MONTANA STATE UNIVERSITY

Some experiments on the freezing and hardening of the adults of the Colorado potato beetle, Leptinotarsa decemlineata say
by Reginald Wilson Salt

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the Degree of Master of Science In Entomology
Montana State University
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Abstract:
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SOME EXPERIMENTS ON THE FREEZING AND HARDENING OF THE

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Leptinotarsa decemlineata Say.

by

REGINALD W. SALT

A THESIS

Submitted to the Graduate Committee in partial fulfillment of the requirements for the Degree of Master of Science in Entomology at Montana State College

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SOME EXPERIMENTS ON THE FREEZING AND HARDENING OF THE ADULTS OF THE COLORADO POTATO BEETLE, *Leptinotarsa decemlineata* Say.

INTRODUCTION

Only during the past few years have entomologists devoted much time to the details of the freezing and hardening of insects, with one notable exception. Reamur (1736)* published an account of his experiments on the freezing of insects, using his newly invented thermometer. The translation of this account is included for reference in this paper in order to show the remarkable clearness, accuracy and value of Reamur's experiments. His conclusions, two centuries old, are now considered much nearer the truth than many theories which have been proposed only recently.

The literature on the subject is not voluminous, and only a small part of it is the result of fundamental research. The parallel subject of the freezing and cold-hardening of plants is much older and more advanced. The writer's interest in the subject developed from a consideration of the measurements of water-binding in insects. This subject appears to be founded on so many unstable assumptions that it was thought necessary to go back and try to find out just what physical and physiological processes are involved in the freezing and hardening of insects. The following work is the result of this attempt to learn anything at all about this extremely complicated subject.

The writer wishes to acknowledge his gratitude to Dr. A. L. Strand

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*-Reference is made by author and year to Literature Cited.

for the proposal of the problem and for constant help and advice during its development; and to Dr. W. McK. Martin, Prof. G. A. Mail, Dr. R. M. Melaven, Dr. A. J. M. Johnson, and Prof. W. D. Tallman. for helpful criticisms and assistance.

REVIEW OF LITERATURE

The subject of cold-hardiness and the freezing of insects has developed from a theoretical and practical study of insect hibernation. The history of these studies is presented by Payne (1926) in a short article in which she mentions such important developments as Reamur's recording (1736) of the fatal temperature of wood-boring larvae with his newly invented thermometer; Kirby and Spence's work (1818) on the hibernation of bees; Vaudoner's discovery (1827) that some insects displayed periodicity and could hibernate in the presence of high temperature and abundant food; Nobili and Melloni's (1831) use of the thermocouple to determine the temperature of insects; and Scudder's discussion (1887) of the subject in his "Butterflies of Eastern United States and Canada". Reamur's contributions are so important that a translation is included here.

"Memoirs Pour Servir A L'Histoire Des Insects. by M.de Reamur
(Translation of pages 140-147, Vol.2:1736)

"While the larvae are very small, in spite of the various layers which make up their nests, they remain quite exposed to the rigors of winter. For after all, a nest attached to branches which no longer have leaves, and about which the air circulates freely on all sides, ought not to be long in acquiring in its interior the same degree of coldness of the air surrounding it. These extremely small larvae, then, which thereby seem to be very delicate, must therefore be strong enough to resist the cold. I have been curious to find out what degree they could resist, and above all, what degree of cold was capable of killing them. There was at least a small consolation, while winter makes us feel a very severe cold, of knowing that it saves us from insects which are multiplying too fast, and which would have defoliated our trees in the spring and to the end of the summer. But the experiences that I have had have taught me that we have nothing to hope in this country for the destruction of this kind of caterpillar by the cold of our worst winters, since they are in a state of resisting a greater cold than that of 1709.

"We know how to make ice in any season, by surrounding with ice mixed with salt the thin vessel in which is the water one wishes to freeze. Physicians know also that the degree of cold that one can produce by suitable mixtures of ice and certain salts, is much superior to the degree of cold of water which is beginning to freeze. The thermometer of which I described the construction in the Memoires of the Academy in 1730 ought to go down as far as the greatest cold of 1709, about 14 1/4 degrees below the point where the freezing of water begins (-17.9°C.). Toward the end of February and during the first days of March, I placed a thermometer in the middle of a mixture of crushed ice and sea-salt; the liquid of the thermometer dropped to 15 degrees (-18.8°C), i.e., about 3/4 of a degree below the point where the greatest cold of 1709 would have made it drop. At the same time that I sank my thermometer into this mixture of salt and ice, I immersed there a small glass tube in which I had placed seven or eight of our small larvae; it was closed at the lower end, and its upper end which was above the ice, was open; I left it there nearly half an hour. When I took the small larvae out of the tube in which they had suffered excessive cold, they appeared dead. I warmed them little by little, beginning by placing them in ordinary ice; in a quarter of an hour they were in such a state that I could see that they were alive: they stirred and walked.

"The next day I put them to a still more rigorous test; I surrounded the glass tube in which I had put them with a mixture of ice and rock-salt which made the liquid in the thermometer drop to more than 17 degrees below freezing (-21.25°C.). In this second trial, the larvae had then to resist a degree of cold nearly three degrees greater than that of 1709; it killed none of them. The sudden passage of air sufficiently tempered (for when I carried on these experiments the liquid of the thermometer was about eight or nine degrees above freezing (10-11°C.), the passage, I say, of air, tempered by air of such excessive coldness, should be for them a much more rigorous test than that of the same coldness of longer duration, which would become such only by successive accumulations made during a great number of days, as happens in winter. Also I have made these larvae sustain a cold of 19 degrees without having them perish.

"Lister has already remarked that caterpillars are in a state of resistance to very great cold; he reports that he has found them stiff with ice, and so rigid that in dropping them in a glass they made a noise like that which would be made by a small stone, or a small stick which is dropped; that in this state, however, they were alive, and that they had given incontestable proof when he had warmed them, that they had walked. This was a great astonishment; if an insect whose blood, of which all the liquids had been frozen, came back to life, this was a true resurrection; for since all circulation, all movement of the liquids are stopped, the animal is a dead animal; at least, we have no other conception of the state of death. I believed it ought to be proved if the caterpillars whose liquids have actually been frozen, come back to life, as it were. Our common caterpillars are not the only ones on which I have made these tests. I wished to know if those of other species had the ability to resist such a great cold. One of those whose resistance against cold I wished to test was the Pine caterpillar, of which we shall speak soon; and of those which were hatched and raised on this species of tree in the vicinity of Bordeaux. I put several of them in a glass tube and made them suffer, like the common ones, a cold of 15 degrees below freezing (-18.8°C.). When I took them out of the tube they were stiff, hard as a stone, or like harder ice. I broke several of them as one breaks a soft stone; their whole inside was completely frozen; also I re-heated those which I left whole; they did not come back to life; they were too well dead.

"A degree of cold much less than that which affects the common ones is sufficient, therefore, to kill those of the Pine. In other experiments, a degree of 10-11 degrees of cold (-12.5to -14°C.) was sufficient for the latter ones. I have taken from the tube which had attained 8 or 9 degrees of cold (-10 to -12°C.), some which were already quite hard, which upon falling into a porcelain cup, made quite a noise; and which after having been held for some time in a temperate atmosphere, gave signs of life, and soon regained their former vitality. But these larvae had not been frozen completely. Although they had a certain amount of rigidity when taken from the tube, they still had a degree of elasticity. Places pressed gave way under the finger, which did not happen in the case of those which were completely frozen, and which died. Perhaps even, that the little stiffness which they had, only came from vapor which was frozen around them; a vapor similar to that which freezes on the outside surface of the vessel which contains the mixture of salt and ice.

"What is certain, is that I have never seen larvae which were really frozen, whose liquids had turned to ice, which were not killed. Starting when all movement of their liquids ceased, they were perfectly dead caterpillars, just as any other animal in a similar case would be a dead animal. But there remain always these peculiar facts, that in spite of the small amount of heat in the body of certain species of caterpillars, however delicate they might seem, because they are extremely small, the liquids which fill their bodies cannot be frozen by a degree of cold considerably more than that of our hardest winters. That there are species of caterpillars much larger, and in appearance much stronger, whose liquids can be frozen by a degree of coldness much less than that which does not affect the liquids of others. The kind of blood, the liquids which circulate in the vessels of different species of caterpillars, are therefore in comparison to others as with alcohol; or a very strong brandy compared to a very weak brandy. The latter will be hardened, reduced to ice, by a degree of cold much less than another degree of cold, under which a very strong brandy will all remain as a liquid.

"It is known that movement of water is an obstacle to freezing; quiet water, that of a ditch or pond, freezes, while the water of a river remains a liquid; the more rapid the current, the less chance of solidifying. If the circulation of the liquids of our small common larvae were more rapid than the circulation of the Pine caterpillars, from that alone, it must take more cold to fix the first ones in their canals than to fix the second ones in theirs; but this consideration has little or no part in the effect we are considering. I cut off the head of three of our small caterpillars; I put them in a glass tube with others of their kind which were alive and healthy; I lowered the tube into a mixture of ice and salt which made the liquid of the thermometer drop to 15 degrees below freezing (-18.8°C.). When I took the caterpillars out of the tube, those which had had their heads cut off were pliable and soft like the others; their liquids had not been frozen. From whence it follows that these liquids do not need to be in the movement of a rapid circulation in order to conserve their fluidity against a degree of cold of 15 degrees below freezing (-18.8°C.). We are not surprised that of the inflammable or spirituous liquids, and of the liquids charged with salts which resist very great cold without freezing, we have hundreds and hundreds of examples; but it ought to appear to us very peculiar that a liquid which is not at all inflammable, which seems to us very insipid and quite watery, that such a liquid, I say, as the blood of some species of caterpillars, can preserve its fluidity in spite of great cold. That liquid is not, then, so simple that we judge it by the same standards we usually use to discover the nature of liquids.

"The blood of large animals, birds, quadrupeds, and ourselves, easily coagulates; besides, they are more easily frozen than the blood of insects. The blood of a pigeon, which was made to flow warm into a glass tube, was reduced to hard ice by a degree of cold of 7 or 8 degrees below freezing (-9 to -10°C.), and could have been frozen by a less cold. The blood of a lamb sustained three degrees of cold (-3.75°C.) without freezing, but a cold of 5 degrees (-6.2°C.) converted it into ice. Large animals have in their bodies a heat and a principle of heat which is not found in those of insects. Big animals, then, have no need of having a blood which freezes as difficultly as that of insects.

"Whoever made insects seems also to have constituted their blood differently according as they are exposed to endure greater or less cold. We have seen, besides, that numbers of species of insects, after having lived in the form of caterpillars, pass the whole winter in the form of chrysalids, and that there are chrysalids which during this harsh season are attached to walls, eaves of houses, and leaves of trees; and which are 'awarmed' there, i.e., they are not covered by a cocoon, be it of silk or some other material. Such is the chrysalis of the most handsome of the cabbage caterpillars, and such are numbers of other chrysalids of the kind which have the industry to suspend themselves by means of a band of silk threads. I have subjected several of these chrysalids to very great degrees of cold, cold of more than 15 to 16 degrees below freezing (-18 to -20°C.), without their freezing. We know that other chrysalids pass the winter down in the ground; there, they are not exposed to as great a cold as they are in any part of the air. I have subjected to a cold of 7 or 8 degrees below freezing (-9 to -10°C.) several of those which stay underground; it was sufficient to make them perish. Thus the insects which remain exposed to great cold are in a position to withstand it. Those which are more sensitive to the impressions of cold act as if they foresaw what would take place during the winter on the surface of the ground, and which they could not resist. I say that they act as though they foresaw, because it is not the approach of winter or the actual cold which causes them to enter the ground; we have seen that there are caterpillars which burrow in July and August, and still others in the early spring. A short time after having entered the ground, they transform into chrysalids; and it is not until the following year that the butterfly leaves its chrysalis."

Uvarov (1931) gives an excellent review of the subject, in which is included a table listing the effects of extreme low temperatures on about thirty insects of various orders and stages, with the references. In his discussion of the theories of cold resistance in insects, Uvarov starts with Reamur's explanation in 1736 of the resistance of an insect to apparently complete freezing. Reamur explained that while an insect may appear to be completely frozen, there remain some fluids in the body which freeze at a much lower temperature, and that death occurs only when all of the fluids are frozen. This viewpoint, considerably older than that of Bachmetjew, is nevertheless much more accurate.

Although Bachmetjew (1901) made a few technical errors in his work which led to false conclusions, his work is considered monumental. He based his theory of cold resistance in insects on observations of their body temperature by means of the thermoelectric method. His observations showed that an insect can be cooled gradually to a low "critical point", about -10°C. At this point, which is called the under-cooling point by present-day workers, ice begins to form in the tissues and the temperature rises due to the liberation of heat of fusion. The point to which the temperature rises is not designated by any particular name by Bachmetjew, but it is, of course, below 0°C. This point will be referred to in this paper as the "rebound point". It has been erroneously referred to as the "freezing point" by several authors, but this nomenclature will later be shown to be wrong. Further gradual cooling causes the insect to become frozen quite hard. In this latent or anabiotic state no metabolic processes are possible, but the insect can be reanimated by warming. It is only when the insect is cooled to a certain "fatal point" that it is killed, and Bachmetjew places this point at the same temperature level as the "critical point". The theory is based on the purely physical conception of the supercooling of fluids, but it has no bearing on the freezing of insects, a fact which has been demonstrated not only by later workers, but by much of Bachmetjew's own data. A graphical representation of Bachmetjew's conception is given in Fig. 1.

He considered that the "critical point" depended on several conditions: (1), the velocity of cooling; (2), the development and sex of the specimen; (3), the nutritional state of the insect; (4) the repetition of cooling; (5), the time of exposure, and (6), the "sap coefficient".

Bachmetjew's theory was severely criticized soon after its appearance by Kodis (1902), according to whom the super-cooling of body fluids has nothing to do with the freezing of water in the protoplasm, with which the fatal effect is connected. Unfortunately this criticism escaped notice and was ignored by Bachmetjew in later works in which he repeated and developed his views.

Maximov (1913) offered the following serious criticism of Bachmetjew's theory. By determining the quantity of ice formed in the pupae of _Celerio euphorbiae_ L., Bachmetjew found that all the fluids in them froze completely at -4.5°C. while the critical point was -10°C. These figures seem to support the theory. However, the specific heat of the frozen pupae within the limits -6.7 to -16.3 was found by Bachmetjew to be 0.917, very little lower than that of the body fluids, (1.01). Since the specific heat of ice is only half that of water, Maximov concluded that freezing was _not_ complete at -4.5°C. The same conclusion is reached by considering the fact that of the 4% salt content

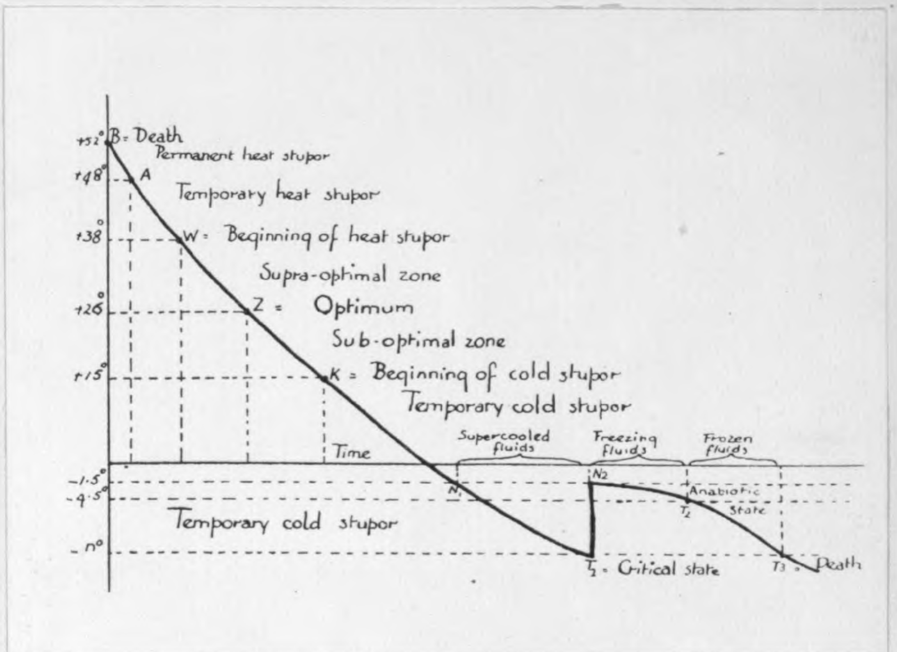

Fig. 1. Zones of vitality and death of an insect.
(Re-drawn after Bachmetjew, 1907)

of insect blood, about 80% is sodium chloride, which has a eutectic point of about -22°C. Thus, even if no other salts were present, the body fluids could not possibly freeze completely at -4.5°C. Recent experiments by Sacharov (1928, 1930) show that it is impossible to freeze completely the body fluids of certain insects even at -21.2°C. Payne (1927) found that complete freezing occurs only in the neighborhood of -40° or lower.

Uvarov points out that Bachmetjew's principal mistake lay in his regarding the body fluids of an insect as a homogeneous liquid which follows the relatively simple physical laws of supercooling and freezing. This viewpoint is obviously incorrect since apart from dissolved electrolytic substances, there are insoluble fats, proteins, and other colloids which are bound to affect the physical properties of the fluids. Bachmetjew's theories, however, though superseded, are widely known, and are still repeated by many writers.

More recent work along these lines has clarified the subject in many details, although the actual physiological phenomena, which after all form the true basis of the problem, are as yet very vague. Periodicity in cold resistance has been one of the main points of attack, and in this connection more work has been done with plants than with insects. Chandler (1913) and Rosa (1921), showed that certain plants exhibit periodicity and that cold hardiness can be induced in them. Harvey (1918) induced hardiness in plants by exposing them to moderately low temperatures. Gueylard and Portier (1916) were the first to point out a seasonal variation in the cold resistance of insects. They observed that larvae of *Cossus cossus* L. survived repeated freezing at -20° in winter, but larvae of the same species taken in spring succumbed at -17°. Knight (1922) supercooled *Perillus bioculatus* in winter to -17°, and in one case even to -26° without freezing, yet in March and later a temperature of -10° caused freezing and death. Bodine (1921, 1923) found that the total water content of certain grasshoppers decreased during hibernation and was later restored to normal proportions.

Probably the most extensive studies along this line are those of Payne. In one of her first works (1926a) she found that in the case of oak borer larvae, which are normally subjected to extremes of temperatures, freezing points vary with individuals and seasons. She found an excellent correlation of freezing and undercooling points with average monthly temperatures. In the fall, the undercooling point falls ahead of the outside temperature, acting as a factor of safety. In the spring, the hardiness is lost with rising temperatures and it is at this time that a sudden cold snap is most fatal. Following the idea of previous authors as already discussed, Payne attempted to induce cold hardiness artificially by holding non-hardy insects at a moderately low temperature, and to break up hardiness by holding hardy insects at a moderately high temperature. The attempt was very successful. She also induced hardiness by dehydration, showing the effect of free-water content on hardiness. Payne measured hardiness in these experiments in terms of undercooling and freezing points; low undercooling and freezing points indicated a high cold resistance, and vice versa. ("Low" and "high" are used in this paper in a geometrical sense, not arithmetical; i.e. the sign is considered.) The points were determined by means of a thermocouple and a pyrovolter, but the author does not state the method of freezing. It may be pointed out here that Payne's use of the term "freezing point" is not exactly correct, the true freezing point being slightly higher than the "rebound point", as will be pointed out later.

Payne (1926b) soon afterwards selected for comparison three ecological groups: (1) the oak borers, normally exposed to extremes of temperature; (2) the aquatic insects, never exposed to temperatures below 0°C., and (3) stored product insects, representing, supposedly, a tropical or a subtropical group. In these experiments the oak-borer larvae showed marked periodicity, as already stated. The aquatic insects showed no periodicity, nor was there any significant difference among individuals, species, orders, or stages of development. The mean undercooling of all the specimens used, representing 14 genera in 4 orders, was 1.52° ± 0.3°, and the mean freezing point 0.57° ± 0.03°.

In the case of the third group, the stored product pests, Payne found no periodicity, but found more variation in undercooling and freezing points than in the aquatic group. Robinson (1926) working on the granary weevil, <u>Sitophilus granarius</u>, and the rice weevil, <u>Sitophilus oryza</u>, tried to harden them by a moderate lowering of the temperature over a long period of time, but the result was death.

The natural conclusion of these workers was that those insects which are normally subjected to temperature extremes acquire a cold resistance in the fall and lose it in the spring, while those which are never subjected to extremes are incapable of adapting themselves when artificially exposed, even when this exposure is made gradual.

The hardening of certain insects in the fall, or when placed artificially at moderately low temperatures, as evidenced by a drop in their undercooling and freezing points, led Robinson to apply to insects the "bound-water" theory already developed by Newton and Gortner for plants. According to this theory, under certain stimuli, (in this case low temperatures), the hydrophylic colloids present in the insect body are capable of adsorbing or "binding" water. The water, on being "bound", loses most of the typical physical properties of water. For example, the freezing point of bound water is greatly depressed, and indeed it is on the assumption that at -20°C. none of the bound water but all of the free water is frozen, that Robinson's (1931a) method of determining the bound water content of a system is based. The method as applied to an insect, is briefly as follows: The insect, of known weight, is frozen at a constant temperature of -20°C. for several hours and then transferred quickly to a calorimeter, where a determination is made of the number of calories required to melt the ice formed within the tissues. This determination is based on the fact that to melt one gram of ice without raising its temperature requires 80 calories of heat. By calculation, the amount of free water per gram of solid is determined. The final step is to dry the material to constant weight, (100°C. or in a vacuum oven at 60-65°), as a measure of total water content. The difference between the total and free water values is a measure of the bound water in the specimen.

The theory of water binding is of great importance in winter hardening. The adsorption of the water occurs on the surface of the colloidal particles. Because of their small size, (0.1-0.001μ), these particles present a relatively large surface. Under a falling temperature, the particles attract water and adsorb it as "films" around themselves, the Helmholtz "double layer". The thickness of the film may increase until it is greater than the diameter of the particle. The water on the inner layers is held by inconceivably high pressures due to surface energy, often running into thousands of atmospheres. Many of the physical properties of this water are changed in the process, e.g. it will not donduct electricity; it will not dissolve such substances as sugars; it can be considerably compressed; and its freezing point is greatly lowered. With falling temperatures of autumn and adsorption of water by the colloids in the insect tissue, it is obvious that the remaining aqueous solution will be more concentrated and the freezing point will drop. A certain degree of protection against cold weather is thereby established.

Newton and Gortner (1922), working with hardy varieties of winter wheat, established the fact that for plants there is a direct correlation between winter hardiness and percent of bound water. Robinson (1927), was the first to show that the same held for certain insects. He tested the hardy Telea polyphemus, the moderately hardy Callosamia promethea, and the non-hardy granary weevil, Sitophilus granarius, in arriving at the same conclusions as Newton and Gortner. He hardened the first two species both naturally, outdoors, and artificially in a constant temperature cabinet held at -13°C., just above their freezing temperature. In a non-hardy condition in which the first two species started, only 9-10% of the water was bound. This increased during the experiment to 42-52%. It is interesting to note that the total water content remained the same.

Robinson stresses the importance of the per cent of water bound before equilibrium is reached. If an insect in a non-hardy summer condition is placed in a refrigerating cabinet representing winter conditions, it is exposed to an unnaturally abrupt change and may be killed before it can begin to protect itself. He suggests, therefore, a study of (1) water-binding

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capacity, to show the percentage of water adsorbed and how quickly; (2) water-holding capacity, to show the ability to retain bound water under conditions of rapid rises in temperature, which is especially important in spring mortality.

It has just been stated that Robinson (1927) in his experimental hardening of Telea polyphemus and Callosamia promethea found that the total water content remained the same. Payne (1926b) states that the most pronounced feature of hardening was the low moisture content. The oak-borer larvae in fully hardened condition had a low moisture content, but in a non-hardy condition they had a high moisture content. Periodicity was thus exhibited in moisture content. The total water content of Synchroa punctata varied from 31.1% in February to 54% in August. That of Dendroides canadensis varied from 57.4% to 73.5%. The larvae were baked for four hours at 50°C. The adequacy of this method of desiccation will be questioned.

In the same paper Payne describes a multiple freezing experiment. Repeated freezing of the same insect or tissue exhibited no hysteresis, and the rebound and undercooling points remained the same. Samples of blood from the aortae showed definite crystals, while transparent larvae were also seen to have crystals within at the time the freezing point was recorded.. The process of freezing in this group was interpreted as crystalloidal, the first or primary freezing point being that of the blood.

Payne found further that the hardened oak-borer larvae survived freezing, and that on lowering the temperature still more, second undercooling and freezing points were recorded. The secondary freezing point occurred near -40°C. for the oak-borer group and was always fatal. The tissue freezing at this temperature was not definitely isolated, but the nervous tissue and fat were suspected. Payne went so far as to state that non-hardened insects are killed when the primary freezing point is reached, while fully hardened insects are not killed until the secondary freezing occurs. Experiments were run by the same author to determine the relationship between the freezing point and the survival of insects when exposed to low temperatures for as long as twelve hours. It was found that insects with high freezing points were never able to withstand long exposures to low temperatures. However, insects with low freezing points could be killed by long exposure when a short exposure would not be fatal. This author also dissected out the central nervous systems of 50 oak-borer larvae and froze them. The undercooling point recorded was -48°; the rebound point -45°.

Apart from the seasonal variation in cold hardiness, there exist variations during the individual development of an insect. Ludwig (1928) found considerable difference in the ability to withstand low temperatures among the various instars of Japanese beetle larvae. Their hardiness increased at first, then decreased to a minimum which occurred just after the first molt. Hardiness increased considerably during the second and third instars in which stages the winter is usually passed.

Payne (1927a) calls attention to the fact that two factors of heat energy are involved in the study of cold hardiness: (1) the Quantity Factor; (2) the Intensity Factor. Cold hardiness may thus be either the ability to withstand long periods of moderately low temperature, (the quantity factor), or the ability to withstand short periods of intensely low temperatures, (the intensity factor). In her experiments, aquatic insects, considered highly specialized along the quantity factor, endured long periods at 0°, but none survived freezing, even though the freezing point was only about 1° lower. Another group that may be specialized along the quantity factor is that group of soil insects normally living below the frost line. Most of the stored product insects cannot withstand dormacy. The oak-borers develop ability to survive dormacy in September and October, but at that time are still non-hardy to the intensity factor and are killed by freezing. Upon further low temperature exposure, or else dehydration, they become hardy to the intensity factor.

In considering cold hardiness to the intensity factor only, Payne (1927c) brings out the importance of the water content. She found Isia isabella and Diacrisia virginica to be self-dehydrating in the fall, whereas Popillia japonica did not exhibit this phenomenon. She considers that the first two species lost all of their free water. Robinson (1927) found that in hardening Telea polyphemus and Callosamia promethea, the total water content remained the same. It therefore appears that certain insects are dehydrated in the hardening process while others are not. Payne reports a drop in total water content from August to December of 5% for Popillia japonica, 17% for Dendroides canadensis, 24% for Synchroa punctata, and 20% for Romaleum rufulum. The oak borers are self-dehydrating, she states, but never lose all of their free water.

Payne in the same paper plotted blood conductivity readings against survival temperatures for Popillia japonica, Diacrisia virginica and Dendroides