A STUDY OF THE SUSCEPTIBILITY TO LOW TEMPERATURES

AND OF THE RATIO OF THE BOUND-FREE WATER CONTENT

OF THE CODLING MOTH LARVA.

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INTRODUCTION

The present study was undertaken to determine what low temperatures are fatal to the overwintering larvae of the codling moth, Carpocapsa pomonella L., and to ascertain whether any difference in susceptibility to low temperatures exists among larvae indigenous to widely separated fruit districts. In view of the part played by bound water in connection with the resistance of insects to low temperatures, a study was also made of the bound-water free water ratio by means of the heat of fusion and dilatometric methods.

The codling moth. -- The codling moth or "apple worm" is the
most injurious insect pest of the apple. It destroys annually,
or renders unfit for commercial purposes, millions of dollars
worth of fruit, despite present efforts to control it by spray-
ing and supplementary measures. In the absence of suitable
control measures, the codling moth will frequently infest from
10 to 95 per cent or more of the fruit, depending upon the
locality, winter and seasonal conditions, size of crop and other
factors.

The codling moth passes the winter in the
larval stage, enclosed in a silken cocoon, which is about three-
fourths of an inch in length. The overwintering larvae normally
cocoon beneath bark scales on the trunk and larger limbs and in
tree crotches. If, during the winter season, the temperature
becomes sufficiently low, the larvae will be killed. It is there-
fore of importance to the fruit grower to know whether killing
temperatures have occurred since the damage to be expected from
the insect during the following season is, to a considerable ex-
tent, dependent upon the percentage of larvae which have success-
fully passed the winter.

<u>Nomenclature of climatic effects</u>. — A number of terms, many
of which have been suggested by Pierce (8) are appropriate in
connection with the effect of low temperatures upon the codling
moth larva.

Upon completion of its feeding period within the fruit
some of the codling moth larvae of the early or summer broods do
not transform and hence become overwintering individuals. Neither
temperature or humidity, however, is responsible for this differ-
entiation since larvae of the same parentage, and reared under
identical conditions, exhibit differences as to the time of pupa-
tion. The overwintering larvae of the summer broods spin a heavier
cocoon than those which transform during the same season as hatch-
ed and upon its completion become inactive. This sluggish condition
during the summer season is generally known as estivation.

Practically all of the larvae which leave the fruit sub-
sequent to the first part of September become overwintering larvae.
They likewise construct heavy cocoons for the impending hibernation
period. The larvae are inactive throughout the hibernation period,
during which they encounter the low temperatures of the winter
season.
The dormant state of the larvae during the winter season
may be expressed as "rhigonochelia" (nochelia from the Greek word
γωχελεια, meaning sluggishness).

As shown in this paper, there is a minimum fatal temperature for each overwintering larva. Death from cold may be expressed by the word "rhigoplegia" (plegia from the Greek word πλη γη, meaning stroke).

Climatic environment. — The factors of environment are probably
the most dominant of the influences which affect favorably or
otherwise the destinies of the insect world. The environmental
conditions, however, are of such diverse character that relative-
ly little is as yet known concerning the role played by, or the
interrelations of, the environmental factors. From the time of
the birth of an insect, the struggle for its existence begins and,
in fact, in the case of the insect about which this paper has been
written, the defenseless egg may never hatch, either because of
unfavorable weather during the normal incubation period, or the
presence of an egg parasite, the prevalence of which is likewise
largely dependent upon its environmental conditions.

Especially during the past two decades,
a rather intensive study of the relationship between environment
and insect development has been commenced by students of ecology.
Attempts have been made to segregate and evaluate in particular
some of the more obvious and important of the factors contributing
to the life-processes. With the sole exception of suitable food,
nothing plays a more important role in the life of an insect than
that of the weather. And the effect of weather, in its broadest
aspect, of temperature, humidity, rain, snow, wind, etcetera, has
provided the economic entomologist with a problem, the complete
understanding of which would be of pronounced commercial importance.
In fact, the appreciation of the need for such information has
instigated considerable research, the results of which have been
enhanced by information and technique furnished by chemists and
physicists.

It is not within the scope or purpose of this paper
to describe the relationship of weather to the codling moth,[I]
nor to discuss the subject of temperature except in so far as
it may be limited to low fatal temperatures. And even the facts
given, in connection with the studies of low temperatures, leave
still unexplored the field of the physiological effects upon the
insect as a result of exposure to low fatal temperatures.

The importance of low temperatures, especially with
reference to their effect on insect survival, was the subject
of a considerable number of papers during the past century.
But even before this period, careful observers correlated, in
a general way, the abundance and scarcity of noxious insects with
climatic phenomena.

[I] A fairly complete list of publications, issued since 1895, is
given at the end of this thesis.

Resistance to low temperatures. -- In general there is a great
variation in the minimum temperatures which insects can survive.
Insects native to the tropics are so constituted that they are
destroyed by the lower temperatures of the more temperate zones
unless protected by living within houses or warehouses as household
and stored-product pests.

Certain insects, native to the
colder climates, pass the winter in different stages; some in the
egg stage as the gipsy moth Porthetria dispar L; some as larvae,
as in the case of the codling moth; some as pupae, as in the case
of the grape-berry moth Polychrosis viteana Clem.; while others,
as the Colorado potato beetle Leptinotarsa decemlineata Say. spend
the inactive winter season as adults. In each instance, it is
probable that the stage in which an insect hibernates over winter
is its most cold-hardy stage. However, this subject has not been
very extensively investigated and it would therefore offer a further
field of study in connection with the resistance of the different
stages of insects to low temperatures. It is of interest to note,
that closely related species overwinter in different stages. The
reason for this is not apparent, but some light might be thrown
on these phenomena by a study of the effect of low temperatures
on the stages of these insects.

Again we have insects whose
life-cycles extend over more than one year. The smaller chest-
nut weevil, Curculio auringer Fab, for example, spends at least
two winters in the soil. During the first winter it exists as a
pupa, while during the second it is in the adult stage, never

having changed its location during this period. It would be of
interest to know whether this insect is equally resistant to the cold
in the pupal and adult stages. The fact, however, that the chestnut
weevil is protected by a covering of from 4 to 12 inches of soil
might well safeguard either stage against death by freezing tempera-
tures.

Although as a rule, all insects exposed to low winter
temperatures may be considered hardy, some individuals, by reason
of their more favorable location will survive, while others of the
same species will be killed. The codling moth is a case in point.
At Yakima, Washington temperatures of -25°F are occasionally ex-
perienced. All of the larvae cocooned on the tree will be killed by
exposure to this temperature, except those on the lower trunk in the
event that they are protected by snow. Thus the matter of location
and the chance of snow covering means much with respect to the sur-
vival of this species in this district during certain winters. In
the State of Maine, where the winters are often quite severe, the
codling moth survives in relatively small numbers. However, the con-
tinued existence of the insect may be attributed not to its greater
inherent resistance to low temperatures but rather to the fact that
certain individuals pass the winter in protected quarters.

According to Imms (3) certain species of insects
can survive winter temperatures of -50°C. He reports, however,
that the winter hardiness of insects is insignificant in comparison
with the resting phase of rotifers and tardigrades, which according
to Rahm of Germany (1922), are able to withstand as low a temperature
as 2° absolute, (-271°C).

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The role of water in insects. -   The part played by water in the
life of an insect is as essential to it as to any other form of
life. To enumerate its various functions and ramifications would
be quite impossible. The cytoplasm of its cells is of a fluid
consistency and the fluid nature is due to the water content.
Water serves as the principal solvent in the life processes. It
has a high dielectric constant, making it of particular value in
the hydrolysis of nutrient materials. Its high surface tension
aids in the formation of the protoplasmic membrane and in retain-
ing the shape of the cells. The functions of the cells, therefore,
may be seriously impaired or totally destroyed when the cytoplasm
is altered beyond the normal favorable limits.

Further, water acts as a regulator of temperature changes
within the cells to a considerable extent, thereby serving as a
protector of the organism against sudden and extreme changes of
temperature.

Water has great heat capacity, the value of its specific
heat at $0^{\circ}C.$, being 1.00874. With the exception of a very few sub-
stances such as liquid ammonia and hydrogen gas, whose specific heats
are 1.047 at $0^{\circ}C.$, and 3.4090 at $12^{\circ}-198^{\circ}C.$, respectively, more heat
is required to raise a given amount of water than of any other sub-
stance known. And conversely, with few exceptions, water gives off
more heat for each degree of decrease of temperature than does any
other substance.

Many of the properties of water are quite different from those of other substances. For example, its greatest density is at 3.98°C and,while practically all substances contract on cooling, water expands between this temperature and 0°C and beyond to its super-cooling temperature. The fact that water in its solid state as ice has a lower density is another unusual property. The expansion of water at 0° Centigrade to ice at the same temperature represents an increase in volume of about one tenth. In this connection, some investigators have advanced the hypothesis that when freezing occurs within the tissues of an insect, the expansion due to the formation of ice causes death through mechanical injury to the cells.

Henderson (2A) has aptly referred to the role of water in the following words: "In physics, in chemistry, in geology, in meterology, and in biology nothing else threatens its preeminence. The physicist has perforce chosen it to define his standards of density, of heat capacity, and so forth, and as a means to obtain fixed points in thermometry. The chemist has often been almost exclusively concerned with reactions which take place in aqueous solution, and the unique chemical properties of water are of fundamental significance in most of the departments of his science. . . . The action of water now appears to be far the most momentous factor in geological evolution. The meteorologist perceives that the incomparable mobility of water, which depends upon its peculiar physical properties and upon its existence in vast quantities in all three states of solid, liquid, and gas, is the chief factor among the properties of

matter to determine the nature of the phenomena which he studies; and
the physiologist has found that water is invariably the principal
constituent of active living organisms. Water is ingested in great-
er amounts than all other substances combined, and it is no less
the chief excretion.   It is the vehicle of the principal foods
and excretory products, for most of these are dissolved as they enter
or leave the body. Indeed, as clearer ideas of the physico-chemical
organization of protoplasm have developed it has become evident that
the organism itself is essentially an aqueous solution in which are
spread out colloidal substances of vast complexity. As a result of
these conditions there is hardly a physiological process in which
water is not of fundamental importance."

Cold Hardiness of Insects.

Bachmetjew (1) was the first entomologist to make a
critical study of the cold hardiness of insects. He employed a
freezing chamber, thermocouple and galvanometer, thus giving him
suitable equipment for his experiments. As a result of his tests,
Bachmetjew announced that one undercooling was insufficient to
kill an insect; that death occurred only by lowering the tempera-
ture of the insect to its undercooling temperature a second time.

That Bachmetjew's conclusions were too broad was later
proven by a number of American and European workers. In this con-
nection it may be pointed out, that in the tests conducted by the
writer, the codling moth larva has never survived after one ex-
posure to its undercooling temperature. However, this insect may be
considered almost a borderline case, since some individuals actually
lived, although in a very weakened condition, for several weeks fol-
lowing their freezing.

Robinson (9) has made many valuable contributions on the
relations of cold to insect mortality. In the case of a certain
species of white grub (genus Phyllophaga) none of the insects re-
covered after it had reached its undercooling temperature. But in
experiments with the larva of the promethea moth Callosamia promethea Dru.
Robinson showed that it was able to survive repeated freezings.

Robinson has further reported that the lowering of the temperature of the non-hardy granary weevil <u>Sitophilus</u> <u>granaria</u> L. to a temperature above freezing, namely, 2°C for one month, results in the death of the insect.

In the course of the present investigation, the writer has examined in a preliminary way other species of insects. It was found that the common house fly <u>Musca</u> <u>domestica</u> L. can be undercooled and that it will survive. This insect winters as an adult in protected places within buildings and hence is not usually subjected to very low temperatures. The writer also found that the American cockroach <u>Periplaneta</u> <u>americana</u> L. is likewise not killed by a single undercooling.

These few illustrations will serve to show that no hard and fast rule can be postulated. Different species of insects are so variable in their life histories, habits and chemical constitutions that it is not surprising to encounter results of diverse character. When more is known concerning the chemistry of the body fluids of insects and of their various tissues, and the application of physico-chemical methods, it would appear that some sort of classification with respect to cold hardiness may be possible. The discovery of the underlying reasons, which would adequately explain the inherent differences in resistance to low temperatures, offers a wide field of research in insect physiology and related branches of science.

A start toward this end, however, has already been made to correlate the hydrophilic colloid content with winter hardiness, based on the theory that these colloids adsorb sufficient water to noticeably lower the fatal minimum temperature. This adsorbed water is called "bound water," while the remainder of the water is known as "free water."

All of the investigators of colloidal systems are in accord that the properties of water associated with colloids have deviated from those which are so characteristic of dilute true solutions. In this connection, Newton and Gortner (5) made use of the cryoscopic method to determine the percentage of bound water in plant sap, acting on the hypothesis that the adsorbed water would not dissolve cane sugar. It was found that the bound water was not a solvent for cane sugar as a result of tests in connection with the freezing point depression. Newton and Gortner used the constant of 2.085°C instead of 1.86°C in view of the findings of Scatchard (15) that sucrose forms a hexahydrate in solution. In the experiments of these investigators, the depression of the freezing point was 2.339° in a molar solution instead of 2.085°C. From this the above workers concluded that the excess depression was due to the fact that a part of the water had been adsorbed by the colloids as bound water.

It is well known that ordinary water is quite incompressible Harkins and Ewing (2), however, have shown as the result of the tremendous pressure due to adsorption that water may be compressed to about three fourths of its volume when in the bound state.

Another difference between bound and free water is that the former does not serve as a conductor of an electric current. This has been demonstrated by Robinson .

According to Thoenes (16) still another characteristic of bound water is that it will not freeze at —20°C. This pronouncement has led to the belief that the larger the quantity of free water that can be converted into the bound state, the more resistant will the insect be to low temperatures. Thus in the studies by Robinson with the hardy promethea, the non-hardy granary weevils and the white grubs of intermediate hardiness, the ability to bind water was in direct proportion to their resistance to cold.

Sources of Codling Moth Material.

Overwintering codling moth larvae were obtained in the fall of the year by banding apple trees with corrugated paper bands. The larvae were obtained from five fruit districts during the falls of 1930, 1931 and 1932, as follows:

(1)   Easton, Maryland.

(2)   Grand Junction, Colorado

(3)   Yakima, Washington.

(4)   Cornelia, Georgia.

(5)   Bentonville, Arkansas.

The bands containing the larvae were shipped to Takoma Park, Maryland and held under natural conditions in an outdoor insectary.

During the summer of 1931, certain of the moths issuing from the overwintering larvae obtained from the States of Washington and Georgia were confined together in rearing cages for cross-breeding purposes. The eggs obtained were incubated and the resulting larvae were provided with apples as food. Upon leaving the fruit, cocooning quarters, consisting of strips of corrugated paper, were provided. The cross-bred specimens were held in an outdoor insectary until used in connection with the temperature studies during the following winter.

Refrigeration Apparatus.

Refrigerator: -   A wide mouth vacuum bottle Fig. 3H, supported within

a cotton-filled glass battery jar was found to be a very convenient

and satisfactory minature refrigerator for the chilling of the insects.

Two sizes have been used in connection with the work at different

times, namely, a pint and a quart bottle. The choice of size was large-

ly dependent upon the type of work being carried out.

While not used directly for the refrigeration of

specimens under test, mechanical refrigerators (ammonia system) Figs. 1

and 2   were employed in various ways, such as the making of ice and

the cooling of calcium chloride brine, etc., for use in the vacuum-jar

refrigerators and constant temperature baths.

Refrigerants: -   In many of the tests, calcium chloride, hexahydrate,

and finely crushed ice or snow were used as an intimate mixture to

produce low temperatures. The hexahydrate was made from a commercial

grade of calcium chloride (77-80 per cent $Cacl_2$) by the addition of

about 8 pounds of the salt to 1 gallon of water. This was heated until

dissolved and, during the cooling, was rapidly stirred to get fine

crystals of the hexahydrate.

In order to secure a relatively low temperature from

the ice and salt mixture, the materials were mixed in the proportions

of the eutectic, namely, one part by weight of the hexahydrate to

7/10 part of crushed ice. Although such a mixture is capable of giving

a temperature of -54.9°C under the best of controlled conditions, the

writer was unable to realize lower than about -37°C. However, this

temperature was sufficiently low to take care of the experimentation.
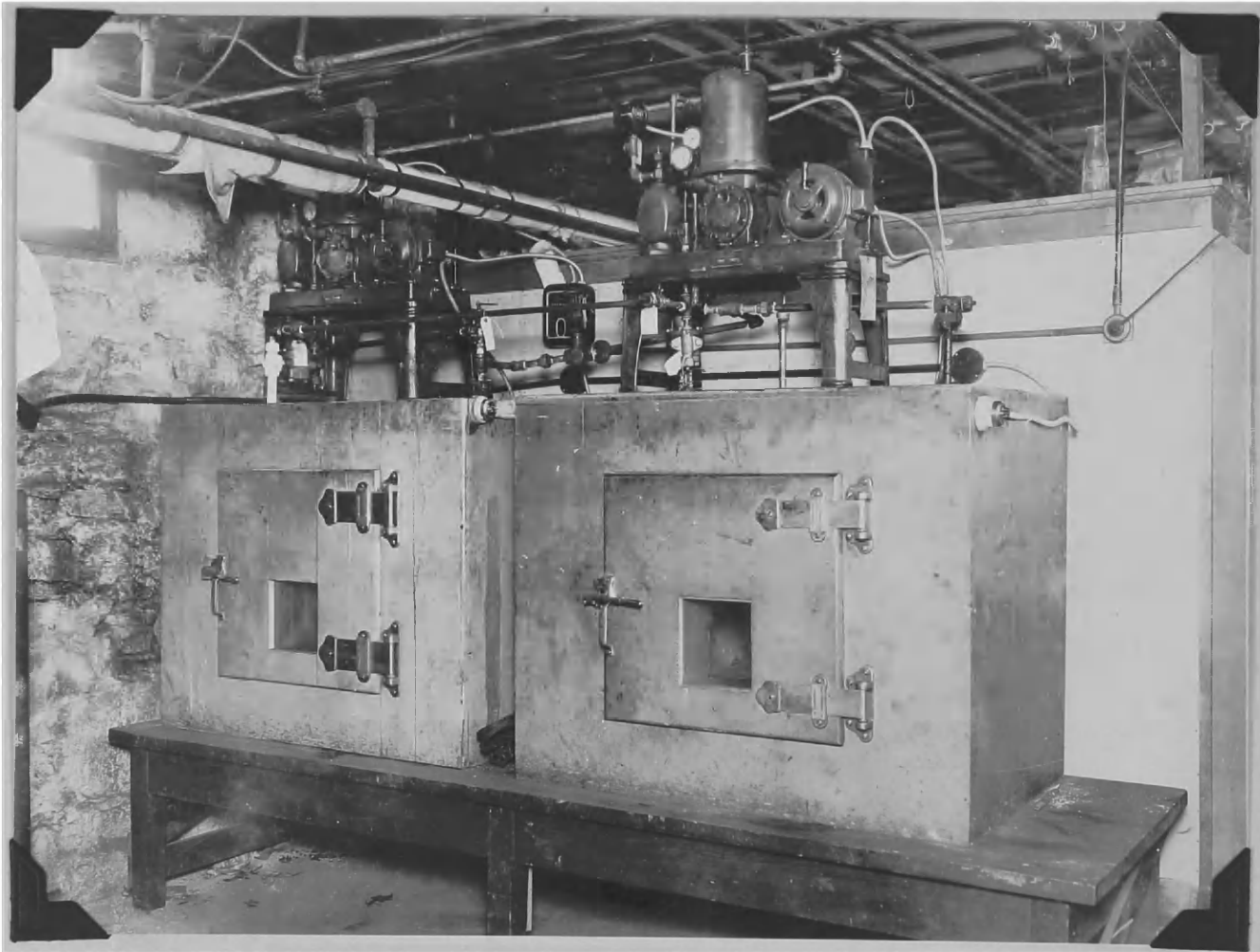
Fig. 1 -   Mechanical refrigerators
(ammonia system).

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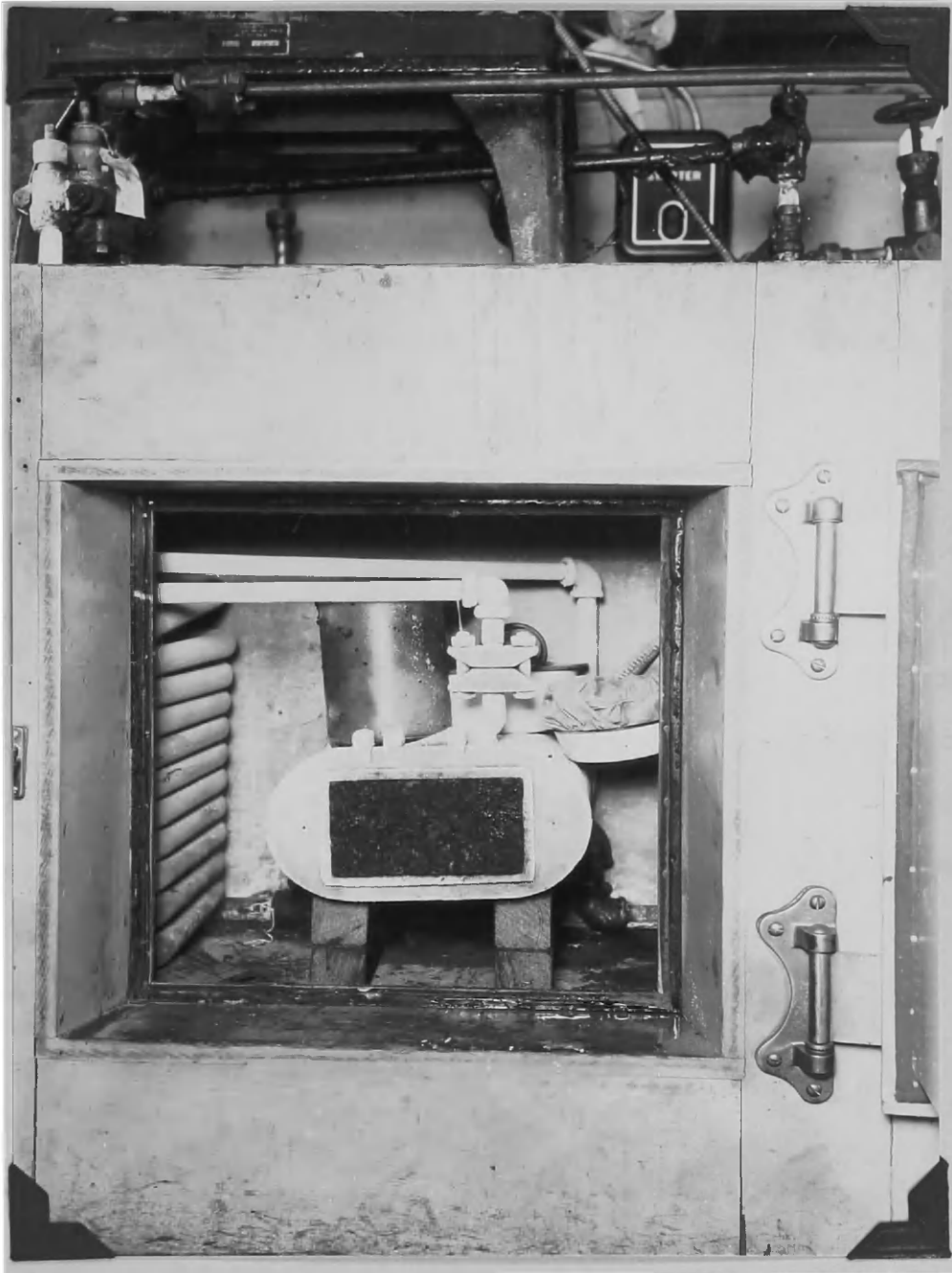
Fig. 2 –   Mechanical refrigerator
            (ammonia system). Inside
            view of box.

Another mixture used in the production of cold was a slush composed of solid carbon dioxide and gasoline. As a matter of convenience, it is sometimes preferable to the calcium chloride-ice combination. Occasionally the solid carbon-dioxide was purchased as "dry-ice" but, as a rule, a cylinder of liquid carbon dioxide was employed. The cylinder was inverted on a stand and a copper tube was attached to the cut-off valve. The solid carbon dioxide was secured by opening the valve and catching the material in a canvas bag of double thickness. The gas expands rapidly as it leaves the tank, cools and solidifies within the bag.

In making the slush of carbon dioxide and gasoline, the former was added to the latter since the gasoline remained permanently in the thermos bottle. Care must be taken to add the carbon dioxide in small quantities at first since there is considerable effervescence of the gas until the gasoline has become quite cold. Experience in making the slush, however, soon serves to guide the operator as to how to add the carbon dioxide in order to prevent loss of the mixture by overflowing from the vacuum bottle.

Solid carbon dioxide will produce a temperature of $-78.5^\circ C$ as it sublimes. Since, however, a temperature as low as this was not required for the experiments, only enough of the material was employed at a time to give the desired refrigeration.

Freezing chamber for insect:— As a rule a double-walled container was used as a container for the insect holder. This consisted of two glass test tubes of different sizes, the smaller being placed within the larger. This arrangement provided an air space between the two test tubes thus preventing too sudden changes of the temperature within the insect holder Fig. 7 . The freezing chamber was inserted through a cork stopper and placed in the freezing mixture contained in the vacuum-bottle refrigerator, Fig. 3H .

Electrical Apparatus

Potentiometer – A type " K " Leeds and Northrup potentiometer Fig. 3A was used for all measurements. Its accessories were a standard cell and a storage battery, whose voltage was maintained by means of a trickle charger.

Galvanometer – A Leeds and Northrup mirror galvanometer, Fig. III was securely fastened to a solid wall to provide against vibrations.

Thermocouple – A thermocouple of copper and constantan wire #32 was used for the determination of the temperatures. The junction used in contact with the insect was soldered, while the free ends were held in a cold junction at $0^{\circ}C$.

Cold junction – A pint thermos jar, Fig. 3F , served as a cold junction unit. A cork stopper, through which was inserted small glass tubes containing a half inch of mercury was used to stopper the thermos. The cold junction ends of the thermocouple were inserted in the glass vials until they made contact with the mercury. The thermos jar was filled with finely crushed ice made from distilled water, sufficient water being added to insure a uniform temperature of $0^{\circ}C$ throughout the upper 2 inches of the melting ice. Care was taken to have the cold junction of the thermocouple at the constant temperature of $0^{\circ}C$ by observing a calibrated thermometer graduated into fifths of a degree.
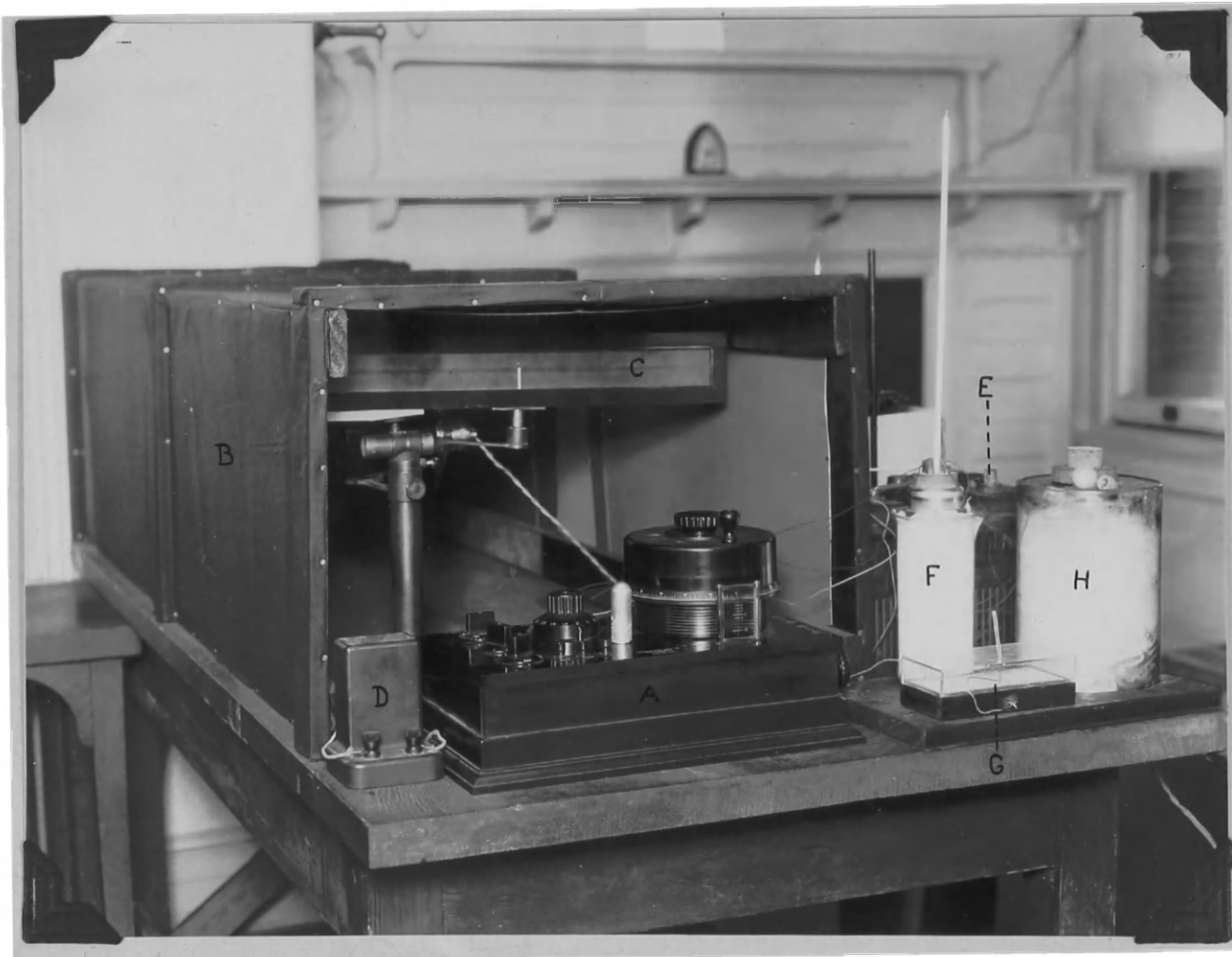
Fig. 3 — Potentiometer and accessories
    A — Potentiometer   E — Battery
    B — Hood for galvanometer and scale   F — Cold junction
    C — Scale for galvanometer reading   G — Mercury switch
    D — Standard cell   H — Refrigerator
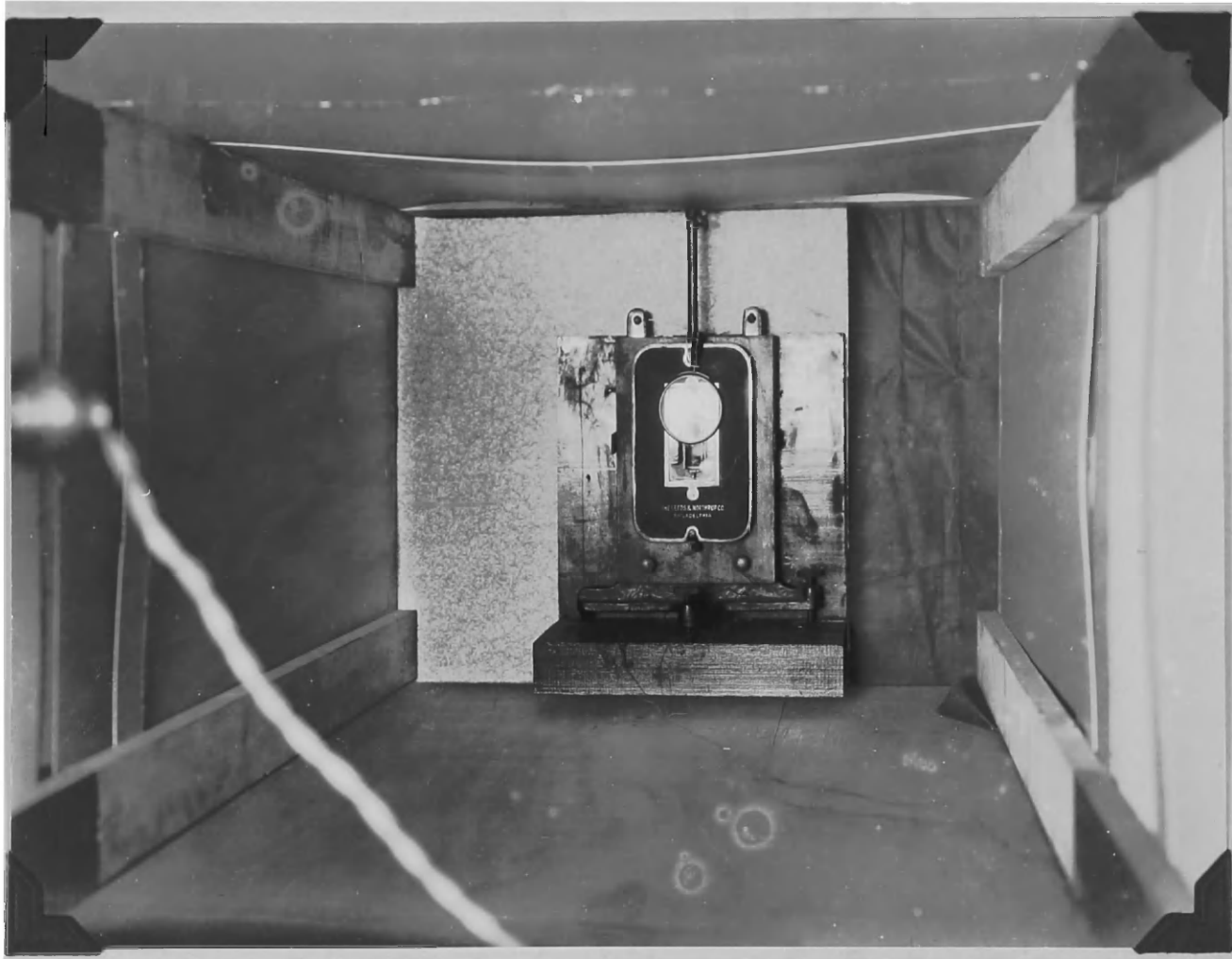
Fig. III — Wall galvanometer enclosed with hood.

Reversing switch: — In order to reduce to a minimum any resistance which might be introduced into the system as a result of a poor contact in a knife switch, a mercury-pocket reversing switch (Fig. 4) was made. This was constructed from a small wooden box which served as a container for paraffin. Melted paraffin was poured into the box, and before it hardened, 3 pockets were provided by means of a test tube pushed into the soft paraffin. Each pocket was partly filled with mercury into which copper leads were inserted. A movable copper wire served as the electrical connection between the mercury pockets. The entire switch was covered with a glass case to provide against dust.
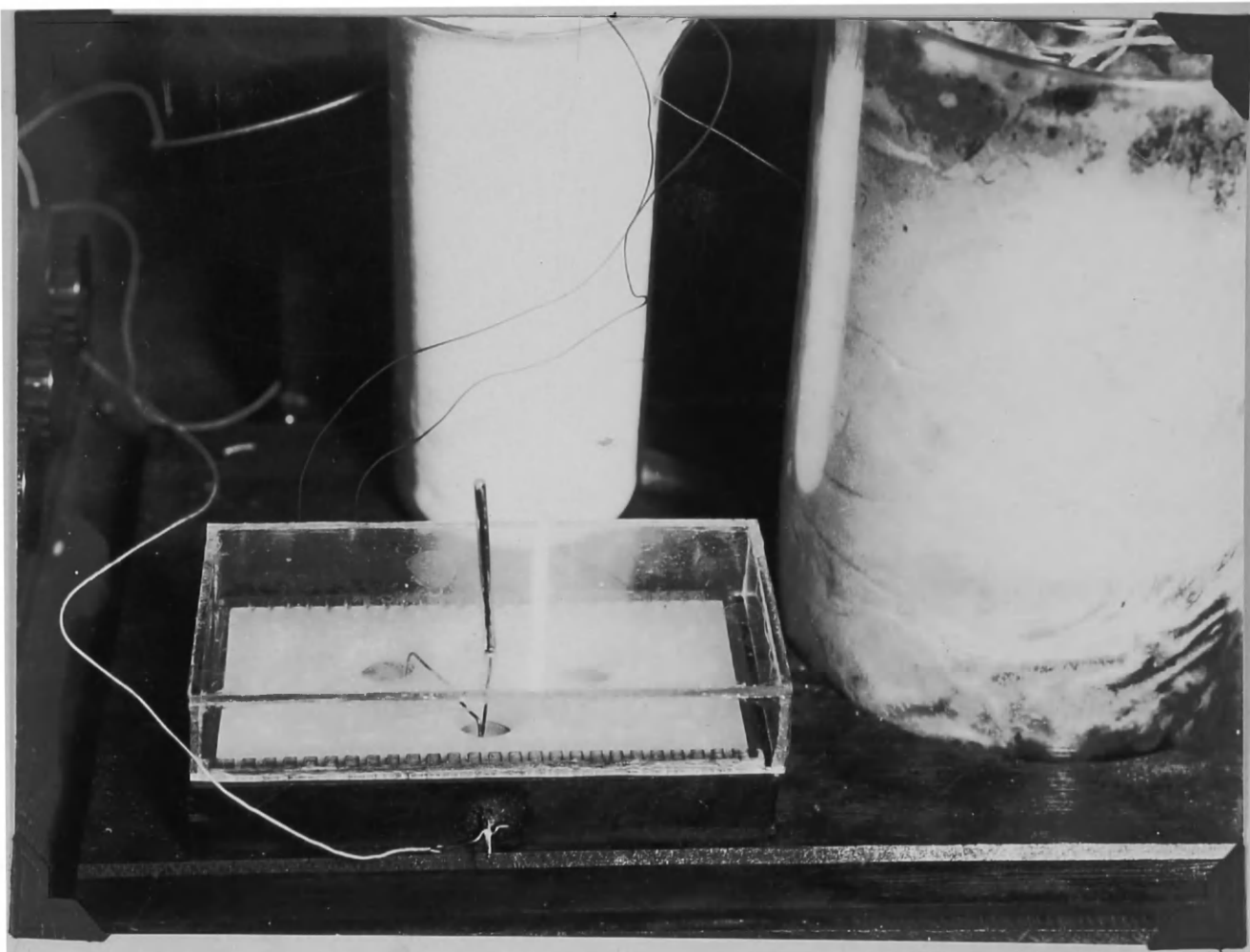
Fig. 4 -   Mercury switch.

Calibration of thermocouple: The thermocouples were calibrated as
to E. M. F.— temperature function in accordance with the following
equation and constants furnished by the United States Bureau of
Standards, which organization likewise kindly provided the thermocouple-
wire used in the experiments herein reported:

$$\text{Equation:} \quad E \text{ (microvolts)} = at + bt^2 + ct^3$$

Constants: 0°C to + 100°C

$$a = + 38.86$$
$$b = + 0.0473$$
$$c = - 0.0000381$$

Constants: 0°C to − 190°C

$$a = +39.29$$
$$b = + 0.0516$$
$$c = - 0.0000215$$

The E. M. F. — temperature function as calculated is
given in Table 1   and Fig. 5.

The calibration curve constructed from the equation
and constants just mentioned was checked and found to be in agreement
with 2 fixed points within the range of temperature concerned in this
study, namely, from 0° to −38.75°C.

Two substances were found to be convenient for calibra-
tion purposes, (1) carbon tetrachloride, whose freezing temperature
is −22.9°C and (2) mercury which freezes at 38.87°C. The carbon
tetrachloride was purified by distillation, while the mercury used
was a high grade vacuumed–distilled material.

The same procedure was used in each case and, as far as is known, is thought to be satisfactory. A 20 c.c. sample was poured into a wide test tube, after which the tube was lowered into a refrigerating mixture of solid carbon dioxide-gasoline slush. By moving it in and out of the cold mixture, the rate of freezing was readily controlled. The outside of the substance under test froze first, leaving in the center a core of liquid material. This was kept agitated by means of the thermocouple and, as the core of unfrozen material reached a relatively small volume, the number of microvolts furnished was observed. The number of microvolts, interpreted in terms of degrees, represents the difference in temperature between the cold junction end of the thermocouple and the measuring end which was in contact with the freezing carbon tetrachloride or mercury as the case might be.

A further check of the accuracy of the thermocouples used was made by comparing them directly, from time to time, with a calibrated thermometer graduated, however, to only .2°C. This was readily accomplished by immersing the thermometer and thermocouple, the junction of the latter being attached to the bulb of the thermometer, in a well-stirred cold bath of calcium chloride brine. The temperature drift of the bath was exceedingly slow on account of the insulating qualities of the container Fig. 17.

The principal on which the thermocouple operates is based on the fact that in a closed circuit of dissimilar metals a current is produced when the junctions are at different temperatures.

When the temperature junction is warmed, electrons
will flow from the element higher in the electromotive force
(in this case the alloy constantan: (60% cu and 40% ni) to the
element lower in the series (in this case copper). The tempera-
ture, which is measured by means of a potentiometer and galva-
nometer, the latter being used as a null instrument, is directly
proportional to the flow of electrons.

Table 1 . - Conversion: Potentiometer reading (thermocouple) microvolts to degrees.

Above freezing of water			Below freezing of water		
Degrees		Potentiometer (microvolts)	Degrees		Potentiometer (microvolts)
C	F		C	F	
0	32.0	0.00	0	32.0	0.00
1	33.8	38.907	-1	30.2	39.238
2	35.6	77.908	-2	28.4	78.373
3	37.4	117.004	-3	26.6	117.869
4	39.2	156.194	-4	24.8	156.333
5	41.0	195.477	-5	23.0	195.157
6	42.8	234.854	-6	21.2	233.877
7	44.6	274.324	-7	19.4	272.494
8	46.4	313.887	-8	17.6	311.006
9	48.2	353.543	-9	15.8	359.414
10	50.0	393.291	-10	14.0	387.718
11	51.8	433.132	-11	12.2	425.917
12	53.6	473.065	-12	10.4	466.712
13	55.4	513.089	-13	8.6	502.002
14	57.2	553.206	-14	6.8	539.887
15	59.0	593.413	-15	5.0	577.667
16	60.8	633.712	-16	3.2	615.342
17	62.6	674.102	-17	1.4	652.911
18	64.4	714.583	-18	-0.4	690.373
19	66.2	755.153	-19	-2.2	727.734

Table   1   (Continued)

Above freezing of water			Below freezing of water		
Degrees		Potentiometer (microvolts)	Degrees		Potentiometer (microvolts)
C	F		C	F	
20	68.0	795.815	-20	-4.0	764.988
21	69.8	836.566	-21	-5.8	802.135
22	71.6	877.407	-22	-7.6	839.176
23	73.4	918.337	-23	-9.4	876.112
24	75.2	959.357	-24	-11.2	912.941
25	77.0	1000.467	-25	-13.0	949.664
26	78.8	1041.665	-26	-14.8	986.280
27	80.6	1082.951	-27	-16.6	1022.790
28	82.4	1124.326	-28	-18.4	1059.193
29	84.2	1165.790	-29	-20.2	1095.490
30	86.0	1208.267	-30	-22.0	1130.849
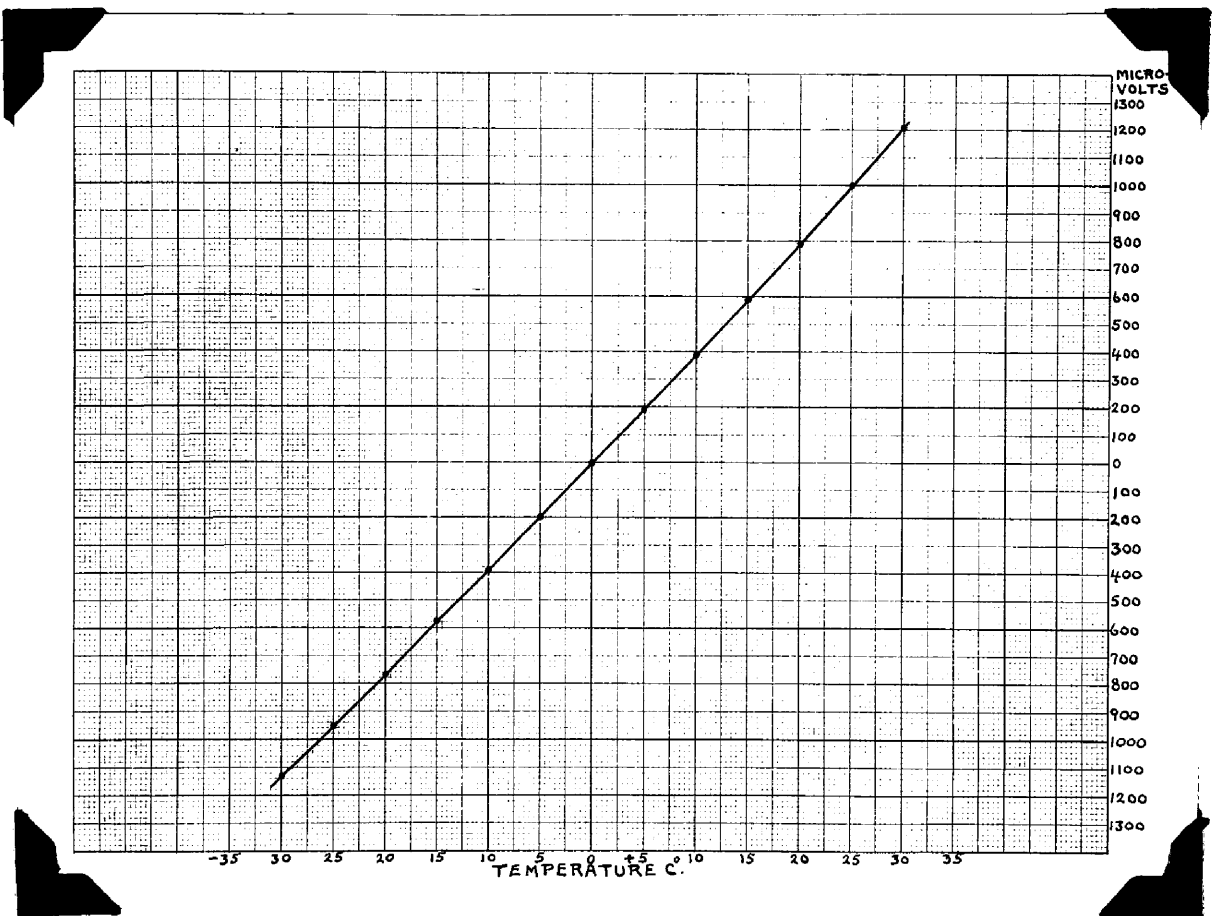
Fig. 5 — Calibration curve for thermocouple.

Insect Holders.

During the course of the investigation, study was given to finding the most suitable means of holding the insect and thermocouple junction in close contact.. Piercing of an insect with the thermocouple could not be adopted since this procedure not only injures the insect but also gives incorrect freezing data.

Glass cell. – It was apparent that the insect must be confined in a small enclosure and that the thermocouple junction should be in close contact with its body. In many of the tests a glass cell and cork, through which the thermocouple was inserted, was used. The larva was doubled over and the thermocouple was placed between the body segments. This device served to give the undercooling temperature without difficulty, but the eutectic halt at the true freezing point was so short as to make it it impossible to get an accurate reading on the potentiometer.

Cork cell. – Provisions were subsequently made to give the specimen better insulation so as to increase the time period at the eutectic. A satisfactory insect cell was made in the end of a cork which in turn was inserted into another cork filled with cotton so as to hold the insect in place (Fig. 6). The thermocouple was inserted through the cork, the junction being exposed in the cell in order that it would be in contact with the body of the larva. With this holder, the insulation was ample to provide sufficient time to take the potentiometer reading at the true freezing point immediately after the undercooling temperature had been observed.
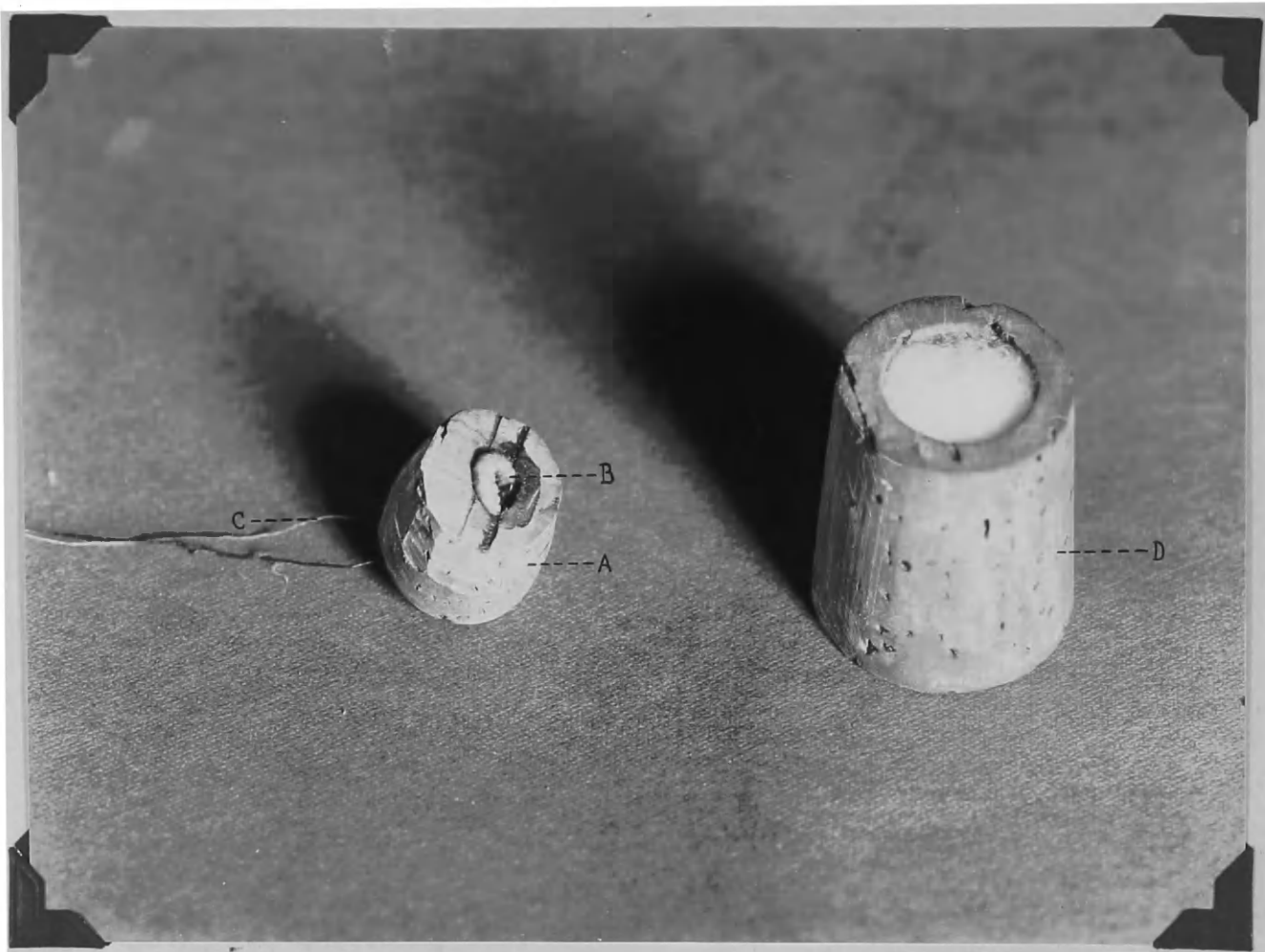
Fig. 6 – Insect holder (open) for determination of freezing
and undercooling temperatures of codling moth larva.

A – Insect cell
B – Codling moth larva
C – Thermocouple wires
D – Container for insect cell.

Fig. 7 –   Insect holder (closed) for determina-
            tion of freezing and undercooling
            temperatures.

During March and April, 1931, a number of tests were conducted with larvae from several states, with a view to determining the undercooling and freezing temperatures. The results are presented in Tables 1A and 2 . It will be noted from the results obtained that there was practically no difference in susceptibility to low temperatures.

Table 1A – Undercooling and freezing temperatures of
Codling moth larvae from several states,
March and April 1931.

Larva no.	Source of stock	Undercooling temperature C°	Freezing temperature C°
1	Maryland	-27.53	-6.45
2	"	-23.15	-5.18
3	"	-27.26	-6.46
4	Colorado	-26.55	-4.63
5	"	-26.42	-8.52
6	"	-28.10	- - -
7	"	-25.84	-6.58
8	"	-25.70	-5.31
9	"	-22.33	-6.08
10	"	-29.21	- - -
11	"	-20.71	-5.94
12	"	-25.81	- - -
13	"	-27.26	-6.33
14	"	-27.13	- - -
15	Arkansas	-27.53	-8.26
16	"	-26.29	-7.26
17	"	-28.21	-6.84
18	"	-27.76	-6.81
19	"	-25.07	-8.00
20	Georgia	-27.76	-3.89
21	"	-22.33	-4.05

Table 1A —   (Continued)

Larva	Source of stock	Undercooling temperature C°	Freezing temperature C°
22	Georgia	-27.53	-6.00
23	"	-27.40	-5.31
24	"	-26.15	-5.42
25	"	-27.26	-5.21
26	"	-27.40	-4.15
27	"	-18.68	-3.89
28	"	-25.47	- - -
29	"	-25.94	-8.00
30	"	-27.53	- - -
31	"	-22.08	-7.53
32	"	-28.07	-7.74
33	"	-26.17	- - -
34	"	-19.37	- - -
35	"	-26.84	- - -
36	"	-26.68	-9.58
37	"	-27.42	-11.26
38	"	-27.79	-12.00
39	"	-26.31	- 6.94
40	"	-26.58	- 6.74
41	Washington	-28.37	- 5.47
42	"	-28.21	- 7.15
43	"	-27.53	- - -
44	"	-28.74	- - -

Table 1A – (Continued)

Larva no.	Source of stock	Undercooling temperature C°	Freezing temperature C°
45	Washington	-28.37	-9.79
46	"	-26.15	- - -
47	"	-29.21	-12.42
48	"	-27.63	- 6.94
49	"	-21.15	- 4.42
50	"	-27.79	-11.63
51	"	-28.89	- 5.79
52	"	-20.21	- 4.42
53	"	-19.15	- 4.15
54	"	-23.94	- 5.00
55	"	-27.15	- 6.21
56	"	-26.84	- 5.58
57	"	-25.84	- 9.26
58	"	-27.79	- 7.74
59	"	-23.84	- 6.10
60	"	-27.26	- 7.37
61	"	-22.47	- 4.79
62	"	-26.15	- 8.10
63	"	-25.10	- 6.58

Table 2 - Summary of Table 1A. Undercooling and freezing temperatures of codling moth larvae from several States, March and April, 1931.

Larvae no.	Source of stock	Undercooling temperature C°	Freezing temperature C
3	Maryland - Average	-25.98	-6.03
	" - Highest	-23.15	-5.18
	" - Lowest	-27.53	-6.46
11	Colorado - Average	-25.91	-6.19
	" - Highest	-29.21	-8.52
	" - Lowest	-20.71	-4.63
5	Arkansas - Average	-26.97	-7.43
	" - Highest	-28.21	-8.26
	" - Lowest	-25.07	-6.81
20	Georgia - Average	-27.03	-6.73
	" - Highest	-28.07	-12.00
	" - Lowest	-18.66	- 3.89
23	Washington-Average	-25.99	-6.94
	" -Highest	-29.21	-12.42
	" -Lowest	-19.15	-4.15

In Table 3 will be found a comparison of the under-cooling and freezing temperatures of 5 larvae from the State of Washington. The observations were made 24 hours apart and the larvae were held at $-10^\circ C.$, during the intervening time. While it will be noted that some variation exists in the data obtained for the first and second undercooling and freezing temperatures, the differences are not extremely large. Such differences, however, are to be expected in working with biological material.

Table 3 , - Comparison of undercooling and freezing temperatures
of some specimens after an interval of 24 hours.
Larvae from State of Washington, April 1931.

Larva no.	First		Second (24 hours after first)	
	Undercooling temperature C°	Freezing temperature C°	Undercooling temperature C°	Freezing temperature C°
1	-23.94	-5.00	-23.84	-6.10
2	-27.15	-6.21	-27.26	-7.37
3	-26.84	-5.58	-22.47	-4.79
4	-25.84	-9.26	-26.15	-8.10
5	-27.79	-7.74	-25.10	-6.58

As mentioned on page 19 stock from the States of Washington and Georgia were crossed in order to study possible inherited resistance to low temperatures. Since, however, the susceptibility to low temperatures of overwintering codling-moth larvae indigenous to different fruit districts is practically the same, cross-bred material should show no variation. Within the limits of the work carried out, such was found to be the case.

The data for the male cross-bred larvae are given in Table 4 , and for the female larvae in Table 5 .

Table 4 .- Undercooling temperature of male cross-bred
codling moth larvae. Female moths from
State of Georgia crossed with male moths from
State of Washington, March 1932.

Larva no.	Weight of larva in grams	Undercooling temperature of larva C°	Time to reach undercooling temperature		
1	0.0363	-27.31	2 min.	28	sec.
2	0.0381	-28.42	3 "	22	"
3	0.0440	-27.74	3 "	22	"
4	0.0327	-29.89	3 "	33	"
5	0.0382	-28.79	3 "	33	"
Average	0.0379	-28.43	2.8 "	27.6	"
Maximum	0.0440	-29.89	3 "	33	"
Minimum	0.0327	-27.31	2	28	"

Table 5 . -   Undercooling temperature of female cross-bred
codling moth larvae. Female moths from
State of Georgia crossed with male moths from
State of Washington, March 1932.

Larva no.	Weight of larva in grams	Undercooling temperature of larva C°	Time to reach undercooling temperature		
1	0.0549	-27.79	3 Min.	14	sec.
2	0.0499	-29.00	3 "	47	"
3	0.0514	-27.26	3 "	32	"
4	0.0519	-29.68	4 "	13	"
5	0.0462	-28.68	3 "	44	"
6	0.0419	-29.47	3 "	29	"
Average	0.0494	-28.64	3 "	29.8	"
Maximum	0.0549	-29.68	4 "	13	"
Minimum	0.0419	-27.26	3 "	14	"

In Tables 6, 7, 8 , the undercooling temperatures are given for male larvae from the States of Maryland, Georgia and Washington; in Tables 9 ,10 , 11, will be found similar data for female larvae from the same States. Apparently neither the environmental factors under which the larvae were reared, nor the weight or sex play an important part with respect to the under-cooling temperature. It should be mentioned that the maximum and minimum weights as given do not belong to the maximum and minimum observations presented in the column "Undercooling temperature of larva C°."

Table 6 . - Undercooling temperature of male codling moth
larvae from State of Maryland, April 1932.

Larva no.	Weight of larva in grams	Undercooling temperature of larva C°	Time to reach undercooling temperature
1	0.0378	-19.10	2 min. 35 sec.
2	0.0338	-28.58	4 " 8 "
3	0.0453	-28.10	3 " 0 "
4	0.0384	-28.21	3 " 10 "
5	0.0386	-27.79	3 " 35 "
Average	0.0388	-26.35	3 " 161.6"
Maximum	0.0453	-28.58	4 " 8 "
Minimum	0.0338	-19.10	2 " 35 "

Table 7 — Undercooling temperature of male codling
moth larvae from State of Georgia, March 1932.

Larva no.	Weight of larva in grams	Undercooling temperature of larva C°	Time to reach undercooling temperature
1	0.0287	-27-84	6 min. 21 sec.
2	0.0438	-26.47	7 " 47 "
3	0.0437	-28.79	7 " 56 "
4	0.0401	-29.47	3 " 6 "
5	0.0405	-27.15	3 " 1 "
6	0.0381	-29.89	3 " 21 "
Average	0.0391	-28.26	5 " 26.3 "
Maximum	0.0438	-29.89	7 " 56 "
Minimum	0.0287	-26.47	3 " 1 "

Table 8 —   Undercooling temperature of male codling
moth larvae from State of Washington,
March 1932.

Larva no.	Weight of larva in grams	Undercooling temperature of larva C°	Time to reach undercooling temperature		
1	0,0402	-28.47	2 min.	31	sec.
2	0.0346	-26.84	2 "	25	"
3	0.0414	-22.89	4 "	5	"
4	0.0484	-26.26	6 "	19	"
5	0.0384	-25.84	6 "	26	"
6	0.0330	-25.15	6 "	2	"
7	0.0488	-27.26	9 "	48	"
8	0.0329	-26.47	6 "	54	"
9	0.0373	-25.94	7 "	15	"
Average	0.0394	-26.12	5 "	25	"
Maximum	0.0488	-28.47	9 "	48	"
Minimum	0.0329	-22.89	2 "	25	"

Table 9 . - Undercooling temperature of female codling moth
larvae from State of Maryland, April 1932.

Larva no.	Weight of larva in grams	Undercooling temperature of larva C°	Time to reach undercooling temperature		
1	0.0413	-23.10	2 min.	46	sec.
2	0.0471	-28.26	3 "	41	"
3	0.0543	-23.10	3" "	12	"
4	0.0668	-26.37	3 "	58	"
5	0.0473	-27.47	4 "	6	"
6	0.0648	-28.00	3 "	29	"
7	0.0394	-27.58	3 "	5	"
8	0.0549	-25.21	3 "	15	"
9	0.0463	-18.94	2 "	33	"
10	0.0360	-27.68	3 "	20	"
Average	0.0498	-25.57	2 "	26.2"	
Maximum	0.0668	-28.26	4 "	6	"
Minimum	0.0360	-18.94	2 "	33	"

Table 10. - Undercooling temperature of female codling moth
larvae from State of Georgia, March 1932.

Larva no.	Weight of larva in grams	Undercooling temperature of larva C°	Time to reach undercooling temperature		
1	0.0538	-27.68	5 min.	47	sec.
2	0.0338	-27.53	6 "	58	"
3	0.0608	-15.91	3 "	21	"
4	0.0466	-28.31	3 "	10	"
5	0.0454	-29.53	3 "	9	"
6	0.0574	-28.79	3 "	36	"
7	0.0528	-22.94	4 "	10	"
8	0.0454	-28.26	3 "	13	"
9	0.0301	-20.47	3 "	15	"
Average	0.0473	-25.49	4 "	4.3	"
Maximum	0.0608	-29.53	6 "	58	"
Mimimum	0.0301	-15.91	3 "	9	"

During February, 1933, further studies of the under-
cooling and freezing temperatures of overwintering codling moth
larvae obtained the previous fall from several states were made.
The results of the tests with the male larvae are given in Tables
12 and 13 ; with the female larvae in Tables 14 and 15 . As a rule,
the undercooling and freezing temperatures were somewhat lower in
February 1933 than in March and April 1931. In general, the under-
cooling temperatures were about the same for the larvae from the
different states, but those from Colorado had a somewhat lower aver-
age freezing temperature.

Table 12 . - Undercooling and freezing temperatures of male
codling moth larvae from different States,
February 1933.

Larva no.	Source of stock	Weight of larva in grams	Undercooling temperature C°	Freezing temperature C°
1	Maryland	0.0567	-27.15	-6.85
2	"	0.0525	-26.40	-10.35
3	"	0.0529	-29.46	-9.19
4	Arkansas	0.0381	-26.79	-7.20
5	"	0.0438	-29.94	-8.84
6	Georgia	0.0382	-31.39	-7.69
7	"	0.0450	-30.97	-15.45
8	"	0.0494	-29.38	-6.85
9	"	0.0559	-28.76	-7.25
10	Colorado	0.0332	-26.73	-12.46
11	"	0.0180	-31.74	-16.31
12	"	0.0395	-29.61	-7.72
13	"	0.0370	-29.22	-8.30
14	"	0.0414	-30.12	-12.41
15	"	0.0386	-29.15	-9.26
16	Washington	0.0367	-26.04	-9.29
17	"	0.0459	-22.84	-5.07

Table 13 - Summary of Table 12 , Undercooling and
freezing temperatures of male codling moth
larvae from different States, February 1933.

Source of stock	Weight of larvae in grams	Undercooling temperature C°	Freezing temperature C°
Maryland - Average	0.0540	-27.67	8.79
" - Maximum	0.0567	-29.46	-10.37
" - Minimum	0.0525	-26.40	- 6.85
Arkansas - Average	0.0409	-28.12	- 8.02
" - Maximum	0.0438	-29.46	- 8.84
" - Minimum	0.0381	-26.79	- 7.20
Georgia - Average	0.0471	-30.12	- 9.31
" - Maximum	0.0559	-31.39	-15.45
" - Minimum	0.0382	-28.76	- 6.85
Colorado - Average	0.0346	-29.42	-11.07
" - Maximum	0.0414	-31.74	-16.31
" - Minimum	0.0180	-26.73	- 7.72
Washington-Average	0.0413	-24.44	- 7.18
" - Maximum	0.0459	-26.04	- 9.29
" - Minimum	0.0367	-22.84	- 5.07

Table 14. - Undercooling and freezing temperatures of female
codling moth larvae from different States,
February 1933.

Larva no.	Source of stock	Weight of larva in	Undercooling temperature C°	Freezing temperature C°
1	Maryland	0.0592	-28.34	-5.00
2	Arkansas	0.0448	-28.06	-8.69
3	"	0.0469	-29.77	-10.30
4	Georgia	0.0535	-26.40	-5.85
5	"	0.0542	-27.75	-7.33
6	"	0.0624	-27.29	-9.44
7	"	0.0521	-28.51	-7.59
8	"	0.0590	-22.27	-4.51
9	"	0.0550	-29.66	-6.61
10	"	0.0472	-28.92	-4.36
11	Colorado	0.0403	-28.86	-12.38
12	"	0.0352	-30.65	-12.94
13	"	0.0359	-28.20	-7.72
14	"	0.0480	-29.92	-14.02
15	"	0.0439	-28.84	-8.79
16	Washington	0.0507	-23.51	-8.00
17	"	0.2333	-29.41	-11.36

Table   15 –   Summary of Table   14   .   Undercooling and
        freezing temperatures of female codling moth
        larvae from different States,   February 1933.

Source of stock	Weight of larvae in grams	Undercooling temperature C°	Freezing temperature C°
Maryland - Average	0.0592	-28.34	- 5.00
" - Maximum	0.0592	-28.34	- 5.00
" - Minimum	0.0592	-28.34	- 5.00
Arkansas - Average	0.0458	-28.91	- 9.49
" - Maximum	0.0469	-29.77	-10.30
" - Minimum	0.0448	-28.06	- 8.69
Georgia - Average	0.0547	-27.25	- 6.52
" - Maximum	0.0624	-29.66	- 9.44
" - Minimum	0.0472	-22.27	- 4.36
Colorado - Average	0.0406	-29.29	-11.17
" - Maximum	0.0480	-30.65	-14.02
" - Minimum	0.0352	-28.20	- 7.72
Washington-Average	0.1420	-26.46	- 9.68
- Maximum	0.2333	-29.41	-11.36
- Minimum	0.0507	-23.51	- 8.00

Effect of dry and humid conditions on codling
moth larvae.

Payne (6), in experiments with Synchroa sp. larvae was
able to demonstrate that both the under-cooling and freezing tempera-
tures of the larvae could be artifically lowered by means of dehydra-
tion. The abstraction of water causes an increase in the concentra-
tion of the protoplasm resulting in a depression of the freezing
temperature.

In another paper by Payne (7) with reference to the measures
of insect cold hardiness, it is pointed out that for insects which are
normally not self-dehydrating, artificial dehydration is especially
effective in lowering their undercooling and freezing temperatures.
This author states that the Japanese beetle larva Popillia japonica Newm.
which normally overwinters in a relatively moist environment, can be
dehydrated to half its body weight. When thus dehydrated this insect
is very cold resistant.

As has already been shown by Payne (6) and (7), the loss
of water in an insect lowers its undercooling and freezing temperatures.
In the instance of the codling moth larva, the insect is largely self-
dehydrating, especially just after completing its growth within the
flesh of the apple. It does not carry an abundance of water as winter
approaches.

Because of the habit of the codling moth larva to spin
new cocoons if disturbed, it was not possible to weigh larvae before
and after dehydration without affecting the normal condition of the
insect. However, a series of tests was conducted in which the insects,
undisturbed in their cocoons, were held at 10°C., in sealed glass
containers under (1) humid conditions and (2) dry conditions. In the

former, an abundance of moisture was supplied by means of lamp wicks
dipping into a beaker of water. In the latter, dehydration was ef-
fected by means of silica – acid gel. The latter was thoroughly
dried out weekly.

In tables $16, 17, 18, 19$, will be found the results.
It will be noted that the average undercooling and freezing tempera-
tures of both the males and females are slightly lower in the case
of those held under the drier conditions than those larvae which
were maintained in the more humid environment. The differences,
however, are not sufficiently pronounced to have significance. It is
believed, therefore that the dormant codling moth larva under natural
field conditions would neither take on or loose water in sufficient
quantity to affect its resistance to low temperatures.

It will be further noted in tables $16, 17, 18, 19$,
that there is no correlation between the weight of the larva and its
low critical temperature, nor is there a significant difference with
respect to the cold hardiness of the sexes.

Table 16 —   Undercooling and freezing temperatures of female
             codling moth larvae from several States placed
             in a humid environment in December 1932.

Date of test 1933	Source of stock	Weight of larva in grams	Undercooling temperature C°	Freezing temperature C°
January	Georgia	0.0569	-28.12	-10.22
April	"	0.0565	-26.89	- 5.67
January	Maryland	0.0624	-27.98	- 6.95
February	"	0.0529	-27.51	- 7.86
April	"	0.0515	-27.31	- 6.25
January	Arkansas	0.0582	-26.94	- 6.95
January	Colorado	0.0490	-26.78	- 8.11
February	"	0.0449	-27.20	- 8.32
"	"	0.0449	-29.98	- 9.08
April	"	0.0536	-28.05	- 8.67
February	Washington	0.0510	-27.31	- 7.09
April	"	0.0471	-28.05	- 8.67
Average (all States)		0.0524	-25.33 27.67	- 7.82
Maximum " ")		0.0624	-29.98	-10.22
Minimum (" ")		0.0449	-26.78	- 5.67

Table 17 — Undercooling and freezing temperatures of female
codling moth larvae from several States placed
in a dry environment in December 1932.

Date of test 1933	Source of stock	Weight of larva in grams	Undercooling temperature C°	Freezing temperature C°
January	Georgia	0.0410	-26.73	-6.48
February	"	0.0549	-26.78	-5.98
April	"	0.0519	-24.70	-4.51
January	Maryland	0.0432	-25.95	-11.78
"	"	0.0755	-27.04	- 6.53
April	"	0.0433	-25.13	- 5.98
January	Arkansas	0.0636	-25.43	- 6.69
"	"	0.0570	-26.81	- 9.29
February	"	0.0514	-31.00	- 9.15
April	"	0.0463	-24.22	- 5.10
January	Colorado	0.0408	-27.20	- 6.69
February	"	0.0424	-29.27	- 7.55
April	"	0.0557	-26.94	- 6.40
"	"	0.0509	-28.97	- 7.33
February	Washington	0.0637	-23.73	- 7.59
April	"	0.0392	-28.61	-12.93
Average (all States)		0.0513	-26.78	- 7.49
Maximum (" ")		0.0755	-31.00	-12.93
Minimum (" ")		0.0392	-23.73	- 4.51

Table 18 -   Undercooling and freezing temperatures of male
              codling moth larvae from several States placed
              in a dry environment in December 1932.

Date of test 1933	Source of stock	Weight of larva in grams	Undercooling temperature C°	Freezing temperature C°
January	Georgia	0.0364	-27.27	-10.87
April	"	0.0120	-26.40	-11.73
February	Maryland	0.0488	-30.33	- 8.48
April	"	0.0342	-22.46	- 4.98
April	Arkansas	0.0332	-29.29	- 6.99
February	Colorado	0.0391	-27.26	-9.86
January	Washington	0.0489	-27.07	--6.48
February	"	0.0417	-12.38	- 7.90
April	"	0.0336	-27.53	- 7.21
Average (all States)		0.0364	-25.55	- 8.27
Maximum (" ")		0.0489	-30.33	-11.73
Minimum (" ")		0.0120	-12.38	- 4.98

TABLE 19 - Undercooling and freezing temperatures of female codling moth larvae from several States placed in a humid environment in December 1932.

Date of test 1933	Source of stock	Weight of larva in grams	Undercooling Temperature C°	Freezing temperature C°
January	Georgia	0.0391	-19.89	- 6.69
February	"	0.0479	-27.73	- 5.77
April	"	0.0451	-26.32	- 5.23
January	Maryland	0.0583	-26.21	- 7.72
April	"	0.0403	-25.48	- 8.00
January	Arkansas	0.0456	-26.04	- 5.73
February	"	0.0377	-28.06	-10.66
April	"	0.0350	-24.16	- 6.25
"	"	0.0462	-26.92	- 6.35
April	Colorado	0.0421	-24.24	- 4.77
January	Washington	0.0470	-27.13	- 8.78
February	"	0.0455	-27.04	-10.63
April	"	0.0447	-24.37	- 5.15
Average (all States)		0.0441	-25.66	- 7.05
Maximum (" ")		0.0583	-28.06	-10.66
Minimum (" ")		0.0350	-19.89	- 4.77

Successive Undercooling and Freezing.

In order to ascertain what effect successive undercoolings and freezings would have upon a codling moth larva, a test was made with an Arkansas-reared larva. The larva was held at room temperature following each freezing and ample time was given to insure melting of the frozen specimen between successive tests.

The results are given in Table 20 and, as will be noted therein, successive exposures gradually raised the undercooling temperature, except in the instance of the first two undercoolings. It has been frequently noted in connection with miscellaneous tests that, as a result of the first and second exposures of the same specimen to its undercooling and freezing temperatures, the temperatures are practically the same provided the time between exposures is brief. Less frequently, however, this has not been true and hence no definite predication can be made. In Table 20 it will be further noted, that the range in the freezing temperature extended from —8.13°C., to —11.44°C., with an average of —9.87°C. The autectic halt was distinct in each of the successive tests.

Table 20 -   Successive undercooling and freezing temperatures
of some codling moth larvae during a period
of six hours.   February 1933.

Source of stock	Sex of larva	Weight of larva in grams.	Test no.	Undercooling temperature C°	Freezing temperature C°
Arkansas	Female	0.0726	1	-30.05	- 8.24
			2	-30.05	- 9.63
			3	-29.72	-10.48
			4	-29.67	- 9.93
			5	-28.73	-11.00
			6	-28.19	-10.11
			7	-27.97	- 8.13
			8	-26.95	-11.44
			Average	-28.91	- 9.87
			Maximum	-30.05	-11.44
			Minimum	-27.97	- 8.13

Cooling Curves

The data for 5 typical cooling curves are given in
Table 21 and are shown graphically in Figs. 8 , 9 ,10 , 11, and 12.
It will be noted that the codling moth larva exhibits a very con-
siderable undercooling. As soon as the undercooling temperature
is reached, the heat of crystallization causes a sudden rise in
the temperature of the larva as shown by the almost vertical slope
of the curve at that point. At the true freezing temperature,
the temperature remains constant until the body fluids solidify.
The eutetic halt ranges from 15 to 20 seconds under the conditions
of the experimentation. The curve then slopes downward as the
temperature of the frozen insect becomes lower.

Table 21 – Cooling-curve data for codling moth larvae, Maryland stock giving undercooling and freezing temperatures. Readings at intervals of 30 seconds.

Range of refrigerator temperature

-33.9°C to -33.2°C	-34.4°C to -33.6°C	-35.8°C to -33.2°C	-30.6°C to -29.4°C	38.6°C to 37.50°C
Test no. 1	Test no. 2	Test no. 3	Test no. 4	Test no.5
Female, 0.0607 g.	Male, 0.0539 g.	Female, 0.0485	Male, 0.0524 g.	Female,0.0573
C°	C°	C°	C°	C°
+ 22.8	+24.9	+ 21.9	+ 22.1	+ 22.3
18.4	20.4	17.4	18.5	18.0
14.1	16.5	13.4	15.2	13.9
8.8	11.2	8.1	9.4	8.4
3.0	5.3	2.3	3.9	2.4
- 2.0	0.0	- 3.2	- 1.4	- 3.5
6.9	- 6.4	7.9	6.3	8.5
12.3	9.1	12.3	10.6	14.3
15.1	13.4	16.2	14.5	17.4
19.2	16.9	19.2	16.8*	20.2*
21.9	19.7	21.9	4.3**	4.5**
23.2	22.1	24.2	5.1	5.5
25.1	24.2	26.2	6.8	8.6
26.4	25.9	27.5	10.9	15.2

Table 21   (Continued)

Range of refrigerator temperature

-33.9°C to -33.2°C	-34.4°C to -33.6°C	: -75.8°C to -33.2°C	-30.6°C to -29.4°C	38.6°C to 37.50°(
Test no. 1	Test no. 2	Test no. 3	Test no. 4	Test no. 5
Female, 0.0607 g.	Male, 0.0539 g.	Female, 0.0485	Male, 0.0524 g	Female, 0.0573
C°	C°	C°	C°	C°
27.5*	27.4	28.1*	16.4	21.1
5.7**	27.8*	6.3**	20.3	25.4
7.8	5.2**	10.7	23.7	27.8
13.2	7.8	17.6	25.6	29.4
18.7	13.9	22.7	27.1	
22.7	19.7	25.9	27.8	
25.5	23.6	27.9		
27.4	26.3	29.4		
28.3	27.9			
29.3	28.6			
30.5	29.3			
31.2	30.5			

*Undercooling temperature reached during regular 30 sec. observation intervals
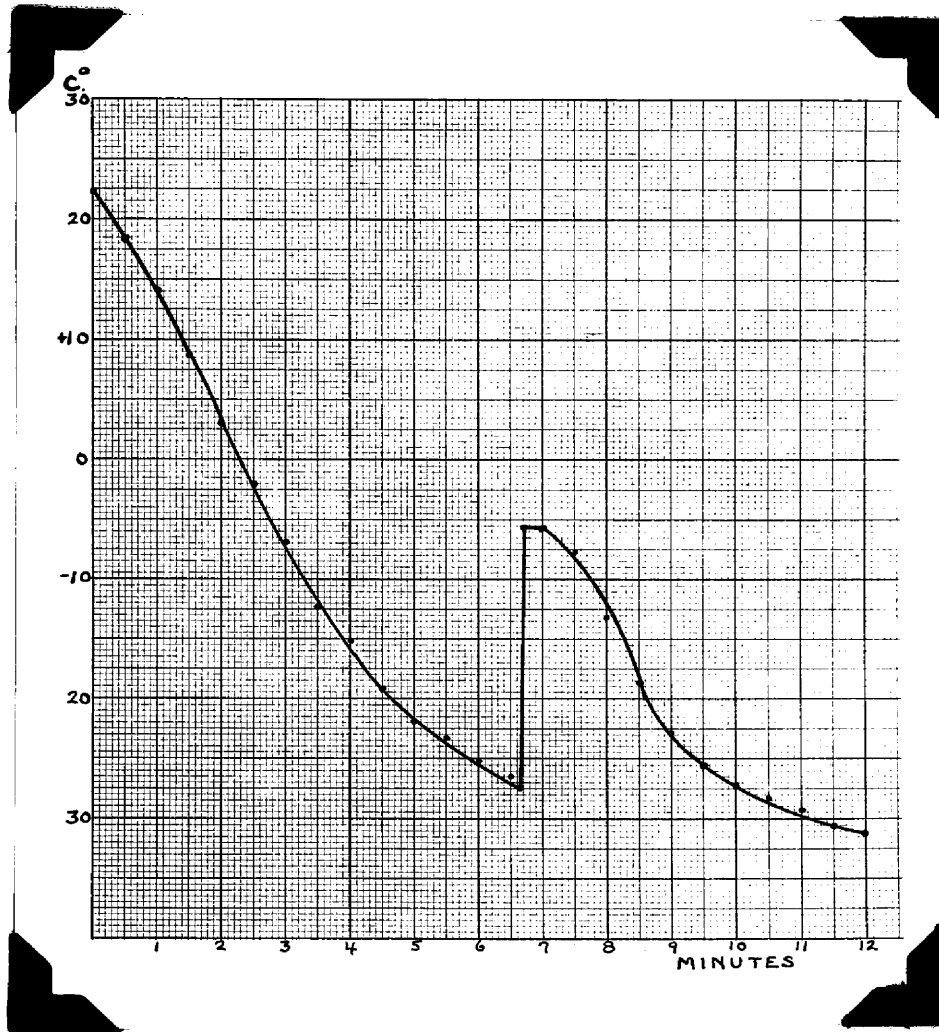
** Freezing temperature.

Fig. 8   Cooling curve, codling moth
larva, showing undercooling
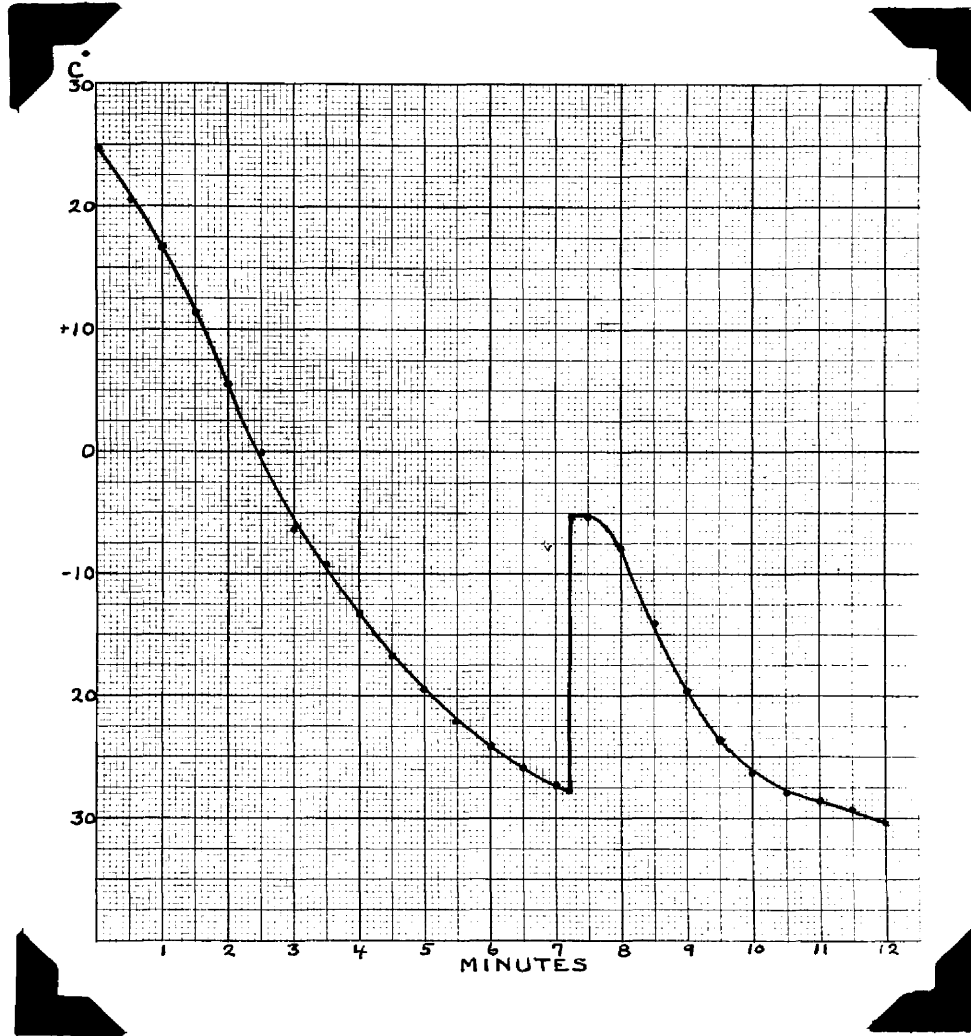and freezing temperatures.
Test no. 1, Table   21   )

Fig. 9   Cooling curve, codling moth
larva, showing undercooling
and freezing temperatures.
(Test no. 2, Table 21   )
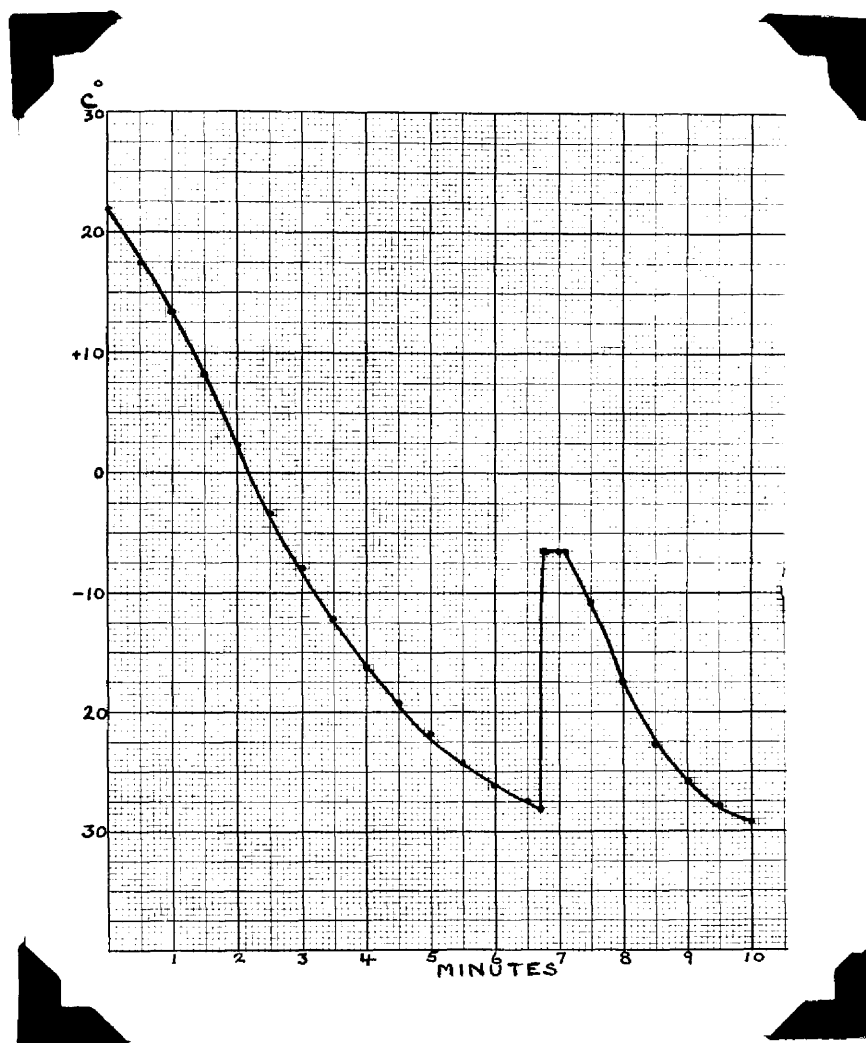
Fig. 10   Cooling curve, codling moth
          larva, showing undercooling
          and freezing temperatures.
          (Test no. 3, Table 21)
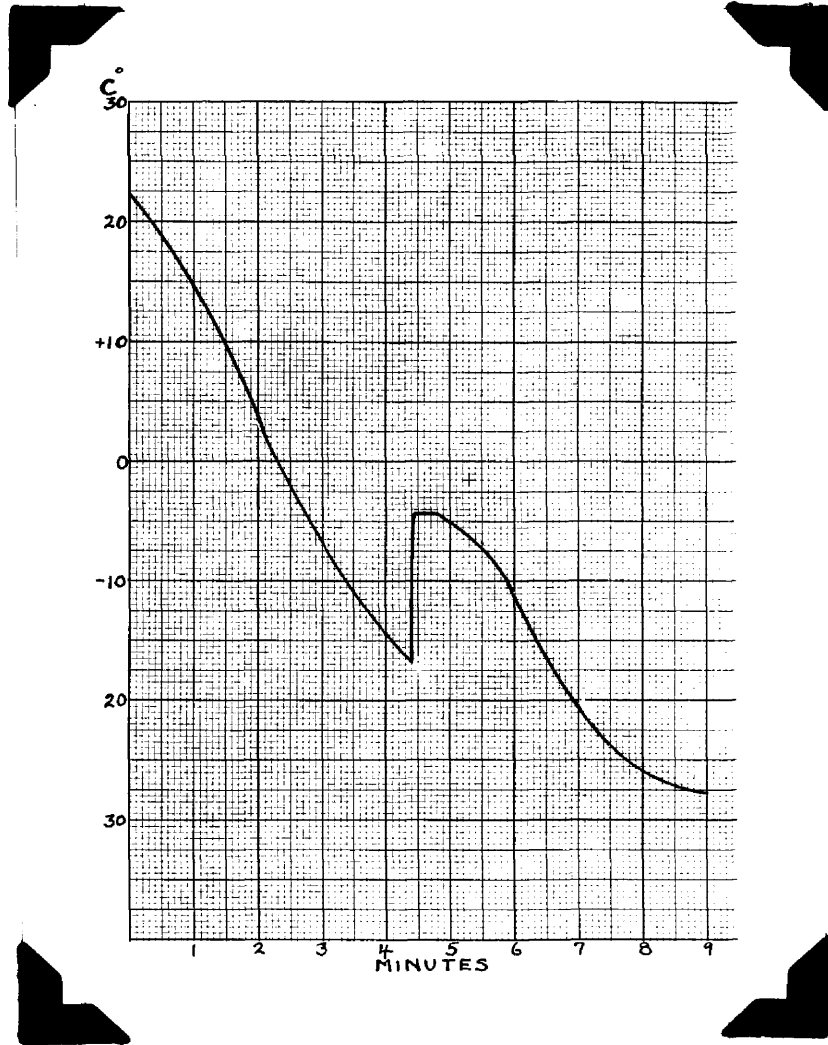
Fig. 11 Cooling curve, codling moth
         larva, showing undercooling
         and freezing temperatures.
         Test no. 4, Table 21)
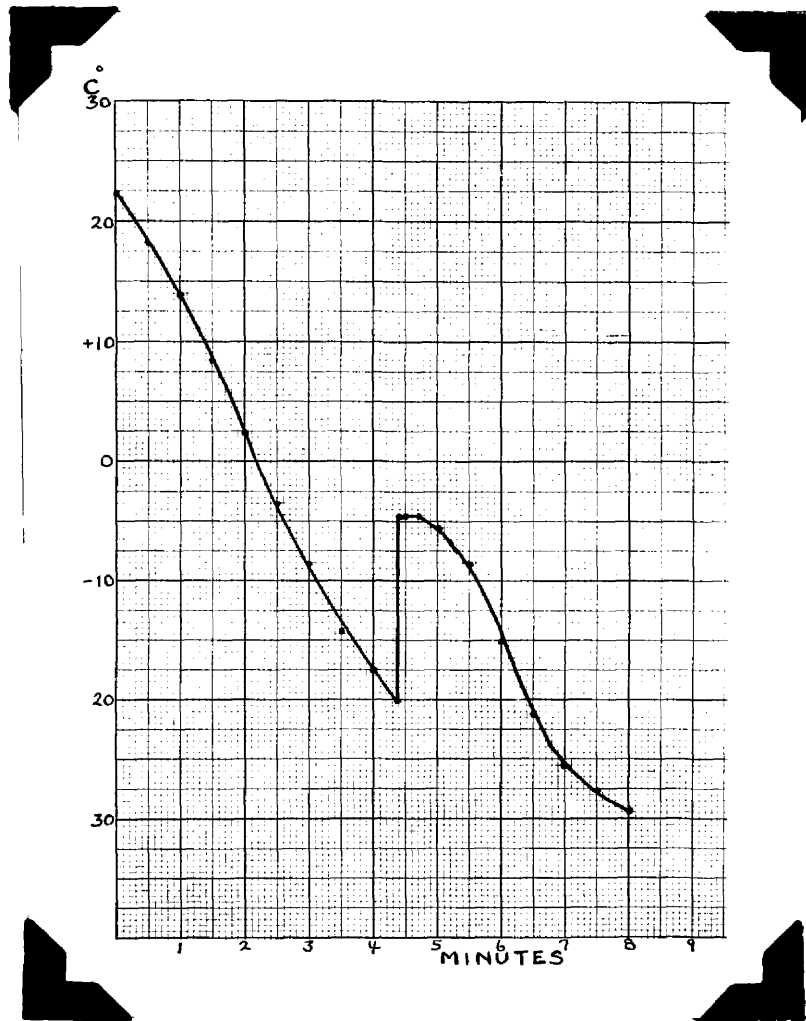
Fig. 12 Cooling curve, codling moth
         larva, showing undercooling
         and freezing temperatures.
         (Test no. 5, Table 21)

Protective value of undercooling for the
codling moth larva.

In most fruit districts the overwintering larva is pro-
tected against death during the winter season very largely because
of the fact that, as the temperature falls, the larva becomes more
and more inactive. It lies perfectly quiet in its silken cocoon
and reduces its metabolic processes to a minimum. Such conditions
favor supercooling of the larva in the same way that quiet condi-
tions permit water, for example, to be undercooled considerably below
zero degrees centigrade.

In the case of the larva, there is no chance for the intro-
duction of a foreign substance into the body fluids to serve as
artificial nuclei for ice formation. Again, the tree, on which the
larva is quartered beneath the bark scales, is relatively free from
vibrations, which, if present, would reduce the number of degrees of
supercooling.

It will be noted from the results given in this manuscript
that, although considerable variation exists in the undercooling
temperatures of different larvae, the temperature required to produce
the first crystal of ice is much lower than the true freezing tempera-
ture. The ability to supercool therefore plays a most important
protective role in the life of the codling moth during the winter.

In order to furnish proof that the quiet condition or state of
the larvae during periods of exposure to temperatures below that of the
true freezing temperature makes it possible for them to supercool,
some tests were conducted in which the larvae were kept in motion by
means of a wire loop as the temperature was lowered.

The results of these tests are given in table 22 . It
will be noted that both the undercooling and freezing temperatures
are very considerably higher than those obtained when the larvae
were undisturbed during their exposures to low temperatures. The fact
that any undercooling occurred at all is evidence that the tendency
to supercool is exceedingly pronounced.

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Table 22 –   Effect of artificial movement in codling moth
larvae upon theit undercooling and freezing
temperatures.   April 1933.

Source of stock	Larva no.	Undercooling temperature C°	Freezing temperature C°
Arkansas	1	- 9.8	- 2.5
"	2	- 4.5	- 2.6
"	3	- 8.2	- 3.4
"	4	- 7.6	- 1.8
Maryland	5	- 6.4	- 2.7
"	6	- 9.9	- 2.9
"	7	-11.9	- 5.4

Bound water

Considerable experimentation has been in progress, especial-
ly during the past decade, in connection with the determination of
bound water in plant and animal tissues. Agreement has not been
reached as to the best method or technique nor as to the conclusions
drawn from the data obtained. However, the methods in common usage,
namely, cryoscopic, calorimetric and dilatometric aim to take into
consideration the total water of the system and to subject it to condi-
tions which render some of the water unaccounted for. Thus a quantita-
tive difference is found between the total water content and that which
exhibits characteristics different from water in its normal free state.
The portion of the water which behaves in accordance with the recognized
characteristics of normal water is termed "free" water, whereas that which
has taken on different properties is known as "bound" water. The latter
is considered as adsorbed water, with the colloids of the system serving
as the adsorbing media. It should be pointed out that the percentage
of bound water, as determined, is probably the minimum quantity. Con-
clusive proof is lacking that the total water may be consistently ob-
tained by taking the difference between the weights of the live and
dessicated material. The conditions under which the material is usual-
ly dessicated, namely, at $100°C.$, or under vacuum at lower temperatures,
may either not remove all of the bound water or may possibly remove some
other volatile constituents, the loss of which has not been appreciated.

Rubner (13), Thoene (16) and Robinson (9), (10)and (12) have
made studies of the bound-free water ratio based on the hypothesis that
bound water would not feeze at $-20°C.$ Thus the materials they investigated

were held in a freezing chamber at -20°C., until the free water had frozen, before being transferred to the calorimeter.

Jones and Gortner (4) have recently made some important contributions to the general subject of bound water studies which are of special interest to the biological workers. They employed the dilatometric method and found in the case of gelatin and also of the white of an egg, that after equilibrium had been established for the frozen free water, the bound water present did not freeze even at temperatures as low as -50°C.

In their conclusions, these authors state "It is emphasized that 'bound' water is an indeterminate term, and that 'bound' water values, as experimentally determined, may be expected to vary from system to system, the variation being due to many factors, not the least of which is the method selected for measurement. If biological cells and tissues are similar in their behavior to gelatin and (probably) to the thick portion of egg white, then 'bound' water is a measurable entity and (using dilatometric procedure) has a constant value at least at temperatures between -6° and -50°C."

The results obtained, by Jones and Gortner (4), Sayre (14) and others with the dilatometer, indicated that the dilatometric method would be entirely suitable in connection with bound water determinations of the codling moth larva. The writer therefore conducted tests with a dilatometer as herein described.

Dilatometer apparatus for Bound Water

Determinations

Dilatometer: The dilatometer (Fig. 15) used in the present

study of bound water consisted of a capillary tube having a bore of

about 1 millimeter and a length of 55 centimeters. The capillary was

fused to a bulb which has a capacity of 25 cubic centimeters. A

graduated scale, as shown in the illustration, was attached to the

capillary tube. A glass stopper, accurately ground into the bulb and

provided with ears for the attachment of rubber bands, completed the

dilatometer.

Dilatometer liquid: Since the substance in the codling moth larvae

under investigation was water, toluene was selected as a suitable liquid

with which to fill the apparatus. Toluene is practically insoluble in

water and has a low freezing temperature.

Thermostat: A thermostat (Fig. 17) consisting of two large glass

containers, separated by a 3 inch layer of ground cork was prepared.

A motor driven stirrer provided a uniform temperature throughout the

calcium chloride solution which was used in the thermostat. The calcium

chloride solution was first cooled to the desired temperature and, as

it became warmer, small additions of slightly colder solution were added

from time to time. The temperature drift upward was exceedingly slow,

thus facilitating a temperature equilibrium between the contents of the

dilatometer and the thermostat solution.

Temperature of specimens: The temperature of the thermostat bath
-7.5°C., was recorded by means of a thermocouple and potentiometer,
the galvanometer being used as a null instrument. The temperature
recording apparatus is shown elsewhere (Fig. 3).

Calibration of dilatometer: The capillary of the dilatometer was
calibrated by the U. S. Bureau of Standards using mercury at 20°C
and water delivered at 20°C. The latter was allowed to drain for
15 minutes. The values given were reported as being accurate to
+ 0.0003 milliliters. The data are given in Table 23, and are shown
graphically in Figs. 13 and 14.

Table 23 –   Calibration of dilatometer

Divisions of dilatometer	Mercury at 20 °C (milliliters)	Water delivered at 20°C (milliliters)
1 - 4	0.1071	0.1057
1 - 7	0.2147	0.2121
1 - 10	0.3223	0.3195
1 - 13	0.4274	0.4240
1 - 16	0.5301	0.5256
1 - 19	0.6315	0.6258
1 - 22	0.7326	0.7252

*Drainage 15 minutes.   Values accurate to ± 0.0003
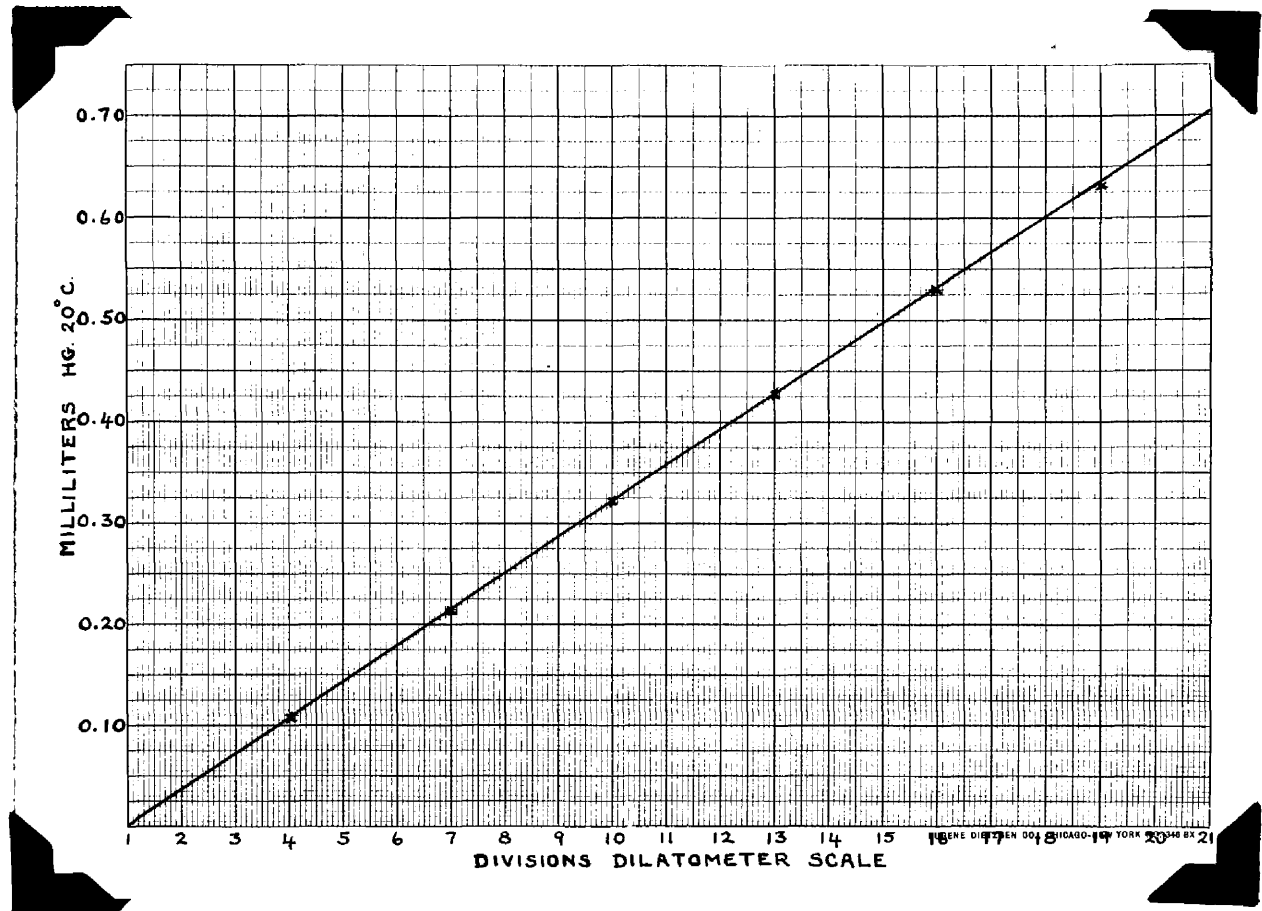U. S. Bureau of Standards.

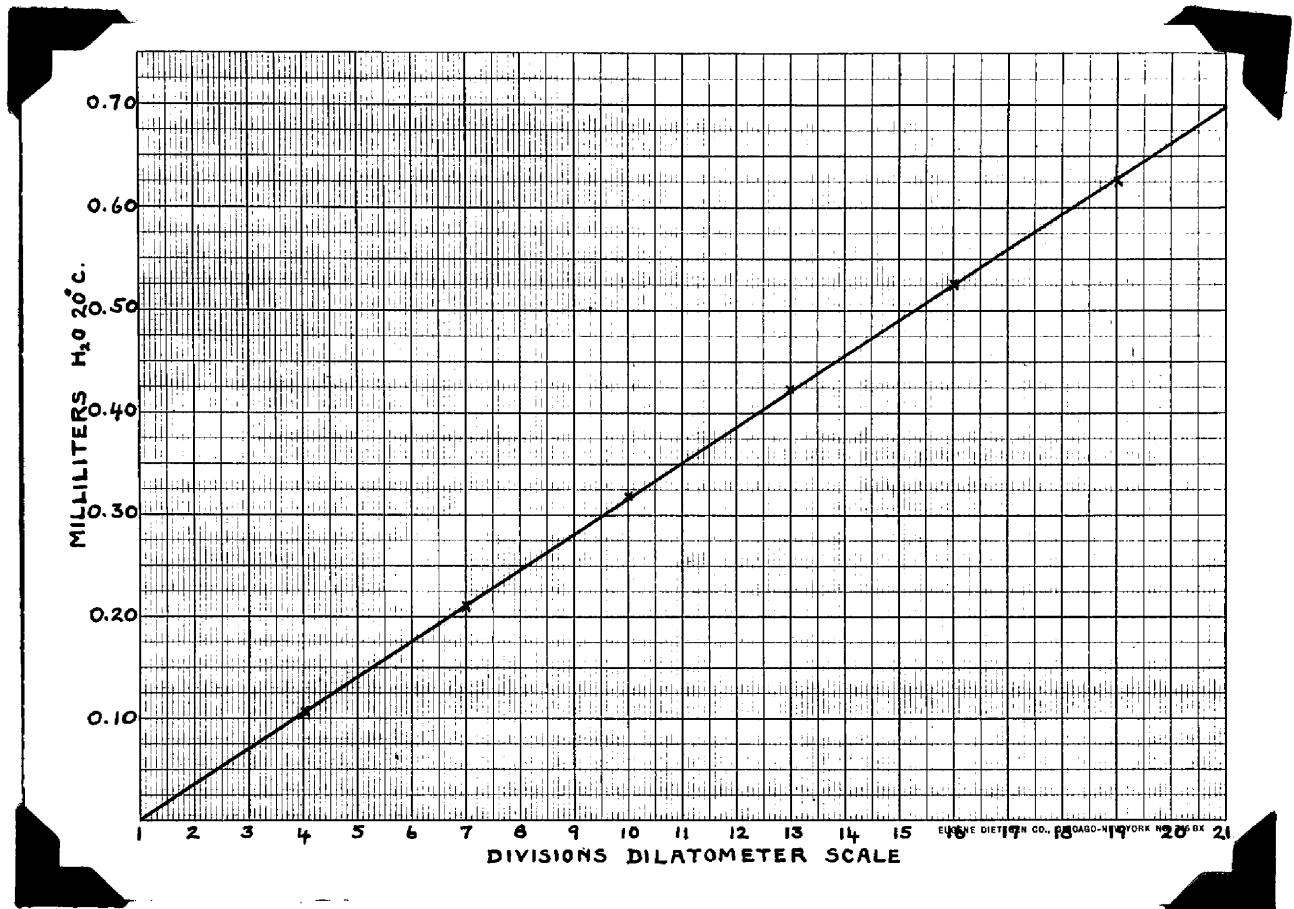Fig. 13 — Calibration curve for dilatometer.
Mercury in milliliters at 20°C.

Fig. 14 . — Calibration curve for dilatometer.
Water in milliliters delivered
at 20°C.

Procedure

In order to have dependable results, a large number of codling moth larvae, approximately 125, with a total weight of slightly over 5 grams, was used in each test. After weighing, the larvae were introduced into the toluene contained in the dilatometer bulb. The dilatomer was then filled with toluene and by means of a water aspirator the toluene was slowly drawn up the capillary to a certain reading on the scale. At the same time the glass stopper, smeared with a thin coat of glycerin, was slowly revolved into the bulb until it had seated. Care was taken to exclude any air bubbles. The ground glass joint held the stopper firmly in place, but as an extra precaution, rubber bands were used to prevent any movement of the stopper. In addition, the stopper was sealed at the top with a cement made from celluloid dissolved in acetone.

During the course of preliminary tests, it was found that the undercooling temperature of the larvae immersed in toluene was considerably raised and hence a temperature of $-7.5°C.$, was selected as the standard temperature of the thermostat bath. It was found that this temperature was safely above the undercooling temperature of the larvae and below the melting temperature of the frozen larvae.

Having established this temperature, it was then possible to conduct the entire study under the same temperature conditions, thus largely reducing experimental errors.

When everything was in readiness, the dilatometer containing the unfrozen larvae (Fig. 15   ) was placed in the thermostat and held therein until it had come into thermal equilibrium with the bath. The reading on the scale was then taken. The dilatometer was next transferred to a freezing chamber of $-35°C.$, and held therein until all the

larvae had frozen. The dilatometer with the frozen larvae (Fig. 16) was then replaced in the thermostat and, when temperature equilibrium was again reached at −7.5°C.

Dessicating the larvae: Upon completing the tests in the dilatometer, the larvae were transferred to an electric oven at 87°−89°C. and dessicated under vacuum by means of a Hy-vac motor driven pump as shown in Fig. 18. The dried larvae were weighed at intervals until approximately constant weights were obtained.

Results: The results obtained are given in Table 24. It will be noted by reference to this table that the range of bound water extended from 40.96 to 44.59 per cent with an average of 42.35 per cent.

Fig. 15 —Dilatometer bulb showing codling moth
larvae, natural color, before freezing.

Fig. 16 – Dilatometer bulb showing codling
moth larvae after freezing. Note
whitish color due to freezing
(compare with Fig. 15   ).

Fig. 17 – Dilatometer and constant temperature
        chamber.

A —   Dilatometer
B —   Constant temperature chamber
C —   Stirrer
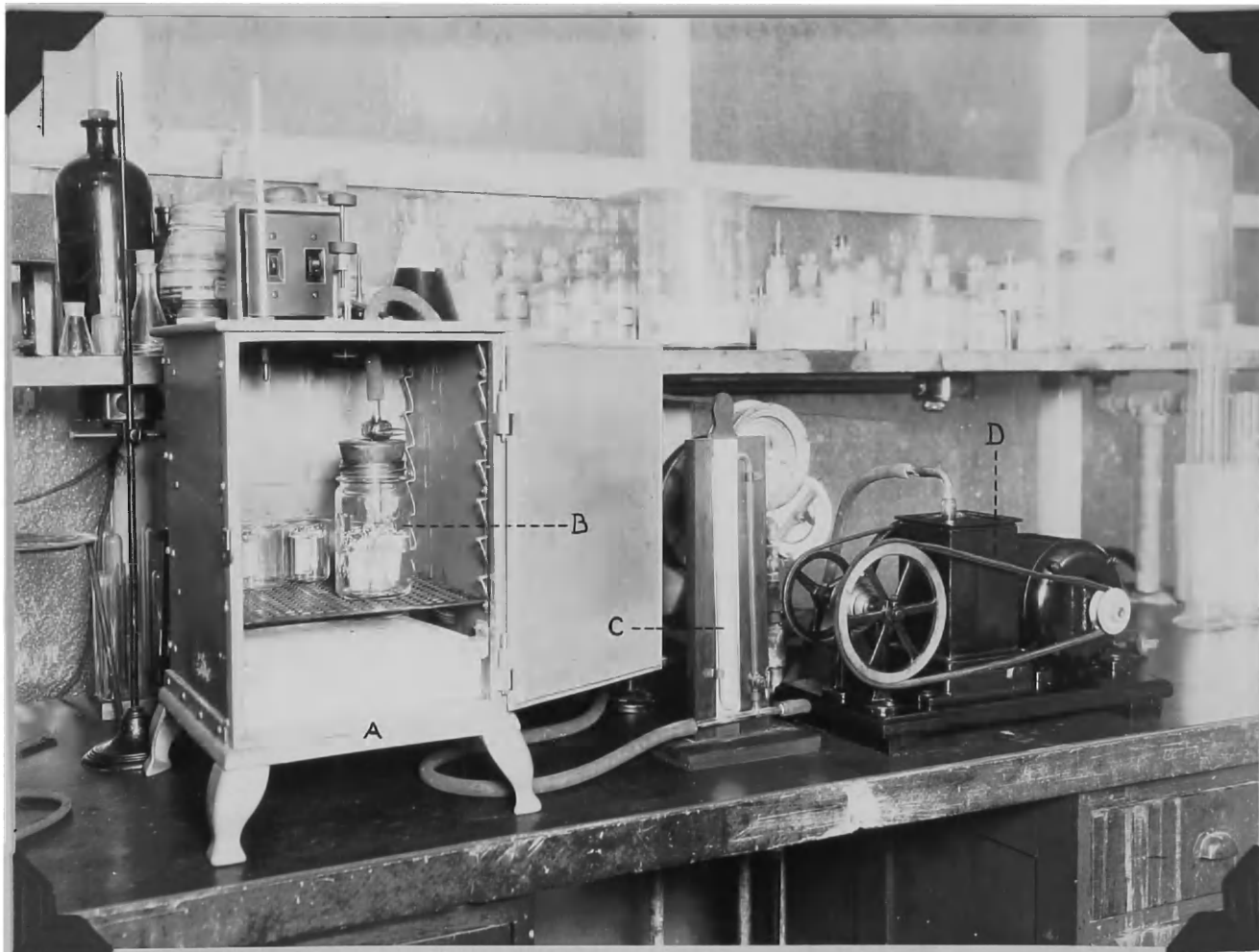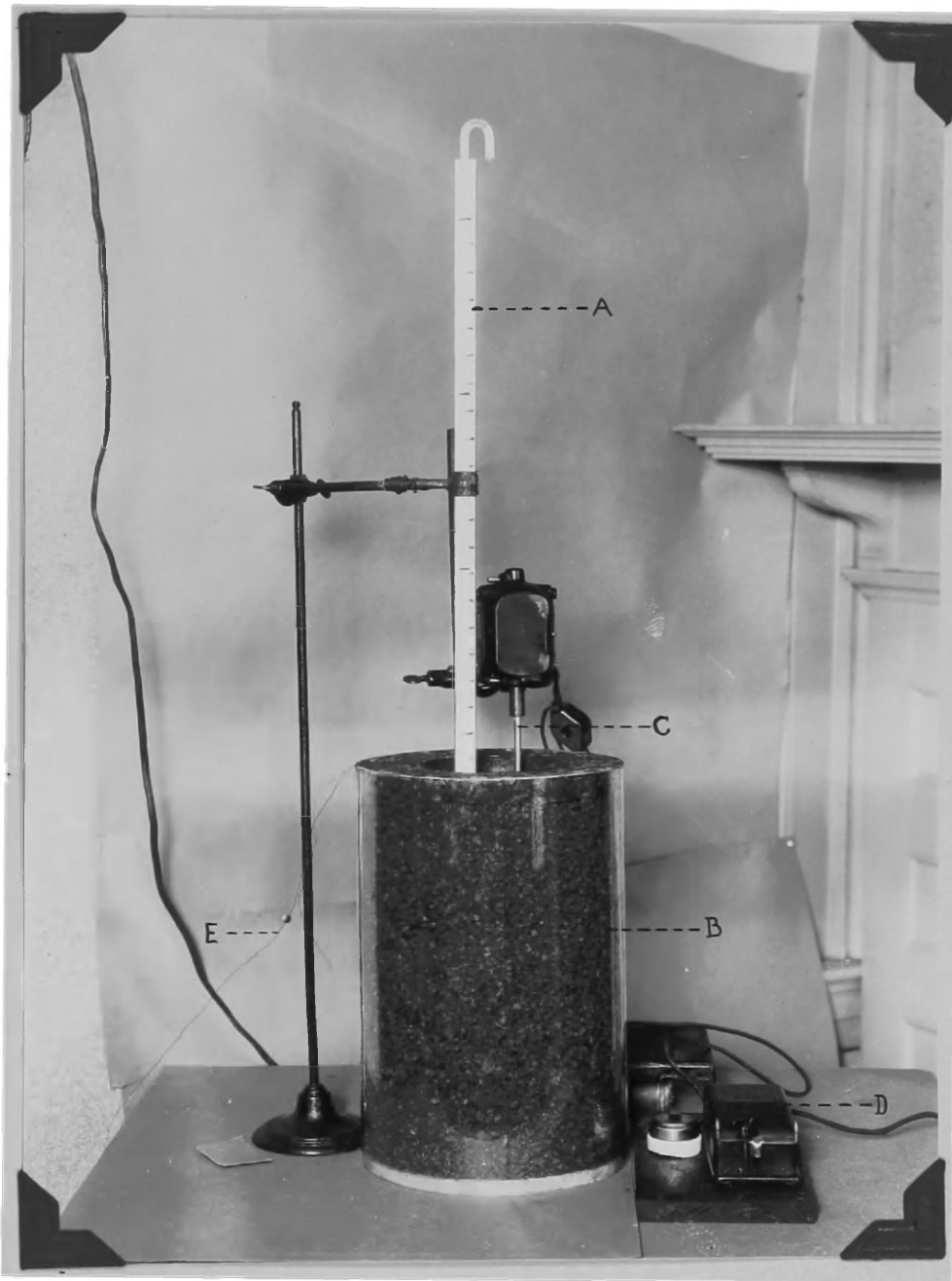D —   Rheostat
E —   Thermocouple

Fig. 18 -   Drying outfit
A -   Drying oven
B -   Glass container for specimens being dried
C -   Manometer
D -   Vacuum pump.

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Fig. 19 — Dilatometer in constant temperature chamber
A — Dilatometer
B — Constant temperature chamber
C — Stirrer
D — Rheostat
E — Thermocouple

Table 24 - Per cent of bound water in codling moth larvae,
dilatometric method, April 1933.

Test no.	Weight of live larvae in grams	Weight of dried larvae in grams	Total water in grams	Per cent of water in larvae	Capillary rise in dilatometer due to expansion of free water on freezing. Milliliters	Per cent of free water	Per cent of bound water
1	5.0211	2.2935	2.7276	54.3	0.1789	59.04	40.96
2	5.1637	2.3344	2.8294	54.8	0.1842	58.61	41.39
3	5.1060	2.2904	2.8156	55.1	0.1792	57.28	42.72
4	5.0981	2.3072	2.7909	54.7	0.1718	55.41	44.59
5	5.0877	2.3262	2.7615	54.3	0.1782	58.08	41.92
Average	5.0953	2.3103	2.7850	54.7	0.1784	57.65	42.35
Maximum	5.1637	2.3344	2.8294	55.1	0.1842	59.04	44.59
Minimum	5.0211	2.2904	2.7276	54.3	0.1718	55.41	40.96

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Calorimeter

For the bound water determinasions, heat of fusion method, a calorimeter, Figs. 20 and 21 , was assembled to take care of the work. The various parts are described below:

Calorimeter jacket: A silvered Dewar flask of 300 c.c. capacity Fig. 20, A, , was used as the calorimeter jacket. Although this is not as satisfactory for precision work as an adiabatic instrument, it served the purpose for the present investigation in view of its relatively good insulation properties.

Water container: A small glass shell vial Fig. 20 ,E ,held in place within the Dewar flask by means of a cork served as a container for the calorimeter water. In all tests the water was carefully pipetted into the vial so as to avoid drops on the side walls.

Stirrer: A mechanical stirrer of glass Fig. 20, B ,   was provided. It was made from a glass tube and was flattened at one end and twisted so as to give adequate stirring without splashing. This stirrer was attached to another glass tube or connecting shaft of the same diameter by means of a small piece of rubber tubing. The rubber tubing served as a universal joint Fig., 20, C , to take care of proper alignment, as well as to effect insulation between the stirrer proper and the connecting shaft, which latter was attached to a $\frac{2}{200}$ horsepower electric motor. The connecting shaft was held in alignment by passing it through a closely fitting lubricated glass sleve, the latter being held in place by a cork Fig.,20 , D .

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In order to check on the efficiency of stirring, a pinch of finely-divided kaolin was placed in the calorimeter water and the motor was started. The particles were observed to circulate freely and no splashing occurred.

Tin-foil containers: The water sample used in the determination of the thermal capacity of the calorimeter and the samples of insects used in the determination of the free water content and specific heat were enclosed in tin-foil containers as shown in Fig. 21. The thermocouple was held in the middle of the samples.
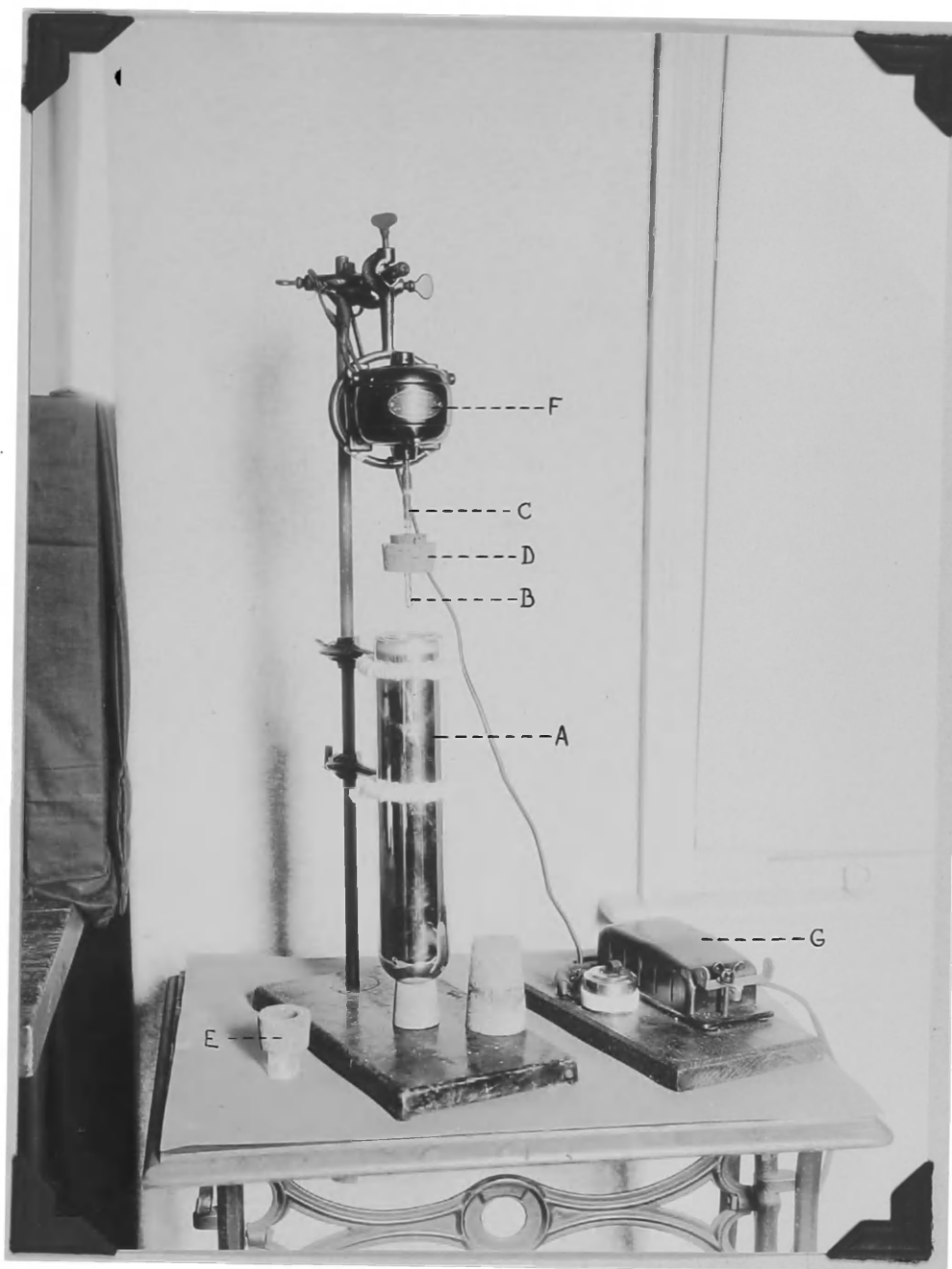
Fig. 20— Calorimeter, open

A — Dewar flask
B — Glass stirrer
C — Universal joint
D — Cork stopper for Dewar flask and bearing for stirrer
E — Container for calorimeter water
F — Motor for stirrer
G — Rheostat for motor
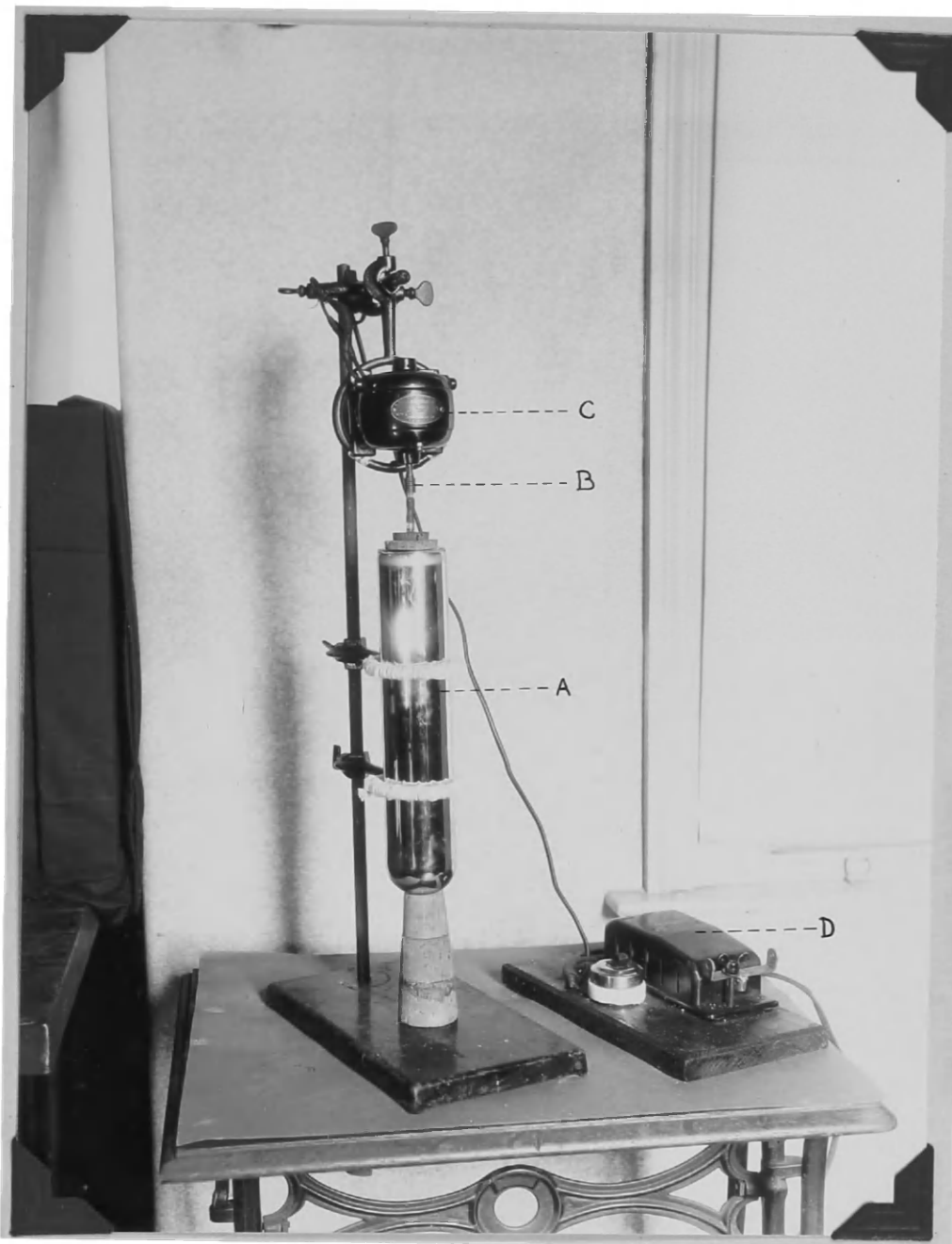
Fig. 21 — Calorimeter, closed
A — Dewar flask
B — Stirrer shaft
C — Motor for stirrer
D — Rheostat for motor

Fig. 21 — Container for specimen used in bound water determinations,
heat of fusion method.
A — Tin foil cylinder
B — Tin foil cylinder after folding for freezing of specimen
C — Tin foil cylinder as folded after specimen was frozen.

, Calculation of Free and Bound Water

All calculations in this paper, pertaining to the free — bound water ratio, were made in accordance with the most recent equations given by Robinson (12).

Thermal capacity of calorimeter: The thermal capacity of the calorimeter was obtained from the data presented in Table 25 and, as shown therein, the average was 1.2182.

$$\text{Equation:} \quad F = \frac{\dfrac{W(80-T_1) + W S R + W_1 S_1 R}{2}}{N(T_2 - T_3)} \quad .$$

F = correction for thermal capacity of calorimeter

N = volume of water in cc. used in calorimeter

T_2= initial temperature of water in calorimeter

T_3= final temperature of water in calorimeter

W = total weight of water

S = specific heat of water

R = range in temperature between T_1 and T_3

W_1= weight of tin foil covering

S_1= specific heat of tin foil which is 0.05

T_1= initial temperature of water sample in freezing
chamber. This being below zero appears to have a
minus value but the formula is constructed so that
the sign may be disregarded.

Table 25 –   Thermal capacity of calorimeter

Test no.	Weight of water sample in grams	Weight of tin foil container in grams	Initial temperature of frozen water C°	Initial temperature of calorimeter Water C°	Final temperature of calorimeter water C°	Thermal capacity calorimeter
1	2.5162	1.0201	-30	25.3	16.6	1.2050
2	2.5417	1.2301	-30	26.4	16.9	1.2097
3	2.5398	1.3081	-30	26.1	16.7	1.2191
4	2.5381	1.2466	-30	25.1	15.7	1.2198
5	2.5072	1.3434	-30	23.1	14.0	1.2107
6	2.5369	1.2726	-30	22.9	13.8	1.2126
7	2.5074	1.3183	-30	22.9	13.7	1.2108
8	2.5036	1.2600	-30	27.0	17.9	1.2539
9	2.5079	1.0670	-30	27.8	17.9	1.2298
10	2.5055	1.1957	-30	28.0	18.5	1.2112

Average   1.2182

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Specific heat of codling moth larvae: The specific heat was calculated by use of the following formula. The water content of the insect was considered sufficiently constant to permit the use of the live larvae in the determination of their specific heat. The data are given in Table 26 .

$$\text{Equation: } S = \frac{F N (T_2 - T_3) - W_1 S_1 (T_3 - T_1)}{T_3 - T_1} \times \frac{1}{W}$$

S = specific heat of codling moth larvae

N = volume of water in cc. used in calorimeter

T_2 = initial temperature of water in calorimeter

T_3 = final temperature of water in calorimeter

W = total weight of codling moth larvae

W_1 = weight of tin foil covering

S_1 = specific heat of tin foil which is 0.05

T_1 = initial temperature of codling moth
larvae in freezing chamber.

Table 26 - Specific heat of codling moth larvae, April, 1933.

Test no.	Weight of larvae in grams	Weight of tin-foil container	Temperature of larvae C°	Initial temperature of calorimeter water C°	Final temperature of calorimeter water C°	Specific heat of larvae
1	2.5094	1.2555	4.28	24.19	23.00	0.7714
2	2.4992	1.2891	4.23	23.07	22.02	0.7160
3	2.5003	1.2599	4.00	24.63	23.41	0.7547
4	2.5133	1.2569	4.02	24.53	23.36	0.7511
5	2.5229	1.2497	3.20	24.61	23.34	0.7677
Average	2.5090	1.2622	3.94	24.21	23.02	0.7522
Maximum	2.5229	1.2891	4.28	24.63	23.41	0.7714
Minimum	2.4992	1.2497	3.20	23.07	22.02	0.7160

Calculation of free water: The free water in grams was calculated by

means of the following equation and the data are presented in Table

Equation: $$Y = \dfrac{F\,N\,(T_2 - T_3) - (WSR + W_1 S_1 R)}{80 - \dfrac{T_1}{2}}$$

Y = free water in grams

F = correction for thermal capacity of calorimeter

N = volume of water in cc. used in calorimeter

T_2= initial temperature of water in calorimeter

T_3= final temperature of water in calorimeter

W = total weight of codling moth larvae

S = specific heat of codling moth larvae

R = range in temperature between T_1 and T_3

W_1= weight of tin foil covering

S_1= specific heat of tin foil which is 0.05

T_1= initial temperature of codling moth
     larvae in insect chamber.

Calculation of bound water: The bound water was obtained by difference between the total water and the free water content. The data, as obtained by the heat of fusion of ice method, are given in Table 27. It will be noted that the average percentage of bound water with this method was 11.15, whereas with the dilatometric method the average per cent was 42.35.

Table 27 – Per cent of bound water in codling moth larvae,
heat of fusion of ice method. April 1933.

Test no.	Weight of live larvae in grams	Weight of dried larvae in grams	Per cent of water in larvae	Weight of tin-foil container in grams	Tempera-ture of frozen larvae C°	Initial tempera-ture of calorime-ter water C°	Final tempera-ture of calorime-ter water C°	Per cent of free water	Per cent of bound wate
1	2.5296	1.1398	54.94	1.6355	-30.0	26.0	20.1	88.84	11.16
2	2.5083	1.1354	54.61	1.3596	-30.0	22.7	17.0	91.57	8.43
3	2.5117	1.0123	56.11	1.2655	-30.0	25.2	19.3	85.23	14.77
4	2.5212	1.0968	56.49	1.2375	-30.0	25.6	19.5	94.66	5.34
5	2.5286	1.1072	56.21	1.2659	-30.0	24.9	19.0	89.51	10.49
6	2.5175	1.0883	56.77	1.2783	-30.0	24.7	19.0	83.66	16.34
7	2.5092	1.0875	56.65	1.2069	-30.0	24.6	18.7	90.50	9.50
8	2.5041	1.1057	55.84	1.2195	-30.0	24.6	18.9	88.13	11.87
9	2.5050	1.1241	55.12	1.2694	-30.0	24.8	19.1	87.53	12.47
10	2.5036	1.0938	56.31	1.2500	-30.0	24.8	19.0	88.84	11.16
Average	2.5139	1.0991	55.91	1.2988	-30.0	24.8	19.0	88.85	11.15
Maximum	2.5296	1.1398	56.77	1.6355	-30.0	26.0	20.1	94.66	16.34
Minimum	2.5036	1.0123	54.61	1.2069	-30.0	22.7	17.0	83.66	5.34

(105)

Discussion and Conclusions.

The question as to whether the low fatal temperature
of the overwintering larva of the codling moth varies in the
different apple growing districts has never been previously de-
termined. The insect has shown a difference in susceptibility
to poisoning by lead arsenate according to toxicity tests conduct-
ed by Hough of the Virginia Experiment Station as well as by the
writer. But, with respect to cold-hardiness, there appears to be
no acquired resistance. The present investigation, however, has
shown an individual variation in resistance to low temperatures.
The reason for this is unknown in the absence of a chemical and
physical examination of the individuals which have exhibited such
differences. The role' played by the hydrophilic colloids in
binding water in individual larvae has not been determined. But
the colloid content might readily account for individual differ-
ences in cold-hardiness.

No correlation was found with respect to the weight and
sex of the larvae and low fatal temperatures.

The overwintering larva of the codling moth has a very
pronounced tendency to undercool. From the practical point of view
it is this phenomenon which protects the larva from almost total
destruction by cold in most of our apple growing regions. If it
were not for the larva's undercooling far below its true freezing
temperature, it would be killed at its freezing temperature a
few degrees below zero centigrade.

The percentage of bound water in the overwintering larvae was determined by two methods, namely, (1) the dilatometric and (2) the heat of fusion of ice method.

The results obtained with the dilatometric method gave an average of 42.35 per cent of bound water and with the heat of fusion method an average of 11.15 per cent. Although toluene, is generally considered an ideal liquid for use in the dilatometer, the fact that both the undercooling and freezing temperatures of the larvae were elevated is possibly against its use in this connection. The effect of the toluene, however, was not different from that of mercury which was tested as an alternative. The experiments with the dilatometer were carried out at $-7.5^{\circ}C$, whereas the temperature of the specimens in the heat of fusion of ice method was $-30^{\circ}C$. The latter temperature was used because it is the temperature just below the natural undercooling temperature of the codling moth larva.

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