows:

	Coefficients	Probable errors
Adults.....	-0.095	0.138
Pupae.....	.190	.129
Larvae.....	+ .015	.134

It is thus seen that the rate of supercooling has no part in establishing the temperature of supercooling; the coefficient is less in each case than the figure required to establish even a bare relationship. It might be added here that the data for larvae offer the best opportunity for verifying this phase of the question. Previous to obtaining the latter half of these data the suction of an ordinary tap pump was used to cool the ether, and differences in rate were slight. Later, a suction pump was obtained which reduced temperature very rapidly, so that there is a much greater variation in the rates.

EXPERIMENTS ON THE UPPER LIMITS OF LOW TEMPERATURES FATAL TO BRUCHUS OBTECTUS SAY

HISTORICAL

Garman (5) subjected weevils to outside temperatures. These are presumably mean temperatures for the periods stated. His general conclusion was that a temperature of about 0° F. for a period of 24 hours was a complete control. According to the data of this paper the eggs are most resistant and the larvae least resistant. This writer suggests that the reason for the freedom of Canadian-grown seed from infestation is due to the inability of the weevils to survive the rigor of the winter. Sanderson (15, 16) discusses this subject in some detail for insects generally.

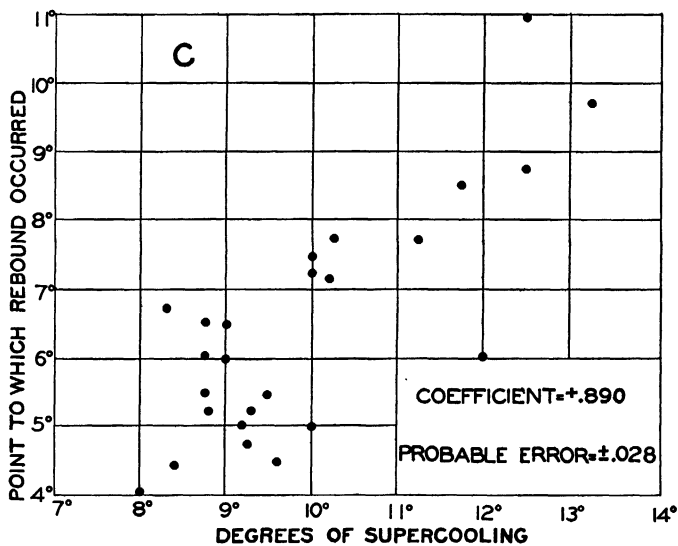
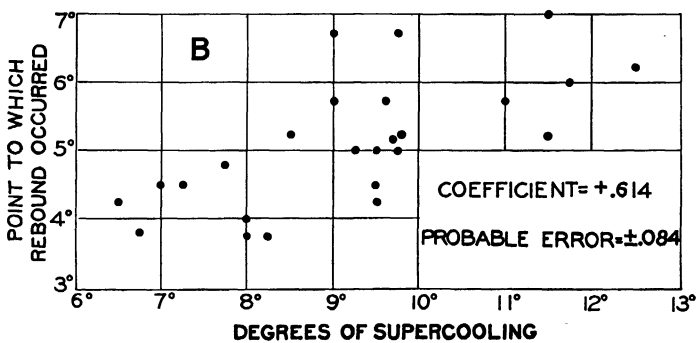
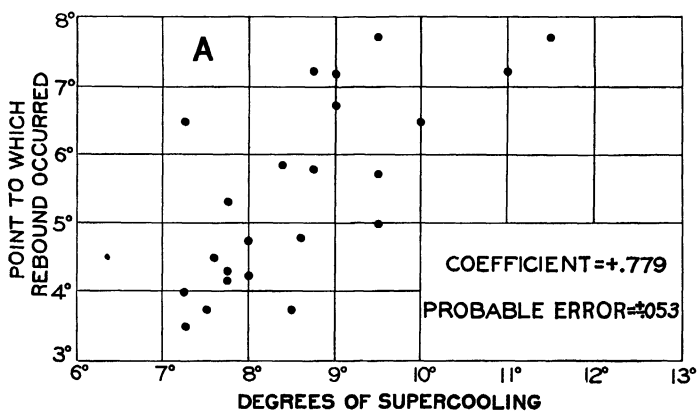


FIG. 2.—Relation between supercooling and rebound. A, adults; B, pupae; C, eggs

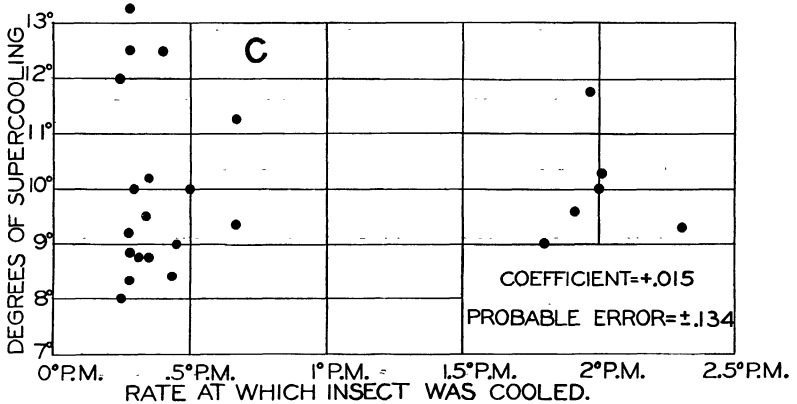
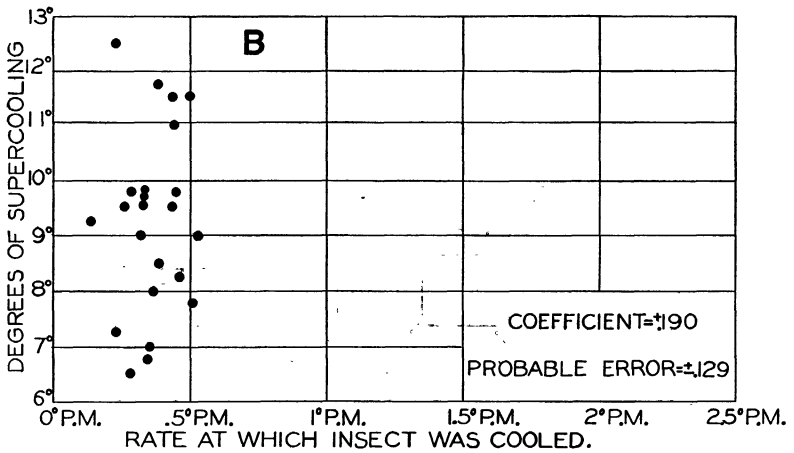
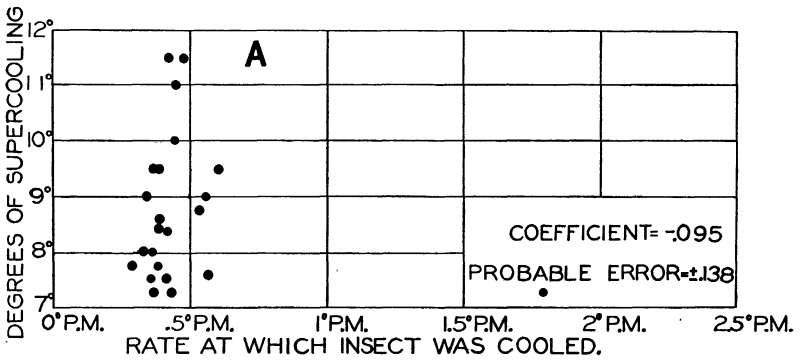


Fig. 3.—Relation between supercooling and the rate at which insect was cooled. A, adults; B, pupae; C, larvae.

Larson and Simmons (10) conducted an extensive series of experiments with commercial cold-storage conditions. They used three temperatures, 36, 32, and 20° F. Perfect control of the weevil infestation was obtained with 56 days' exposure to 32°. Loss of reproductive power was noted after exposure for 22 days or longer. Satisfactory control was obtained with 66 days' exposure at 36°. Unemerged adults were found to be the most resistant, pupae next, and larvae the least resistant. No experiments with eggs of *Bruchus obtectus* are recorded. It is suggested that weevils put into cold storage from lower temperatures obtaining in February could resist longer than those put in at the higher temperatures of November.

De Ong (3) used several species of insects in a study of cold-storage control. His findings are interesting here in that he reports that mature larvae were more resistant than any other stage studied. He also doubts the mortality of the egg at temperatures fatal to other more advanced stages.

METHOD

In these studies a constant-temperature cabinet was used with which it was possible to keep temperatures within less than 1° of the desired temperature for long periods. Glass vials containing beans infested with the several stages of weevil were placed in this cabinet and subjected to the temperatures as stated in the tables for the several periods.

A difficulty experienced in dealing with the immature stages of bean weevil infestation is that of knowing just which stage was being dealt with when the cavity first became visible from the outside of the bean. The cavity may contain either a pupa, prepupa, or even a larva. Another difficulty is in the fact that when beans are heavily infested with weevils in all stages the last larvae to enter the bean often perish through lack of food. This act rather tends to stress a larval mortality which may not be due to the conditions of the experiment. In order to avoid this colonies of known data were used for getting definite data on larvae. The most complete data, however, have been obtained from the use of beans where the weevils were showing black through the bean—in all cases unemerged adults—and those where the work of the weevil was visible from the outside, but the weevils were still cream-colored. The majority of these were early pupae, but larvae were sometimes found within these light-colored cells.

EFFECT OF FATAL TEMPERATURE ON THE APPEARANCE OF THE SEVERAL STAGES.

Eggs shrivel up and the contents become clear. It is not always easy to ascertain whether the eggs were killed by the temperature or were simply infertile. For this reason total mortality and hatching have been the criteria used. Pupae when fatally affected turn rotten and brown. The same is true of larvae. With unemerged adults later emergence was the criterion, and for active adults recovery.

An interesting condition met with was that called, for want of a better name, "arrested development." In this condition development proceeds for some time after return to normal temperatures, but the insect fails to mature and usually fails to complete the stage in which it was at the time of freezing. Pupae often continue their development until they are almost to the unemerged or quiescent

stage. The head and thorax and appendages can become complete, but the abdomen usually remains cream-colored, the insect dying at that point.

Unemerged adults show another phase of this condition. Normally the emerging beetles cut clean circles in the epidermis of the bean, but the treated unemerged adults lose the capacity to do this and endeavor to emerge by pushing through the center of the circle. They are found dead with part of the mouth parts showing through. In the upper limits of the zone of arrested development adults will continue development quite normally if assisted out of the bean. The effect here, then, seems to be an inhibiting of the normal response of the insect, resulting in death by imprisonment.

EXPERIMENTS ON TEMPERATURES FATAL TO *B. OBTECTUS*

Table III gives the results of experiments on the several stages, and following it are Figures 4, 5, and 6, in which the data of the experiment have been incorporated. The table has been condensed, but all the data are given in the figures.

TABLE III.—Limits of fatal temperature-time zones of *Bruchus obtectus*
ACTIVE ADULTS

Temperature	Time	Result	Temperature	Time	Result
°C.	Hours		°C.	Hours	
-2	168	Lived.	-14	1	8 lived; 13 killed. Muscular activity, then death.
	24	Do.		2	
-7	36	Do.	-14	3	Do.
	48	1 lived.		4	Killed.
-8	24	Lived.	-20	5	Do.
	30	Killed.		2	Do.
-9	36	Do.	-20	3	Do.
	1	Lived.			
	.5	Do.			
	1	Do.			
-10	2	18 lived; 10 killed.			
	3	Muscular activity, followed by death.			
	4-11	Do.			
	12	Killed.			
	13	Do.			
UNEMERGED ADULTS					
-8	24	Lived.	-14	1	Lived.
	36	Killed.		2	Killed.
-10	1	Lived.	-17	24	Do.
	2-6	Do.		-20	.25
	7	Killed.			.50
	8	Do.			
PUPAE AND ADVANCED LARVAE					
-2	168	Lived.	-14	1	Lived.
	24	Do.		3-9	Killed.*
-8	36	Do.	-17	24	Do.
	48	1 lived.		36	Do.
-9	56	Killed.*	-20	.5	Lived.
	60	Do.		2	Killed.*
-9	1	Lived.	-20	4	Do.
	1	Do.		6	Do.
-10	3-7	Do.			
	8	Killed.*			
	9-16	Do.			
	22-23	Do.			
	25-30	Do.			
	32-34	Do.			

* Cases where death followed post-freezing development.

TABLE III.—Limits of fatal temperature-time zones of *Bruchus obtectus*—Con.

LARVAE OF KNOWN AGE

Temperature	Time	Result	Temperature	Time	Result
°C.	Hours		°C.	Hours	
-2	168	Lived.	-14	2	Killed.
-8	36	Killed.	-17	56	Do. *
-9	1	Do.	-20	24	Do.

EGGS					
-7	24	Hatched.	-17	56	Killed.
	48	Do.		48	Do.
-8	56	Do.		1	Hatched.
	60	Killed.	-20	4	Do.
-10	4	Hatched.		5	Killed.
	16	Do.			
	2	Do.			
	5	Killed.			
-14	6	Hatched.			
	6	Killed.			
	7	Do.			

* Cases where death followed post-freezing development.

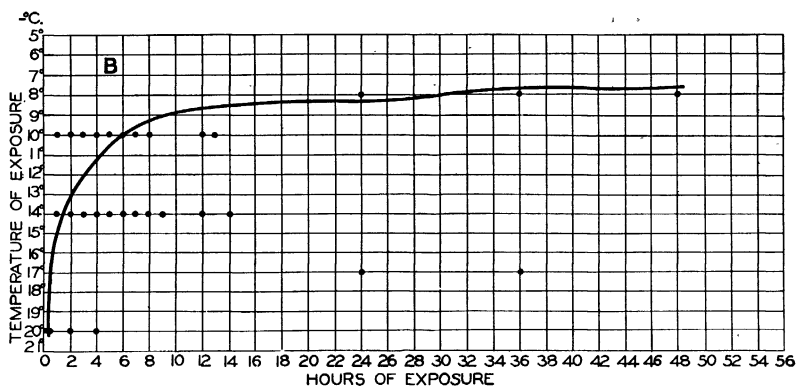
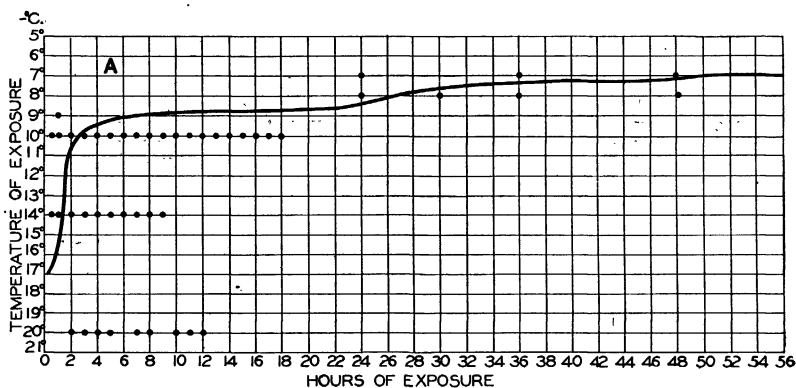


FIG. 4.—Limits of fatal temperature-time zone. A, active adults; B, unemerged adults

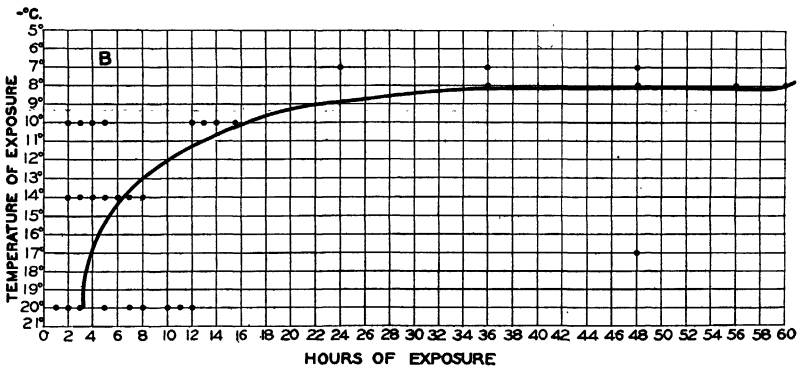
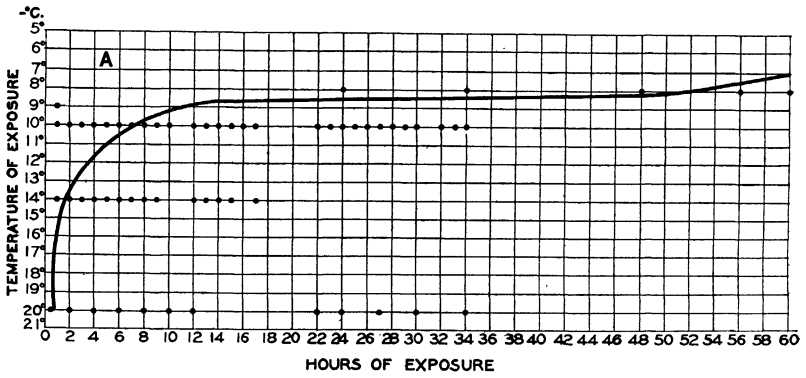


FIG. 5.—Limits of fatal temperature-time zone. A, pupae and advanced larvae; B, eggs

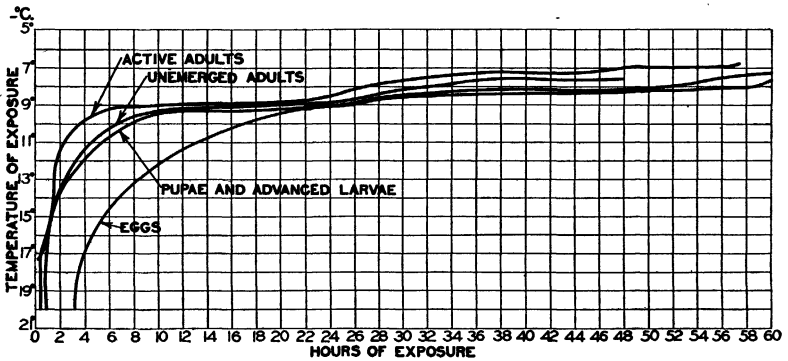


FIG. 6.—Fatal temperature-time zones for *Bruchus obtectus* Say. Active adults; unemerged adults; pupae and advanced larvae; eggs

From these data it is seen that active adults are slightly more susceptible to low temperatures. Unemerged adults and the stages designated as "pupae and advanced pupae" have curves practically similar. The slight difference is in favor of the latter. The eggs of *Bruchus obtectus* can apparently withstand lower temperatures than any other stage. With respect to eggs, however, the criterion used

was whether or not the eggs hatched, not the ultimate development of the insect.

These data further show that at temperatures down to -8° C. a much greater length of time must elapse before fatal results ensue. At that temperature all the curves flatten out at about the 24-hour period. In passing it should be noted that this temperature is approximately the one to which injured specimens supercool in the ether bath used in the electrothermal method.

The highest temperature used, -2° C. for one week, was not fatal to any stage, and Larson and Simmons (10) have shown that a much longer period than one week is necessary at 0° C. to insure a complete kill. The curve showing the limits of the fatal temperature-time zone is therefore very long and with a very gradual slope in the upper 8 degrees of below 0° C. temperature.

It is evident, however, that for lower temperatures a much shorter time is necessary. Referring to the curves again, it is seen that there was no survival at an exposure of -20° C. for a period of five hours, and even this period applies only to the hatching of eggs. Unemerged adults and pupae could withstand only a very short period at this temperature, probably only that period required to permit penetration of the temperature into the bean. Adults died at an exposure of -17° for less than one hour, while one hour at -14° was fatal to them.

From Table III it is seen that pupae and advanced larvae continue their development even after considerable periods of exposure. This is of much interest from the standpoint of physiology, but since such development does not continue long enough to permit the emergence of the insect, the curve has been drawn between the last points of survival and the first points indicating death without regard to whether there was any period of later development or not.

The data for larvae of known age are not as complete as those for the other stages. Table III indicates, however, that the mortality zone is the same as for unemerged adults and pupae and advanced larvae. As sets of vials containing larvae of several ages were used each time, it was possible to show that there was no difference in power of resistance between very young larvae and those that were well advanced, so that the mortality zone for pupae and advanced larvae can safely be applied to larvae of less development.

Larson and Simmons (10) suggest that the temperature at which the colony of weevils was maintained prior to freezing has some effect on resistance. To verify this a colony was maintained at approximately 0° C. for one week in a commercial cold-storage plant. At the end of this time adults, unemerged adults, and pupae were subjected to a temperature of 14° . The results indicate that no hardening had taken place. Both active and unemerged adults were killed by exposure for one hour, while exposures of two hours killed the pupae and advanced larvae. An exposure of seven hours killed pupae and advanced larvae at -10° . These results are essentially the same as with weevils taken from room temperatures.

SUMMARY

The common bean weevil, *Bruchus obtectus*, Say, has been used for experiments on supercooling and resistance to low temperature. Bachmetjew (2) has made the most extensive contributions to the subject of vital temperature in insects, and this study follows in a general way the lines laid down by him. While he believed that an insect's temperature must be lowered again to its supercooling point after a rebound has taken place before death ensued, subsequent works show that an insect is killed if a rebound occurs. A rebound is evidence that heat of crystallization is given off. Crystallization of the lymph is believed to be responsible for the death of the insect.

Comparing differences between individuals, it is found that pupae show more variation from the average trend. This is explained in the fact that pupae of all ages were used, and the physical properties of their lymph varied.

It has been seen that larval, pupal, and adult weevils could withstand lower temperatures when cooled uninjured than when the point of a thermocouple was thrust into them. Active adults withstood temperatures of -10° C., a temperature below the average supercooling point, for a period of three hours. Pupae and advanced larvae withstood the same temperatures for seven hours.

It has not been found possible to obtain the exact freezing point of *Bruchus obtectus* with present-day electrothermal methods.

There is a distinct correlation between the supercooling point and the rebound point, but this is believed to be due to radiation from the insect to the air of the cooling chamber. The exact freezing point is actually much higher, then, than the rebound points recorded.

The temperature-time experiments show that time is a factor in the resistance of *Bruchus obtectus* to low temperature. It is apparent, with freezing points as high as those of this insect must be, that supercooling takes place at temperatures of -10° C. or below. That being the case, the data imply that supercooling is possible for limited times only at certain temperatures.

The relationship between supercooling and the rate at which the insect has cooled has been studied. There seems to be no correlation between these two factors.

A condition of post-freezing development with ultimate death has been observed at temperatures just below the limits of resistance. This has been referred to in this paper as "arrested development." It manifests itself in the adults as failure to emerge, due to the loss of the insect's capacity to cut its way out of the bean; pupae can metamorphose only to adult form in the head and thoracic regions, the abdomen remaining undeveloped; larvae are unable to emerge from the egg, or, if they do emerge, it is often through the side of the egg, not through the operculum. Many that emerge from the egg are unable to enter the bean.

The limits of the fatal temperature-time zones have been determined for four stages of *Bruchus obtectus*. This insect apparently has no capacity for hardening.

The thermal constant, as described by Sanderson and Peairs (16), has not been worked out for this insect. A difference of less than 10° F.—the difference obtaining between the temperature of the laboratory and a cool, dry basement at about 64° —almost doubles the

length of time required for a complete life cycle. This means that even in the South development of the insect could be retarded considerably until fatal outdoor temperatures prevailed.

CONCLUSIONS

Bruchus obtectus Say dies if the heat of crystallization is given off. A definite correlation exists between supercooling and rebound, which is believed due to radiation. The electrothermal method does not give the true freezing point of an insect because of radiation and the effect of injury on the insect. There is no correlation between supercooling points and the time required to bring the insect to these points.

The capacity of *Bruchus obtectus* to resist low temperature is limited by the length of time it can remain in the supercooled condition. This capacity varies with the stage, active adults being least resistant and eggs most resistant.

Growth of *Bruchus obtectus* in beans can be materially retarded by storage in temperatures even as high as 64° F. Temperatures of below -10° C. for a period of 12 hours are fatal to all stages of this insect.

LITERATURE CITED

- (1) BACHMETJEW, P.
1899. ÜBER DIE TEMPERATUR DER INSEKTEN NACH BEOBACHTUNGEN IN BULGARIEN. *Ztschr. Wiss. Zool.* 66: 521-604, illus.
- (2) ———
1901-07. EXPERIMENTELLE ENTOMOLOGISCHE STUDIEN. 2 v. Leipzig and Sophia.
- (3) DE ONG, E. R.
1921. COLD STORAGE CONTROL OF INSECTS. *Jour. Econ. Ent.* 14: 444-447.
- (4) DUVAL, M., and PORTIER, P.
1922. LIMITE DE RÉSISTANCE AU FROID DES CHENILLES DE COSSUS COSSUS. *Compt. Rend. Soc. Biol. [Paris]* 86: 2-4.
- (5) GARMAN, H.
1917. OBSERVATIONS AND EXPERIMENTS ON THE BEAN AND PEA WEEVILS IN KENTUCKY. *Ky. Agr. Exp. Sta. Bul.* 213: 309-333, illus.
- (6) HARVEY, R. B.
1919. IMPORTANCE OF EPIDERMAL COVERINGS. *Bot. Gaz.* 67: 441-444.
- (7) HINE, J. S.
1908. SOME OBSERVATIONS CONCERNING THE EFFECTS OF FREEZING ON INSECT LARVAE. *Ohio Nat.* 8: 258-260.
- (8) HOULBERT, C. V.
1920. LES INSECTES. Ed. 2, 374 p., illus. Paris.
- (9) KNIGHT, H. H.
1923. STUDIES ON THE LIFE HISTORY AND BIOLOGY OF PERILLUS BIOCULATUS FABR., INCLUDING OBSERVATIONS ON THE NATURE OF THE COLOR PATTERN. *Rpt. State Ent. Minn. (1922)* 19: 50-96, illus.
- (10) LARSON, A. O., and SIMMONS, P.
1924. INSECTICIDAL EFFECT OF COLD STORAGE ON BEAN WEEVILS. *Jour. Agr. Research* 27: 99-105.
- (11) MORGAN, J. L. R.
1908. THE ELEMENTS OF PHYSICAL CHEMISTRY. Ed. 4, 539 p., illus. New York and London.
- (12) PEARSON, K.
1897. REGRESSION, HEREDITY, AND PANMIXIA. *Phil. Trans. Roy. Soc. London (A)* (1896) 187: 253-318.
- (13) PIRSCH, G. B.
1923. STUDIES ON THE TEMPERATURE OF INDIVIDUAL INSECTS, WITH SPECIAL REFERENCE TO THE HONEY BEE. *Jour. Agr. Research* 24: 275-288, illus.

-
- (14) ROSA, J. T., JR.
1921. INVESTIGATIONS ON THE HARDENING PROCESS IN VEGETABLE PLANTS.
Mo. Agr. Exp. Sta. Research Bul. 48, 97 p.
- (15) SANDERSON, E. D.
1908. THE INFLUENCE OF MINIMUM TEMPERATURES IN LIMITING THE
NORTHERN DISTRIBUTION OF INSECTS. Jour. Econ. Ent. 1: 245-262,
illus.
- (16) ——— and PEAIRS, L. M.
1913. THE RELATION OF TEMPERATURE TO INSECT LIFE. N. H. Agr. Exp.
Sta. Tech. Bul. 7, 125 p., illus.
- (17) TAYLOR, G. F.
1920. SOME IMPROVEMENTS ON THE NEEDLE TYPE THERMOCOUPLE FOR
LOW TEMPERATURE WORK. Jour. Indus. and Chem. Eng. 12: 797-799,
illus.
- (18) WHITE, W. P.
1910. THE THERMOELEMENT AS A PRECISION THERMOMETER. Phys. Rev.
31: 135-158, illus.