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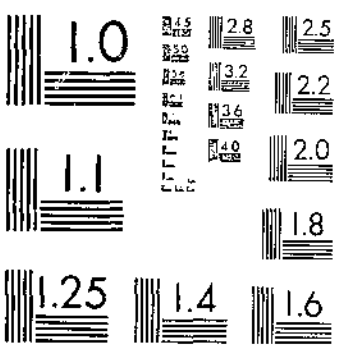

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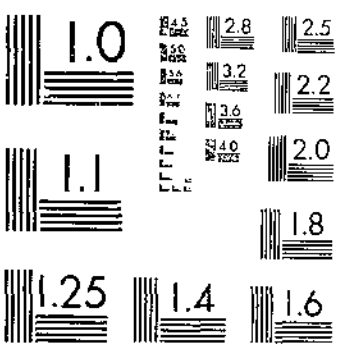

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TECHNICAL BULLETIN No. 400 September 1933

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

OBSERVATIONS ON THE THERMAL DEATH POINTS OF *ANASTREPHA LUDENS* (LOEW)

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THERMAL DEATH POINTS OF *ANASTREPHA LUDENS* (LOEW)

In recent years there has been a trend to apply to insect physiology the laws of physics and chemistry. Before this work can be accomplished, however, the fundamental physiological similarity of the insects to the other members of the animal kingdom must be stressed. The studies to be reported here were undertaken for the following reasons: (1) To see whether insect protoplasm is subject to heat coagulation at temperatures similar to those which affect other animals; and (2) to determine what climatic conditions can be expected to limit or prevent the establishment of *Anastrepha ludens* in regions as yet uninfested.

In the case of this organism, a Trypetid fly, whose developmental stages are passed in such diverse environments, it will be interesting to see whether the temperature limits are identical for all stages. For this reason and for practical considerations of handling the material, the experiments reported here were divided into three main groups, according to whether they dealt with larval, pupal, or adult forms. Of course, it is understood that the so-called "pupal" stage, characterized by the presence of the puparium, includes what is really a larval instar; but since the experimental technique was identical with that applied to true pupae, all phases of the puparial stage have been reported in the section dealing with pupae. The egg stage has not been studied at all as yet, owing to much greater technical difficulties in collecting and handling the material.

LARVAE

The initial experiments on larvae were suggested by the fact that in Cuernavaca, Morelos, where there is a very heavy infestation of

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A. ludens in mangoes, fallen fruits are frequently encountered with all the larvae dead. It was thought that heat from direct sunlight might be the cause of death. In fact, Crawford (*4*) attributes a light infestation in the fall of 1913 in the Tampico region to the high temperatures of the previous summer. If such was the case, then *A. ludens* larvae might not be able to stand the summer temperatures in the Rio Grande Valley.

Accordingly the first experiments were set up with the idea of reproducing field conditions. Eight hours is probably the maximum possible duration of direct sunlight for fruit in the field, while 4 hours would be a more usual situation. Batches of 10–14 infested mangoes were therefore exposed to various temperatures in an incubator for 4- and 8-hour periods. The larvae were removed from the fruits at the end of the exposure, placed on sound fruit pulp, and the numbers of live and dead individuals recorded after allowing time

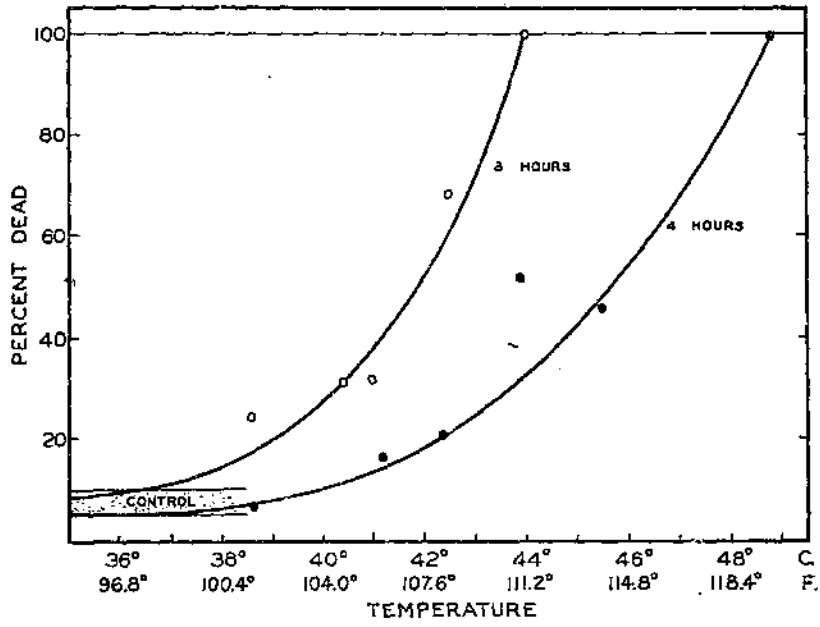

FIGURE 1.—The mortality of A. ludens larvae in original fruit (mangoes) at various temperatures.

for recovery. The results are plotted in figure 1, which shows that the larvae can withstand surprisingly high temperatures even for 8 hours. A single exposure to 41° C. (105.8° F.) for 4 hours would account for only 13 percent, and for 8 hours 37 percent. Grove conditions of shade, evaporation of moisture, and wind would render such a temperature for so long a period highly improbable. It might be stated here, however, that field observations made later on mangoes showed a definite relation between the variety of the mango and the frequency of dead larvae.

The above experiments were somewhat unsatisfactory for three reasons. First, individual fruits vary widely, and frequently contain all dead larvae before any experimental treatment whatsoever. As

the mangoes were used whole, there was no possibility of distinguishing those that contained live or dead larvae at the start. Second, there was a large variation in the texture and physiological state of the various mangoes, and in their degree of infestation. One heavily infested fruit that showed 100 percent mortality at the end of the experiment could overbalance many fruits with a lighter infestation and incomplete kill. In other words, the conditions for all the larvae were not alike, but this would be the situation in the field. Third, a mango reaches thermal equilibrium in the incubator very slowly, because conduction in the fruit is poor and evaporation from the surface keeps it cool. The larvae inside can therefore remain degrees cooler than the incubator temperature for some time after the start of the

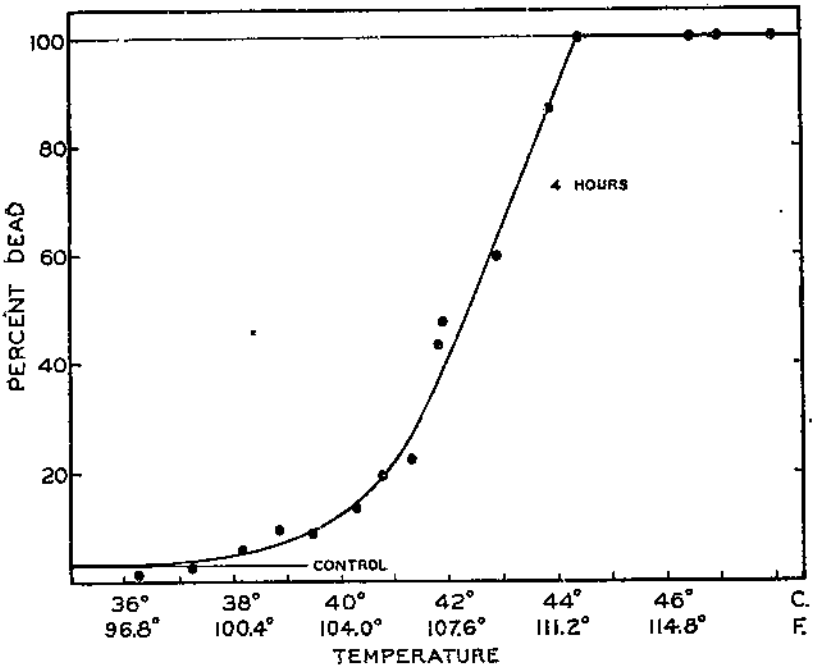

FIGURE 2.—Mortality of A. ludens larvae in sound ripe mango pulp after exposure to various temperatures for 4 hours.

experiment. An attempt was therefore made to standardize the environment of the larvae during exposure by removing them from the original fruit and placing them in covered dishes with pieces of sound ripe mango pulp. This technique permitted the use of a standard number of larvae (200) for each temperature, reduced evaporation to a minimum, and insured the rapid attainment of thermal equilibrium. The results are given in figure 2, for a series of 4-hour exposures.

The curve shows that there is a distribution of resistance to temperature in these larvae, so that at the lower temperatures only a few weak individuals are affected while more and more succumb as increasingly higher temperatures are used. See Brooks (*3*).

The behavior of the larvae under these conditions was interesting. When first removed from the incubators, particularly at the higher temperatures, all the larvae appeared dead; they were fully extended and would not respond to mechanical stimulation. After some hours at room temperature many of them revived and appeared perfectly normal. In order to test the viability of the survivors and see if they would show injury from the higher temperatures, they were kept on fruit in dishes with soil available. The number that pupated were recorded, and the results are given in table I.

TABLE I.—*The effect of exposure to high temperatures on the pupation of survivors*

Temperature		Length of exposure	Environment	Number of larvae survived	Number pupated	Percent pupated
° C.	° F.					
38.6	101.5	8 hours	Original fruit	139	21	15.1
40.4	104.7	do	do	78	34	43.6
41.0	105.8	do	do	114	40	35.1
40.8	105.4	4 hours	Sound pulp	161	85	52.8
41.35	106.4	do	do	157	77	49.0
41.8	107.2	do	do	114	47	41.2
41.9	107.4	do	do	104	13	12.5

Table I shows a high mortality in the larvae previous to pupation. This is undoubtedly due in part to the exposure to heat, but it must be kept in mind that the larvae were of mixed ages (mostly third instar), so that the younger ones had to remain on fruit for some time before full growth was attained. They were, therefore, subjected to many other influences during this period, such as changes of fruit, which may have had injurious effects. Nevertheless, a distinct decrease in the percent pupated is shown with increasing temperature in the last four items of the table. About 75 percent of these pupae emerged, as contrasted with the normal figure of 85 to 90 percent.

All of the foregoing figures are given in terms of the percent killed at a definite number of hours but at various temperatures. It seemed advisable also to know the percentage killed at a definite temperature by various lengths of exposure. A complete series was therefore run at 40.5° C. (104.9° F.). This temperature was chosen because it is at this point that many biological systems show the first signs of heat effects. Enzymes and other organic catalysts cannot be carried above this temperature without being inactivated. The results are given in figure 3. The experimental points fall along a curve whose general form is logarithmic. If, however, the logarithm of the fraction killed be plotted against the time, the figure obtained is not a straight line. A further discussion of this curve will be undertaken in comparison with the other stages.

On the lower end of the temperature scale the experiments were not so successful. Larvae were kept in a constant-temperature room at 3°–5° C. (37.4°–41.0° F.) for 7 days without any injurious effects. It was impossible to cool the room below this temperature for a length of time sufficient to kill.

Another series of experiments was started, in which larvae in U tubes were submerged in a brine tank at —3° C. (26.6° F.). Here the difficulty of supplying air was encountered. The larvae were frozen for 24 and 48 hours, but they survived in such large numbers that it was realized that a better system must be worked out before any consistent results could be expected.

PUPAE

Experimentation with pupae cannot be done satisfactorily without taking into account the morphological changes that take place within

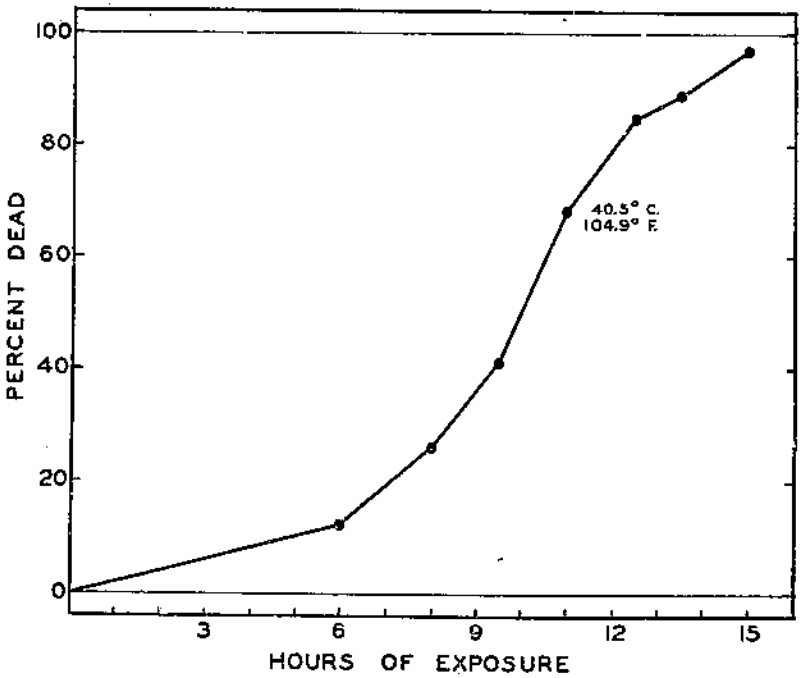

FIGURE 3.—The mortality of A. ludens larvae in sound ripe mango pulp after exposure to 40.5° C. (104.9° F.) for various periods of time.

the puparium. The reorganization of tissues during this period is so far-reaching that it would be absurd to suppose a uniform reaction to outside stimuli throughout puparial life. Fortunately, it has been found possible to determine by direct observation whether this organism is in the last (fourth) larval instar, or has "pupated" in the strict sense of the term. In determining the length of exposure to high temperatures that pupae can survive they were divided roughly into two groups—(1) young pupae, still in the larval condition; (2) selected pupae, with developing adult structures plainly visible, from which all undeveloped and parasitized individuals had been removed. Further subdivision, which requires large numbers of individuals and

very accurate timing at definite temperatures, was not attempted in this series of experiments.

Exposures were conducted in the following way: Larvae from field-collected mangoes were allowed to form puparia in moist soil. They were then removed from the soil, washed, and kept on moist cotton until needed. Those in the young group were used within 4 days at 25°±0.1° C. (77.0°±0.2° F.) after pupation, as repeated observations had shown 4 to 5 days to be the duration of the fourth instar at this temperature. Those in the "selected" group were also kept at 25°±0.1° C. for more than 5 days. Direct observation was then made on them under a binocular to see that they had actually transformed into pupae. At the same time it was possible to eliminate any parasitized individuals.

Enough pupae for at least six points per temperature plus a control were then counted out in lots of 100 or 150, allowed to dry super-

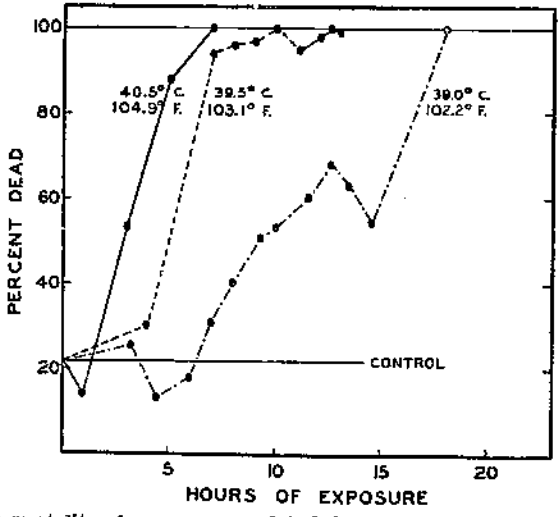

FIGURE 4.—The mortality of young pupae of A. ludens after exposure to various temperatures for various periods of time.

ficially, and placed in small glass-covered Petri dishes. Since this type of container permits some air exchange, it was impossible to supply moisture in direct contact with the pupae. Evaporation would have kept their temperature below that prevailing in the incubator. Some source of moisture, however, was essential, especially in the case of long exposures; and for this purpose a small wad of water-saturated absorbent cotton was placed on the lower surface of each lid, where it adhered perfectly without wetting either the junction between the top and bottom of the dish or the pupae themselves. A whole set (six or more dishes) was usually placed in the incubator at once, and removed, one at a time, after various intervals. Five minutes were allowed for the dishes to come into thermal equilibrium with the incubator before the experiment was considered to have started. The total swing of the incubator, as read from a standard thermometer next to the experimental dishes, was never more than ±0.1° C. (0.2° F.); and except

for variations in the strength of the electric current, which shifted the point of equilibrium slightly, was ordinarily ±0.03° C. (0.05° F.). At the end of each exposure, the pupae were transferred to a cool dish on wet cotton. In these dishes they were incubated with the controls at 25° C. (77° F.) until emergence took place.

It might be significant to state that in all cases pupae were reared in Petri dishes on absorbent cotton moistened with a very dilute solution of $CuCl_2$ (approximately $\frac{1}{1000}$). This technic effectively inhibits the growth of injurious molds and is quite harmless to the pupae, but the emerged flies must be removed at once, as the copper is fatal to adults when imbibed.

In all cases except one 100 pupae were used per exposure at each temperature. The exception was the set of young pupae exposed to 40.5° C. (104.9° F.), where the percentage of parasitism was known to

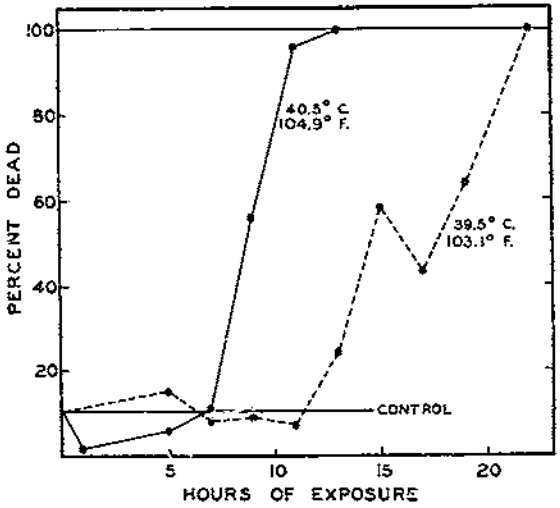

FIGURE 5.—The mortality of selected pupae of A. ludens after exposure to various temperatures for various periods of time.

be about 30 percent. One hundred and fifty (150) pupae were therefore used per point in order to have 100 flies on which to base the percentage of emergence. The controls were handled the same way as the experiments, except that the exposure to heat was omitted. The results are plotted in figures 4 and 5.

These graphs show a distinct difference in sensitivity to heat between the last larval instar and the pupal stage proper, the latter being much less sensitive. For instance, at 39.5° C. (103.1° F.) an exposure of young pupae for 10 hours resulted in a total kill, while selected pupae subjected to the same temperature for the same length of time seemed to be completely unaffected. Or again, at 40.5° C. (104.9° F.) the young pupae were all killed by a 7-hour exposure while the selected pupae were unaffected. The reorganization that is taking place in the young pupae seems to render it highly susceptible to injury by heat.

The results at somewhat lower temperatures were irregular. This is to be expected, as here the role of temperature is not so decisive.

Slight variations in rate of heating, humidity, age, etc., assume greater importance than at more distinctly lethal temperatures and therefore increase the variability of the results.

Some idea of the extent to which the curves will flatten out (approach the base line) at still lower temperatures can be gained from the limits of tolerance for pupal development. While an exposure of 13 hours is necessary to effect a 99-percent kill at 39.5° C. (103.1° F.), an exposure of 13 days is necessary to produce the same results at 31.4° C. (88.5° F.), a difference of only 8° C. (14.4° F.). A 13-day exposure at 31.4° C. resulted in a 99-percent kill of the pupae, while the same length of time at only 0.4° C. less (31° C. (87.8° F.)) killed only 60 percent.

Exposures to cold were conducted at temperatures intermediate between freezing and the lower limit for complete development. Air temperatures at and below 0° C. (32° F.) were impossible to maintain with the equipment available, so attention was confined to temperatures which the refrigerating system could maintain for long periods.

The lower thermal limit at which development can be completed lies slightly below 11.9° C. (53.4° F.), as at this temperature an exposure of 103 days (length of entire pupal period) produces a mortality of 73 percent. Even at 10° C. (50° F.) a few partly developed pupae have been observed after months of incubation. Complete inhibition of development therefore takes place at some point below this.

The mortality resulting from exposures to temperatures lower than 10° C. (50° F.) was determined in newly formed pupae. Field-collected larvae were allowed to form puparia in moist soil at 25° C. (77° F.). The pupae were collected within 12 hours of pupation, and after the customary washing were counted out in lots of 100 (or 125 in cases where the parasitism was high) and placed on wet cotton in Petri dishes. Some of these dishes were placed at once in an incubator at 9.7°±0.2° C. (49.5°±0.4° F.), others in a cold room at 4.2°±1.0° C. (39.6°±1.8° F.), where they were held for various periods of time. At the end of the exposure to cold they were warmed gradually (by passing through 12° and 18° C. (53.6° and 64.4° F.) incubators, 10 minutes each) to 22° C. (71.6° F.) where they were kept until emergence was complete. The results of these experiments are plotted in figure 6.

It will be seen from these two curves that, once below the threshold of development, the effects of different low temperatures are very similar. For instance, at 4.2° C. (39.6° F.) for 5 days 62 percent of the pupae were killed, while at 9.7° C. (49.5° F.) for the same length of time 55 percent were killed. Again, at 4.2° C. (39.6° F.) for 13 days 92 percent were killed, while at 9.7° C. (49.5° F.) for the same 13 days 89 percent were killed. A small percentage of very hardy pupae take somewhat longer to kill at the higher temperature than they do at 4.2° C. (39.6° F.).

This feature of the similarity of the effects of different low temperatures, once they are too low to permit development, has a very practical application. It is extremely costly to hold cold-storage

rooms at very low temperatures, even for short periods. It is much less costly to maintain a moderately low temperature for a longer period. In any case, then, where cold is to be used as a means of killing an insect pest, it would be wise to determine the exposures necessary to kill over the whole length of lethal cold temperatures, starting at the top. A longer exposure at a higher temperature would perhaps be just as effective as a shorter exposure to a temperature some degrees lower. The cost, however, would be very much less in the first case.

A further set of experiments dealt with the effect of the age of the pupa on its susceptibility to cold. The pupae were collected as before, but over shorter intervals (maximum, 3 hours). One hundred pupae

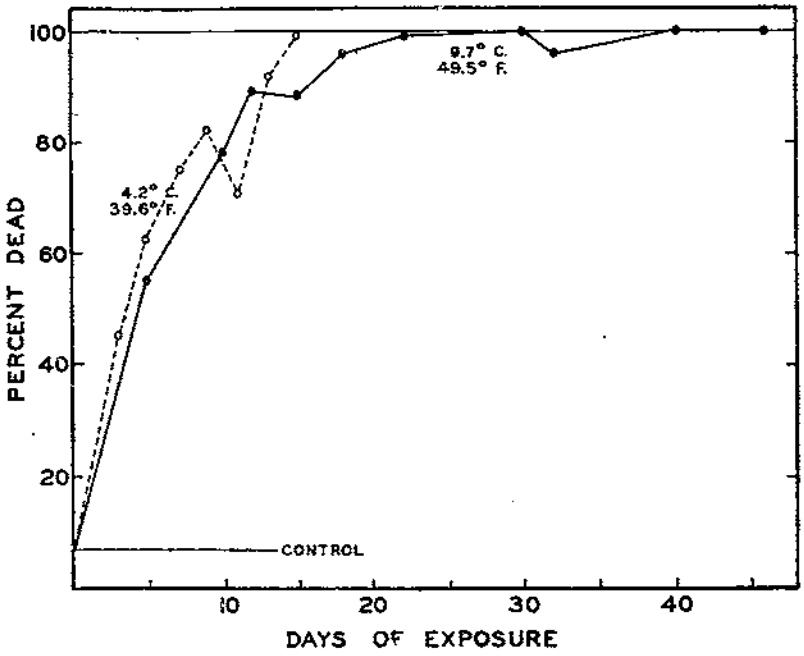

FIGURE 6.—The mortality of young pupae of A. ludens after exposure to low temperatures for various periods of time.

were used per dish. Some of these dishes were placed in the cold room immediately (4.2° C. (39.6° F.)), where they remained for periods varying from 3 to 15 days, exactly timed. The rest were placed in an incubator at 25° C. (77° F.). These latter were divided into several groups, which were incubated at 25° for exactly 2, 4, 6, 8, and 10 days, respectively, before transfer to the cold temperature for exposures of from 3 to 15 days, as above. At the end of its period of exposure to cold each dish was warmed gradually, as explained earlier, to 25°, at which temperature it remained until emergence took place.

The results of these experiments are plotted in figure 7. Each curve represents an age group, respectively, 0, 2, 4, 6, 8, and 10 days, at 25°; and shows the relation between duration of exposure to cold and mortality.

The graph shows clearly that at different stages of development pupae show a widely divergent susceptibility to injury by cold; furthermore, that this susceptibility does not increase or decrease in a regular manner throughout the course of the pupal period but goes up and down in an entirely unexpected way. For instance, 2-day-old pupae were killed off in much less time than newly formed pupae, yet 4-day pupae were not affected at all by the exposures comprised in this experiment. In this connection it will be recalled that 4 days

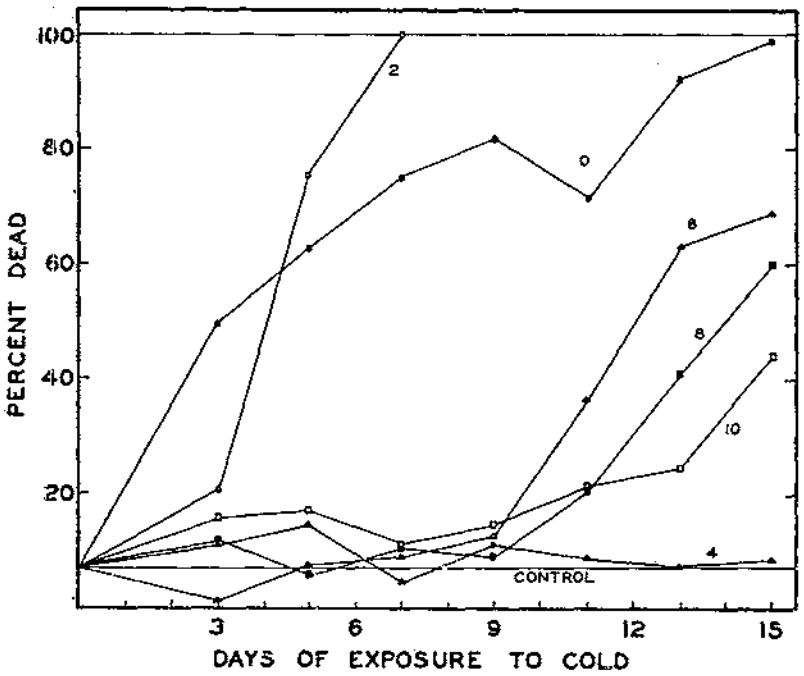

FIGURE 7.—The effect of exposing pupae of various ages to a temperature of 4.2° C. (39.6° F.) for various periods of time. The numerals on the curves represent the age of the pupae expressed in days of incubation at 25° C. (77° F.).

at 25° C. (77° F.) is approximately the age at which the change from the fourth larval instar to the pronymph condition takes place. The basic reasons for the rise in resistance between 2 and 4 days, and its subsequent fall from 4 to 6 days, followed by another rise, are at present entirely obscure. A really adequate study of this situation will have to include a finer subdivision into age groups at critical points, and exposures long enough to effect a complete kill at all ages up to 16 days, when emergence commences.

Another point that came up with the subjection of pupae of various ages to cold was their susceptibility to infections of mold. The percentage of pupae infected by mold was heaviest in those placed

directly in the cold room. The longer they were subjected to the low temperature the more the mold increased. The number attacked by mold, however, decreased steadily when more days were spent in the 25° incubator before transfer to the cold room. Very few pupae are attacked by mold when kept continuously at 25° C. (77° F.). As all the pupae were similarly washed and placed on cotton moistened with a copper chloride solution, the difference has to be attributed to the cold treatment.

A few direct observations were made on the origin of this mold. It is an internal growth that later escapes to the outside, visible first at the anal end of the puparium. From the anal end it spreads over the entire pupa. The growth of the mold is apparently not inhibited by the low temperature. The cells of the pupa at higher temperatures combat the mold successfully, and quite possibly this mold under such conditions is symbiotic. But at lower temperatures the cells are dormant (as shown by the length of time necessary for development at 25° C. (77° F.) after being subjected to cold for several days), and the mold, taking control of the organism, finally kills it.

ADULTS

Experiments in which adult flies were exposed to heat were conducted in the following way. Normal adults at least 3 days of age were transferred without anesthesia into large Petri dishes (diameter 6 inches, depth 1½ inches). Twenty-five pairs of flies were placed in each dish and the dishes then placed in an incubator at the desired temperature. The incubator was equipped with a fan, and when in equilibrium had a variation of not more than a tenth of a degree either way; but, of course, some cooling took place when the dishes were put in. In order to allow for this, and to allow the dishes themselves to warm up to the incubator temperature, the time when the incubator thermometer returned to its proper reading was taken as the beginning of the experiment. This was usually about 5 minutes after the dishes were put in. No water was supplied to the flies during the experiment, as evaporation would have furnished a location cooler than the experimental temperature. Controls kept at 22° C. (71.6° F.) in identical dishes and in the absence of water lived for at least 2 days before any of them died; whereas the longest of the experiments reported here lasted only 5 hours.

At the end of the exposure the flies were generally found lying motionless on their backs, and were immediately transferred to a cool dish, with a sop of wet cotton available. Recovery was rapid in the case of short exposures; but after the longer ones, sometimes a day or more would elapse before some individuals would respond to mechanical stimulation. For this reason, definitely live flies were separated from the rest as soon as they had had time to cool down to room temperature (22°–25° C.) (71.6°–77° F.) and at intervals thereafter. Those that had shown no response to mechanical stimulation by the end of 48 hours were recorded as killed. Exposures were made of various lengths at several different temperatures, from

39.6° to 43.1° C. (103.3°–109.6° F.). The results are placed in graphical form in figure 8.

The differences between the three higher temperatures are small, the slopes of the curves roughly similar. But below 40° C. (104° F.) the curve has a more gradual slope. Similar experiments were run using temperatures between 36° and 39° C. (96.8° and 102.2° F.), but gave irregular results. The cause for this irregularity is the same as that discussed in connection with pupae; namely, that at moderately injurious temperatures other variables can assume much more significance than at distinctly lethal temperatures.

The maintenance of sufficiently severe cold for the length of time necessary to kill was much more difficult than the keeping of a hot incubator. Only one series of exposures, therefore, was run at the lower extreme.

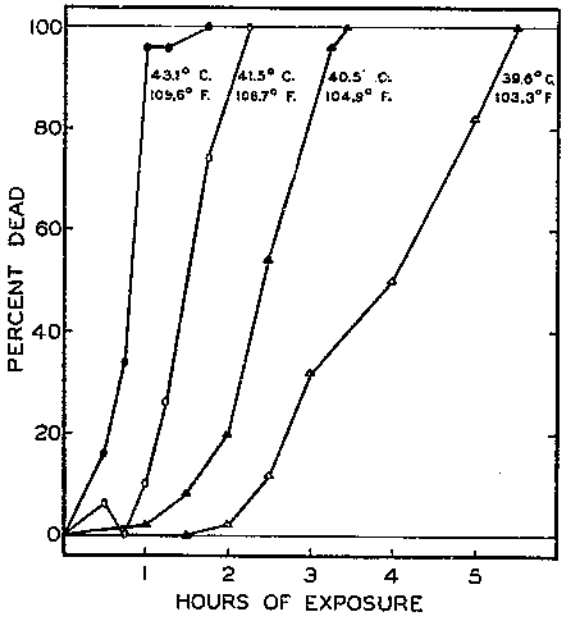

FIGURE 8.—The mortality of adults of A. ludens after exposure to various high temperatures for various periods of time.

Cheesecloth cages, with 50 pairs of flies in each, were kept in a refrigerator room for various periods of time. The room was kept as cold as possible, with a thermograph in it, from which the mean temperature over the various intervals was subsequently calculated. These means, with the variations in temperature, are to be found in table II. The flies were at no time below freezing, yet from the time they were put in the cold to their removal they were quite immobile. Some individuals even lost hold of the cage and fell to the floor, although most of them adhered to the walls. One cage of flies was transferred from the cold to a normal room (22° C. (71.6° F.)) at the end of each interval, and final counts were made 48 hours after transfer. The results of this series are plotted in figure 9. The control consisted of 200 pairs of comparable flies, kept under normally

satisfactory conditions of food and water at 22° C. (71.6° F.). The record of their dying off is also plotted in figure 9.

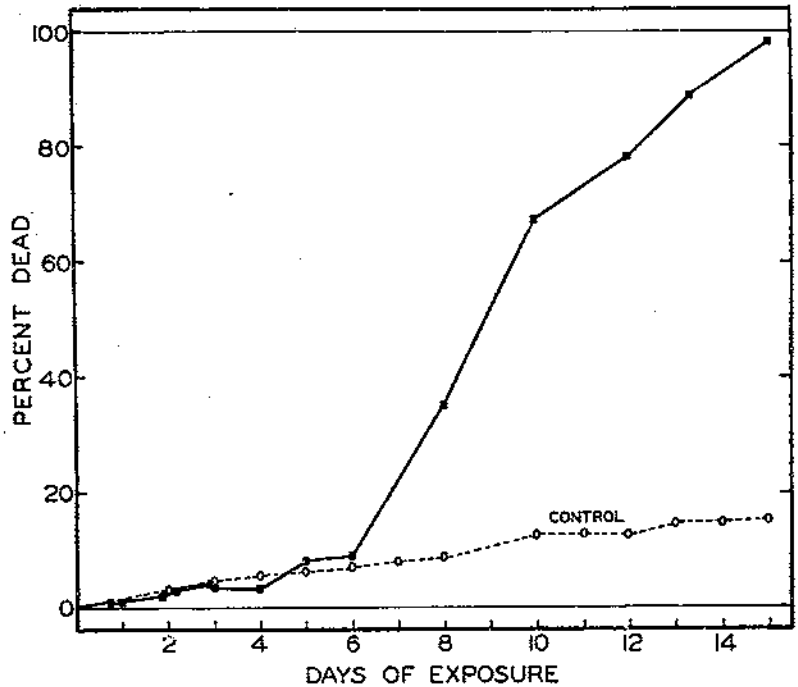

FIGURE 9.—The mortality of adults of A. ludens after exposure to cold for various periods of time. (See table II for temperatures.)

TABLE II.—*The effect of exposure to cold on adult A. ludens*

Temperature, ° C.			Days exposed	Number of flies used	Percent dead
Mean	Maximum	Minimum			
5.03	6.75	4.25	¾	100	1.0
5.03	6.75	4.25	1	100	1.0
5.10	7.00	4.25	1⅚	100	2.0
5.49	7.00	5.00	2⅛	100	3.0
5.61	8.00	5.00	2⅓	100	4.0
4.96	5.50	4.00	3	300	3.7
5.64	10.00	2.75	4	100	5.0
2.98	4.50	2.00	4	200	2.5
5.06	10.00	2.75	6	150	8.0
3.03	5.00	2.00	6	100	9.0
3.09	5.00	1.50	8	100	35.0
3.25	5.00	1.50	10	100	67.0
3.08	7.00	2.00	12	100	78.0
3.23	7.00	2.00	13⅓	100	89.0
3.36	7.00	2.00	16	100	98.0

It will be seen from the foregoing graph that *A. ludens* can withstand long continuous periods of low temperature. In the field the intensity of cold would vary with the time of day and would not be continuous. In the Rio Grande Valley at the Weslaco Experimental Station, Weslaco, Tex., the lowest air temperature re-

corded for 1930–31 was 1° C. (33.8° F.), but it lasted for only 2 hours. At noon of that same day the temperature was up to 16° C. (60.8° F.).

Two sets of flies chilled for 4 days at mean temperatures of 5.64° C. and 2.98° C. (42.2° and 37.4° F.) showed that once the temperature is low its exact value is immaterial, at least if it does not actually freeze the flies. Moreover, it must be remembered that the freezing point of protoplasm is not 0° C. (32° F.) but below, as the dissolved substances in living tissue depress the freezing point below that of water. It seems, therefore, that *A. ludens* can withstand considerable cold before death results.

DISCUSSION

There are two phases of the effect of temperature on this organism that require consideration: (1) The effects of high or low temperatures artificially produced; and (2) the effects of high or low temperatures met with in the field. In the former the attempt is made to find a temperature that will kill the organism and yet not injure the fruit; or, as in the case of the ovens used years ago in the State of Morelos, Mexico, to produce a high temperature merely to kill the larvae or pupae regardless of its effect on the fruit. Crawford (*4*) showed that heating fruit infested with larvae of *A. ludens* to 46° C. (114.8° F.) killed all the larvae within the fruit. The writer, unfortunately, was unaware of this work, but his results check those of Crawford. Subsequent to the first reports of the experiments on larvae described in this paper, the use of high temperatures was developed extensively in the campaign against the Mediterranean fruit fly in Florida. The subject was gone into so much more thoroughly there that more and fuller information (on the Mediterranean fly) will be obtained from their papers when published. It is sufficient here to say that high temperatures can be used against the larvae of fruit flies. The pupal and adult stages are not susceptible to such treatment for purposes of control.

The second phase to be considered has to do with the effects of temperatures met with in the field. In Cuernavaca, Morelos, they are never such as to exert a harmful influence on any stage of the fly. The low temperatures used in the experiments reported above are never experienced in this part of Mexico. Indeed, the fairly low temperatures in the field only serve to increase the organism's chances of survival. They prolong the larval and pupal periods, and permit the fly to emerge later when a new crop of fruit is just becoming available. Adult flies under such conditions merely suspend activities during the cold hours, resuming them later in the day when it warms up.

The low temperatures met with in the Rio Grande Valley are more severe than those in Cuernavaca, but not of lethal extent. Larvae have been carried to much lower temperatures for long-continued periods without harmful effects. Pupae would be harmed if the air temperature which sometimes occurs from 6 to 8 in the morning obtained also in the soil. But soil temperatures, recorded and furnished by W. H. Friend, of the Weslaco Experimental Station, Tex., while they show a much greater constancy than the air temperatures,

never reach and maintain the lethal point of 10° C. (50° F.) for several days.

As regards the adults, the air temperature would render them immobile for periods in the morning and thus possibly leave them at the mercy of their enemies. This does not take into account, however, that low temperatures have a similar effect on all protoplasmic systems and would also render the enemies sluggish. Of the possibility of direct effect, there is no trace shown by the experiments.

The maximum temperatures at Cuernavaca are also within the lethal limits. The rainy season, coming over the months of June, July, August, and September, cools down the air markedly (July is cooler than March) and supplies moisture for evaporation as well. Moreover, the elevation of Cuernavaca is about 5,000 feet, and the reduced pressure brings about a rate of evaporation much higher than that at sea level. Direct sunlight in these large shaded mango groves cannot last for many hours; while the final proof of the favorable nature of the climatic conditions is that 99 percent of the mangos are heavily infested at all times of the year.

In the Rio Grande Valley the temperatures are higher than they are in Cuernavaca. In the month of August 1930 the soil temperature at Weslaco, Tex., reached and stayed above 31° C. (87.8° F.) for over 2 weeks. The pupae in the soil at such a time would have been killed. Strangely, the air temperature was not very much higher, going only to 37° C. (98.6° F.) and not remaining there for long. Adults, therefore, could have sought shade in the center of the tree and remained unharmed by the heat. Larvae inside the fruit, especially under direct sunlight, would certainly have been adversely affected.

In the previous sections of this paper, no attempt has been made to compare all of the stages as to their susceptibility to temperature injury. They were, however, all subjected to a single temperature (40.5° C. (104.9° F.)) and the data on the differences between their reactions are placed in graph form in figure 10.

First, it will be seen that the various stages differ widely in their tolerance for 40.5° C. (104.9° F.). The adults are the most sensitive, followed by the young pupae, old pupae, and larvae. The reactions to heat, however, fall into two groups; the form of the curve for larvae is similar to that for young pupae, and the curve for old pupae is similar to that for adults. There are two factors that seem to be concerned—the position of the threshold of resistance, and the character of the chemical reaction that is involved in the killing process.

From the larvae to the young pupae, the threshold of resistance is changed—the larvae show a much greater resistance than the young pupae. But once this threshold is crossed, the curve for the killing off of the larvae is similar to that of the young pupae. Similarly, there is a change of threshold between the old pupae and the adults, again followed by a similarity of the curves.

It is clear that the resistance of the organism does not have a constant value, but that it alters from time to time. This is also borne out by figure 9 on the cold treatment of pupae. Back and Pemberton (*1*) report a difference of resistance to cold in the various larval instars of *Ceratitis capitata* (Wied.). But strangely these same

authors (*2*) state that there is no similar condition existing in the pupae of various ages. However, if their data are plotted, they will be found to show variations in resistance to cold depending on the age of the pupae. But in such work it is necessary to control the age (degree of development) of the pupae very carefully by collecting them at exact hours and holding them at constant temperatures. When these precautions were taken, clean-cut differences in resistance to both cold and heat were observed in *A. ludens* depending upon the age. In the sequence of age as regards heat the third-instar larvae are the most resistant. Young pupae, however, are very delicate, followed by older pupae with increased resistance. These in turn are followed by the adults with greatly diminished resistance to high temperature.

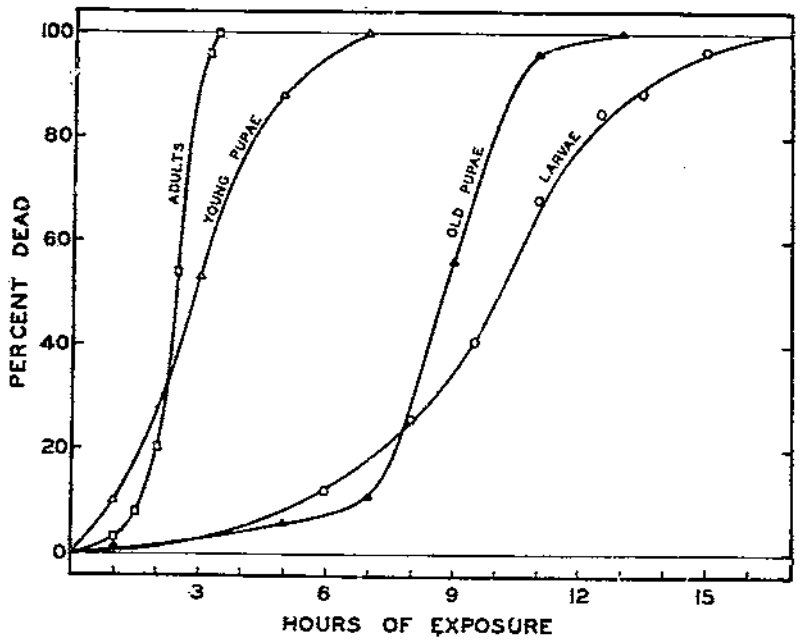

FIGURE 10.—The mortality of A. ludens at various stages of development after exposure to 40.5° C. (104.9° F.) for various periods of time.

As regards the similarity of the curves in figure 10, it will suggest to those who favor the master-reaction theory that reactions of similar type are involved in the killing of larvae and young pupae, and other reactions of a slightly different type in the killing of old pupae and adults. But when it is remembered that young pupae are really fourth-instar larvae within a puparium, and that old pupae are morphologically adults within a puparium, the similarity of these pairs of curves is noteworthy. Furthermore, it focuses attention on the change that takes place on the fourth day of pupal life at 25° C. (77° F.). The implication is difficult to avoid that there is a shift in the type of chemical system at this critical point. I have found that the CO_2 production at this stage in development undergoes a radical change.

Another feature of these experiments is that the criterion used was the actual death of the individual. This is by far the most satisfactory method, for when an organism is dead there is no question of further propagation. But the definiteness of this method of judging should not obscure another type of criterion, which might be very valuable in connection with the sterilization of food products, etc. This other criterion is "race death." When an organism is so injured that propagation is no longer possible, race death ensues. For instance, an organism can be rendered sterile by exposures to X-rays. The adult continues to live, but no offspring are produced.

Experiments in which the sterility of the adult is used as the criterion of the effectiveness of the treatment are of necessity longer than those using immediate death, since the entire life of the adult has to be observed. The value of race death as a criterion lies in the fact that lower temperatures (or shorter exposures) can be used to produce it. In this way deleterious effects on the food product might be avoided entirely. Injury, as Osterhout (*5*) has shown, is seldom followed by complete recovery—the more drastic the injury, the less the recovery. Should the temperature necessary to kill an insect outright be very close to that which destroys the host, it might still be possible to use temperatures that would produce race death with perfect safety to the host.

The question asked at the beginning of this paper, as to whether the protoplasmic system of the insects reacts to temperature in the same manner as that of other classes of animals, can now be answered in the affirmative. Many organisms show marked injury at temperatures of 40° C. (104° F.) and above. One degree below 40° C., however, is very much less toxic and much longer exposures are necessary to produce death. This difference holds true in the case of *Anastrepha ludens*, as can be seen from figures 2–4, especially.

In considering the organism as a whole, it would only be necessary to dislocate one part of the machine to wreck the entire system. In a recent paper Wigglesworth (*6*), working on the digestion of the cockroach, reports a tryptase closely resembling pancreatic trypsin. Since *Anastrepha* feeds on yeast, it must have tryptase of some type similar to pancreatic trypsin to digest proteins. The latter (trypsin) begins to show inactivation at 39° C. (102.2° F.). The tryptase of the fly would be destroyed by temperatures of 40° C. (104° F.) or more, and protein digestion would thus be eliminated. The above is given as an example of how the destruction of only one simple system could result in the death of the organism.

SUMMARY

1. The larval, pupal, and adult stages of *Anastrepha ludens* have been studied with reference to their reactions to high and low temperatures.
2. The lethal temperatures of the various stages of *A. ludens* have been determined and, similarly, the limits of tolerance of this organism.
3. Definite variations of response have been shown to depend upon the age of the organism.
4. Some further applications of these findings to the sterilization of vegetable products have been suggested.

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U.S. GOVERNMENT PRINTING OFFICE: 1933

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