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CONTENTS

THE EFFECT OF HIGH TEMPERATURE ON THE CONFUSED FLOUR BEETLE

M. J. Oosthuizen[1,2]

The application of high temperature has long been recognized as an effective control measure for insects infesting stored products that are not harmed by heat treatment. More and more it is being utilized for this purpose in flour mills.

The most important pest of flour mills in many regions is the confused flour beetle, *Tribolium confusum* Duval. While it is well known that the development of this species is favored by temperatures of about 32° C. (= 89.6° F.), exact data relative to the effects of higher temperatures are meager. There is here presented a detailed study on the effects of various such temperatures on the death rate of different stages, and on the fecundity and fertility of adults. The data obtained have both a practical and a scientific bearing.

Some of the more important conclusions are as follows. The eggs of this insect are killed in less than three days at 105° F. At a temperature no higher than 100° F. there is a considerable reduction in the percentage of eggs that will hatch. The pupa is the stage most resistant to heat. Exposure to high temperatures produces varying degrees of sterility in female beetles, depending upon the temperature and the length of exposure. Males recover from such exposures sooner and more completely than the females.

Practical questions frequently arise regarding the heat resistance of certain stages of the flour beetle, such as the egg. It is sometimes desirable to use a temperature somewhat lower than that usually recommended in order not to injure certain products. How much longer must heat be applied in such a case? Does a different moisture content of the air result in a deleterious effect upon the results one may obtain from heat treatment of an infested building? Or can one change the humidity to make the treatment more effective? These are some of the questions that have been answered, at least in part, by the results reported in this bulletin.

[1] Removed January, 1935, to the Department of Agriculture, Pretoria, Union of South Africa.

[2] The study here reported was conducted under the supervision of Dr. H. H. Shepard. For his constant interest and many helpful suggestions, the writer is deeply indebted.

PART I. THE RATE OF MORTALITY OF THE VARIOUS STAGES OF THE CONFUSED FLOUR BEETLE EXPOSED TO HIGH TEMPERATURES

INTRODUCTION

The resistance of insects to heat varies with the species, with the individual, with the developmental stage, and with the environment. The optimum temperature for the development of *Tribolium confusum* lies from 27° to 32° C. (80.6° to 89.6° F.), but any temperature much above this range will produce harmful results and eventually death.

High temperatures fatal to insects are reported often in the literature, but in most cases percentages of mortality in a considerable population are not given. Often the information is practically without value because the duration of exposure is lacking. In order that a quantity of heat may act to produce death of an insect, there must be a certain intensity of heat (temperature) acting for a period of time. Death is not instantaneous, altho, at very high temperatures, it may result from almost immeasurably short exposures. The actual quantity of heat (as measured in calories) required to kill an insect has never been determined. Quantity of heat is proportional to intensity over a period of time. Time-temperature relations, therefore, have much the same significance as well as the advantage of being readily comprehended by the average person.

THE HISTORY OF THE USE OF HIGH TEMPERATURE IN THE CONTROL OF INSECTS IN STORED PRODUCTS

Duhamel du Monceau and Tillet (1762), working in western France during a severe outbreak of what is now known as the Angoumois grain moth, *Sitotroga cereallela* Oliv., reported that a temperature of 69° C. suffices to destroy caterpillars in grain spread out in a thin layer and left in the oven for three days. They gave illustrations of several ovens that can be used successfully in the heating operations. Prompt threshing, exposure to high temperature, followed by storage in bulk, were suggested as successful control measures for this pest.

John Curtis (1860), speaking of the same insect under the name, *Butalis cereallela* Oliv., the little corn moth, said: "It appears that, of the various attempts made to prevent or diminish the ravages of this moth, the most effective method is to subject the infested grain to the heat of an oven or a very warm room. . . . . It is not, however, so much the intensity of the heat, as its continued action for a certain period which kills the caterpillars and chrysalides in the grain so that from 45° to 50° R. (= 56° to 62° C.) during 24 to 36 hours produces more effect than 76° to 90° R. (= 95° to 112° C.) for one hour." He mentioned two machines called "insect mills" which were invented by Marcellin and Dubillon in France for heating grain. Concerning the control of *Sitophilus granarius* Linn. (the granary weevil) and *S. oryzae* Linn. (the rice weevil), he quoted a certain Mr. Mills, who, while in Maderia in 1835, had noticed that a man named Wilkinson "has now established a heated room with hot water pipes, in which he receives as many as 800 bags of wheat at a time; these become heated through at about 135° F. (= 57° C.) and the wheat, when resifted, is perfectly cleansed from these noxious insects, and makes quite as good bread as before. I also tried some of it in the ground, that had been subjected to this heat, and it came up."

The first reference to the use of heat against stored product insects in the United States was apparently made by Lintner (1885). For the control of the rust-red flour beetle (*Tribolium ferrugineum* Fabr.), he recommended an exposure to heat as the best known method and suggested a temperature of 120° to 130° F. (= 49° to 54° C.) continued for a few hours for eggs, larvae, and pupae, and 150° F. (= 66° C.) for adults.

Webster (1883) recommended the use of heat for the destruction of the Angoumois grain moth. A temperature of 140° F. (= 60° C.) for nine hours "literally cooks the larvae and pupae," for five hours this temperature proves fatal, while 110° F. (= 43° C.) for six hours is only partially effective. He showed that wheat could be heated to 150° F. for eight hours without injuring its germinating properties.

In 1910 and 1911 heat treatment was applied in several mills in Kansas, Ohio, Iowa, Illinois, and Canada.

Goodwin (1912) concluded as follows with regard to heat treatment: "It is the most thorough method of treatment for the control of insects infesting flour mills; it requires but one treatment per year to completely rid the mill of insect pests, and no preliminary cleaning is necessary. . . . . The cost of treatment after the heating system is installed, is less than one-fifth of that of hydrocyanic acid gas fumigation. High temperature, as compared with other methods of treatment, will pay for the average heating system required in a flour mill in less than five years." Later he gave (1922) recommendations for successful heating plants such as had been installed in more than 30 flour mills in Ohio and Pennsylvania.

Dean (1913) gave temperature records and other data from mills that had used heat successfully. He concluded: "Many mill insects do not yield readily to hydrocyanic acid gas, but no mill insect can withstand, for any length of time, a temperature of from 118° to 122° F. (= 48° to 50° C.). The baking tests of all these experiments showed conclusively that the heat had absolutely no deleterious effect on the baking qualities of the flours."

de Ong (1919), testing the effects of temperatures up to 158° F. (= 70° C.) for two hours on about 50 kinds of grains, legumes, and nuts, has conclusively demonstrated that heat sterilization is a safe practice for both grains and legumes and affects germination of these in no way.

Dean and Schenk (1929) stated: "The writers believe that the possibilities of heat as a practical and an effective means of controlling grain-infesting insects are very promising, and that suitable apparatus or appliances will be developed whereby grain can be treated with heat on a smaller scale than by the large commercial driers. For several years the heat method has been used extensively in the United States, Egypt, and other foreign countries, for treating seeds."

EXPERIMENTAL METHODS

The different stages of the confused flour beetle were reared in a special grade of whole wheat flour in which about 95 per cent of the bran was ground fine enough to pass through the bolting cloth sieves used to separate the eggs from the flour. Most of the bran in ordinary whole wheat flour would be removed by such sieves. Before exposure to high temperature, the insects were sifted from the flour.

Air cabinets in which the relative humidity ranged from 20 to 25 per cent were used in which to expose the different stages to constant temperatures of 38.0° ± .5° C. and 41.0° ± .5° C. After exposure for various known periods of time, the insects were removed and placed in flour at 32° C. Daily observations of survival (hatching, pupation or emergence) were made.

The apparatus used in the exposure of the different stages to constant temperatures of 44° and 46° C. consisted of a wooden water bath having internal dimensions of 48 by 20 by 20 inches and a capacity of about 84 gallons. Three movable wooden frames, each having five circular equidistant holes large enough to fit around the necks of quart jars, were made to fit lengthwise in the bath. The temperature of the bath was controlled by means of electrical heating units, a mercury thermostat, and a relay. Each jar was fitted with a two-hole rubber stopper through which was passed a thermometer and a glass tube one-fourth inch in diameter. The thermometer bulb and the lower end of the glass tube were kept at the same level. A section of glass tubing one and one-half inches in diameter and about one and one-fourth inches long was made into a cage by shellacking silk bolting cloth to one end, which served as the bottom of the cage, a two-hole cork stopper being fitted to the other end. The lower ends of the thermometer and the one-fourth inch tube were passed through the cork stopper so as nearly to touch the bolting cloth. In this manner the glass-walled cage was suspended in a quart jar in which the humidity could be controlled. Relative humidities of 0 and 30 per cent were obtained by placing in the bottom of each quart jar concentrations of sulfuric acid made up according to the tables of Wilson (1921). A relative humidity of 75 per cent was obtained by using a saturated solution of sodium chloride, and one of 100 per cent by using distilled water. The test insects were introduced into the cage through the small glass tube, after each jar had been brought to a constant temperature in the water bath. The insects, not being able to crawl up the glass side of their cage, were confined to the level of the cloth floor near the thermometer bulb. The upper end of the small tube was closed with a cotton plug during the exposure. The racks were made to hold 14 jars immersed up to their shoulders in the bath (with an extra hole for stirrers and other equipment), enough to insure running a long series of experiments at one time. Sometimes a slight drop in temperature within a cage was noticed after introduction of the insects. The body of water in the bath was, however, so great that a return to the desired temperature was soon attained. After exposure for the desired period of time, the rubber stopper with attached cage and insects was removed, and the exposed individuals placed in pill boxes containing flour at 32° C. Observations as to survival were made daily. After each series of exposures, air at about the same relative humidity as that in the jars was passed through each one to remove the carbon dioxide produced by the insects during their exposure.

The percentage of mortality was calculated in the case of exposed eggs, larvae, and pupae according to the formula of Abbott (1925):

$$\frac{x - y\ (100)}{x},$$

where x is the number of survivals in the check lot and y that in the exposed lot. There is no mortality of adults of this species in a check lot from a vigorous culture of medium age.

EXPERIMENTAL RESULTS

The rate of hatching, pupation or emergence; the survival in individual experiments; and the fecundity and fertility of individual females have been omitted in most cases from this bulletin. Detailed original data are contained in manuscript copies of a report of the work, however. Scientific workers desiring to see this material should correspond with the Division of Entomology and Economic Zoology of the Minnesota Agricultural Experiment Station.

Eggs.—Eggs, one to six hours old, laid at 32° C., were selected under a binocular in four groups of 100 each and placed in separate pill boxes at a temperature of 37.5° to 38.5° C. until hatched. Observations were made twice daily to determine the numbers hatching and the mean incubation period.

Table 1.—The Hatching of Eggs of *Tribolium confusum* Kept in an Air Cabinet at 37.5° to 38.5° C. (= 100° F.)

Box No.	Numbers hatched						Per cent hatched
	3d day	4th day		5th day		6th day	
	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	
1	1	1	13	39	3	3	60
2	2	1	14	36	6	1	60
3	2	..	18	42	..	3	71
4	4	..	15	42	3	1	65
Average hatch							64.0

The average hatch of 64 per cent obtained at this temperature, when compared with 88.5 per cent in the check lots held at 32° C. (= 90° F.), is indicative of a considerable reduction in viability at the higher temperature. Chapman and Baird (1934) found the mean duration of the egg stage of this beetle to be 4.4 days at 32° C. The mean duration is apparently a trifle longer at 37.5° to 38.5° C.

Exposures of eggs from one to twelve hours old in duplicate series of 100 each for each of the periods 24, 48, and 60 hours, to a temperature of 40.5° to 41° C. (= 105° F.), showed average percentages of mortality of 86, 99, and 100 per cent, respectively.

Eggs, kept at 27° C. until three days old, were exposed in duplicate series of 100 each for each exposure at 44° C. (= 111° F.) and either 0 or 75 per cent relative humidity. After exposure the eggs were placed at 32° C. to be observed for the number hatching in each lot. The data are shown in Figure 1, each point representing the average of each pair of duplicate experiments. In many cases, at either relative humidity, larvae were observed trying to back out of the egg instead of emerging head first as in the hatching of normal eggs.

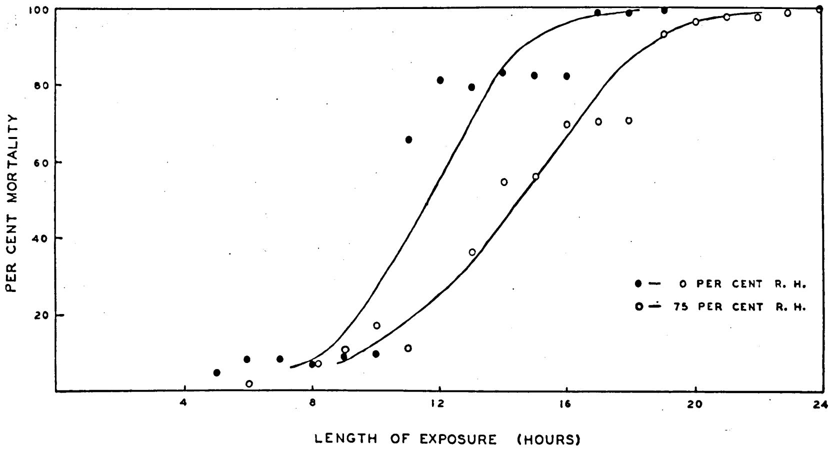

Fig. 1. The Mortality of Eggs of *Tribolium confusum* Exposed at 44° C. to Relative Humidities of 0 and 75 Per Cent

Eggs three days old were exposed to a temperature of 46° C. (= 115° F.) and four different relative humidities (0, 30, 75, and 100 per cent) in four groups of 50 eggs each for each set of conditions. The exposed eggs were placed in pill boxes at 32° C. and observations made twice daily at 12-hour intervals from the time hatching was first noted. The results are shown in Figure 2.

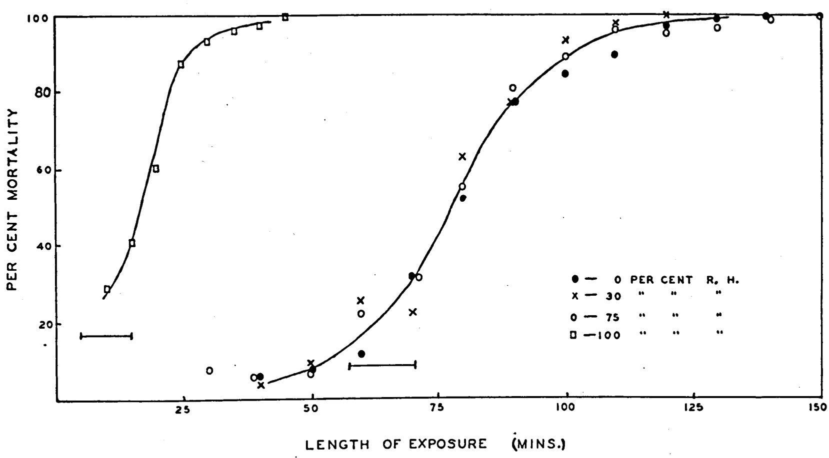

Fig. 2. The Mortality of Eggs of *Tribolium confusum* Exposed at 46° C. to Relative Humidities of 0, 30, 75, and 100 Per Cent

The horizontal bars indicate, respectively, the mortality in the control kept at 100 per cent and the average mortality in the controls kept at the other humidities.

Larvae.—Four groups, each including 50 larvae from one to six hours old, were kept at 37.5° to 38.5° C. The average pupation in these lots was 92.5 per cent. The mean larval life was 22.6 days, as compared to 17.4 days at 32° C. reported by Chapman and Baird (1934). The higher temperature, therefore, retards larval development.

Larvae exposed to a temperature of 40.5° to 41° C. from the time they were one to six hours old were unable to complete their development. Mature larvae were also exposed to this temperature; 18 per cent mortality was produced in a lot of 250 individuals exposed for 36 hours, 52.7 per cent in one of 450 individuals exposed for 48 hours, and 78 per cent in one of 300 larvae exposed for 60 hours.

Mature larvae in duplicate groups of 100 each were exposed to a temperature of 44° C. and to two different relative humidities, 0 and 75 per cent. After each exposure, the larvae were placed in pill boxes with flour at 32° C. The results are shown in Figure 3.

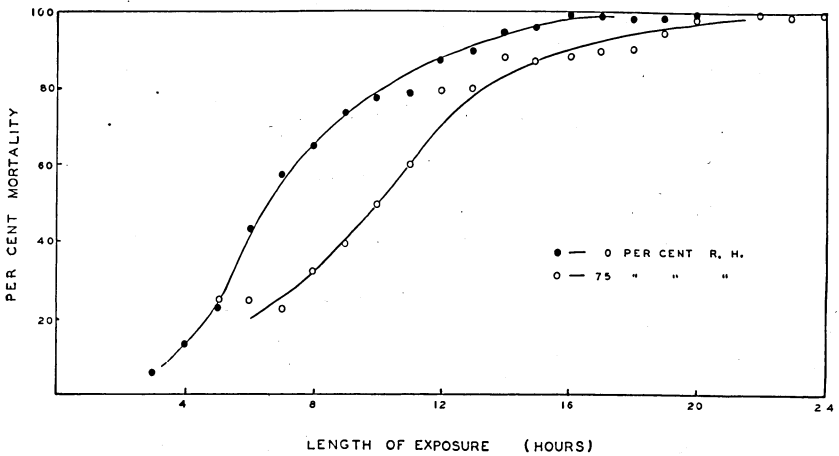

Fig. 3. The Mortality of Mature Larvae of *Tribolium confusum* Exposed at 44° C. to Relative Humidities of 0 and 75 Per Cent

Table 2.—The Retardation of the Pupation of Mature Larvae by Exposure to a Temperature of 46° C. (= 115° F.)

	Per cent relative humidity			
	0	30	75	100
Check, per cent pupation within 6 days	99.0	95.0	100.0	97.0
Exposed 30 minutes, per cent pupation within 6 days	68.9	76.3	87.0	51.1
Exposed 30 minutes, per cent mortality	2.0	3.0	3.5	6.0

Mature larvae were exposed in groups of 50 individuals each, four groups under each set of conditions, at 46° C. (= 115° F.) and relative humidities of 0, 30, 75, and 100 per cent. The exposed lots were placed at 32° C. for observation as to pupation and mortality. The mortality data are shown in Figure 4. That pupation of the survivors is retarded is shown in Table 2. The averages are corrected for the mortality of the check at each humidity.

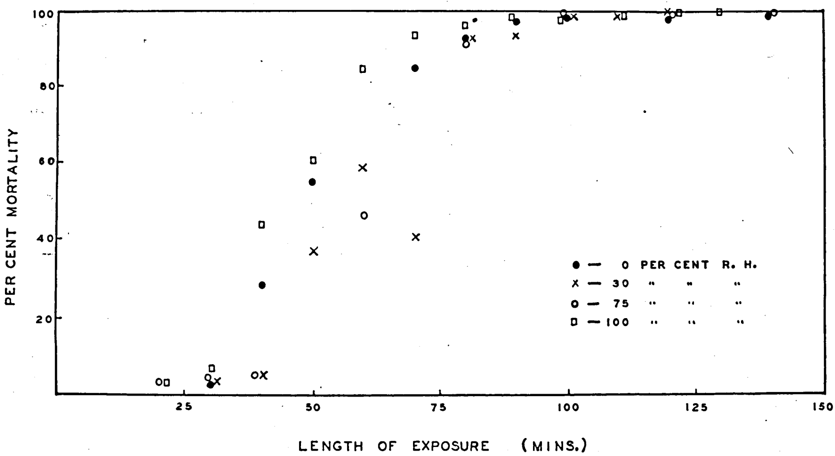

Fig. 4. The Mortality of Mature Larvae of *Tribolium confusum* Exposed at 46° C. to Relative Humidities of 0, 30, 75, and 100 Per Cent

Pupae.—Bodenheimer (1934) figured a mortality curve from data provided by the writer for pupae exposed at 58° C.

Pupae three days old when exposed to a temperature of 37.5° to 38.5° C. showed no apparent injury. There was no retardation in pupal development as compared to the control.

When pupae were kept at 40.5° to 41° C., the emerging adults often possessed transparent elytra, which did not become dark brown as in normal adults. Some of the adults emerging at this temperature were deformed, appearing to possess abdomens like those of pupae. These abnormal adults died after a short time.

Pupae three days old were exposed in duplicate series of groups of 100 pupae each at 44° C. and relative humidities of 0 and 75 per cent. After exposure they were kept at 32° C. until adults began to emerge when daily observations were made. As the pupa appears to be the most resistant stage of the confused flour beetle, complete mortality data are given for it in Tables 3 and 4.

Table 3.—Mortality of Pupae Exposed at 44° C. (= 111° F.) and 0 Per Cent Relative Humidity; Start June 5, 1934

Length of exposure	Daily adult emergence										Per cent mortality
	June							Groups		Total	
	7	8	9	10	11	12	13	A	B		
Control	18	21	55	69	37	..	..	100	100	200	....
7 hours	8	12	55	49	49	17	..	94	96	190	5.0
8 "	7	18	44	57	41	23	..	94	96	190	5.0
9 "	5	10	31	47	49	43	..	91	94	185	7.5
10 "	3	14	35	38	40	37	..	85	82	167	16.5
11 "	2	7	13	30	30	38	6	67	59	126	37.0
12 "	..	5	6	18	21	39	6	50	45	95	52.5
13 "	1	2	1	3	12	18	5	22	20	42	79.0
14 "	..	1	1	2	8	18	8	22	16	38	81.0
15 "	..	..	3	2	4	20	7	19	17	36	82.0
16 "	..	..	1	..	8	12	1	7	15	22	89.0
17 "	..	..	..	..	..	2	1	2	1	3	98.5
18 "	..	..	..	..	..	2	1	3	..	3	98.5
19 "	..	..	..	..	..	2	..	..	2	2	99.0
20 "	..	..	..	..	..	..	..	..	..	..	100.0

Table 4.—Mortality of Pupae Exposed at 44° C. (= 111° F.) and 75 Per Cent Relative Humidity; Start March 30, 1934

Length of exposure	Daily adult emergence								Per cent mortality
	April					Groups		Total	
	2	3	4	5	6	A	B		
Control	107	65	28	..	..	100	100	200	....
10 hours	23	78	95	..	..	96	100	196	2.0
12 "	10	62	120	..	..	96	96	192	4.0
14 "	9	69	112	..	..	95	95	190	5.0
16 "	27	46	110	..	..	90	93	183	8.5
18 "	23	35	53	39	2	77	75	152	24.0
19 "	24	39	45	33	3	77	67	144	28.0
20 "	12	19	40	35	1	51	56	107	46.5
21 "	1	18	19	26	5	37	32	69	65.5
22 "	..	9	16	22	7	26	28	54	73.0
23 "	2	11	19	23	4	26	28	59	70.5
24 "	..	1	17	28	9	20	35	55	72.5
25 "	..	1	3	10	7	9	12	21	89.5
26 "	..	..	4	10	2	4	12	16	92.0
27 "	..	..	2	13	4	11	8	19	90.5
28 "	..	..	3	11	4	9	9	18	91.0
29 "	..	..	..	7	..	4	3	7	96.5
30 "	..	..	..	2	1	3	..	3	98.5
31 "	..	..	..	..	..	..	..	..	100.0

The rate of pupal development at 44° C. appears to be slightly retarded. Many adults from pupae exposed to this temperature have pupa-like abdomens or deformed wings, but die after a few days.

Pupae from two to three days old were separated according to sex and exposed at 44° C. and a relative humidity of 0 per cent at the rate of 200 of each sex for each exposure. The results are given in Table 5.

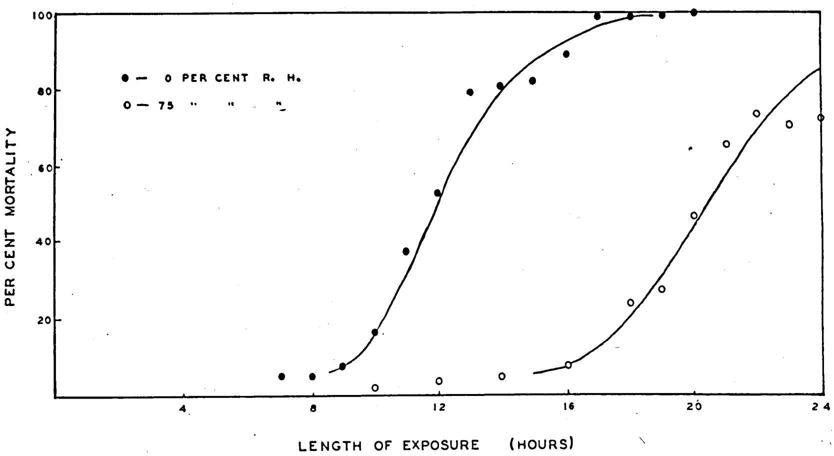

Fig. 5. The Mortality of Pupae of *Tribolium confusum* Exposed at 44° C. to Relative Humidities of 0 and 75 Per Cent

Table 5.—Mortality of Male and Female Pupae Exposed at 44° C. (= 111° F.) and 0 Per Cent Relative Humidity

Length of exposure	Number emerged		Per cent mortality	
	male	female	male	female
8 hours......................	170	171	15.0	14.5
10 "	159	134	20.5	33.0
12 "	94	88	53.0	56.0
14 "	57	52	71.5	74.0
16 "	29	38	85.5	81.0

There seems to be no significant difference in the resistance of pupae of the two sexes to dry heat.

Table 6.—Mortality of Pupae Exposed at 46° C. (= 115° F.) and 0 Per Cent Relative Humidity; Start January 15, 1934

Length of exposure	Daily adult emergence									Total	Per cent mortality
	January					Groups					
	19	20	21	22	23	A	B	C	D		
Control	71	126	3	..	..	50	50	50	50	200	....
40 mins.	49	116	29	..	..	49	48	48	49	194	3.0
50 "	41	118	25	1	..	50	47	45	43	185	7.5
60 "	37	79	28	1	..	38	36	31	40	145	27.5
70 "	11	58	45	2	1	35	30	25	27	117	41.5
80 "	5	44	33	3	..	19	16	23	27	85	57.5
90 "	2	31	29	4	..	18	14	15	19	66	67.0
100 "	4	13	33	5	..	11	13	19	11	54	73.0
110 "	2	10	23	3	2	14	11	9	6	40	80.0
120 "	2	4	16	3	1	8	5	4	10	26	86.5
140 "	1	1	..	..	..	1	..	1	..	2	99.0
160 "	..	1	..	..	..	..	1	..	..	1	99.5
180 "	..	1	..	..	..	..	..	1	..	1	99.5
200 "	..	..	..	..	..	..	..	..	..	..	100.0

Pupae two to three days old were exposed in four groups of 50 each for each combination of conditions, at 46° C. and relative humidities of 0, 30, 75, and 100 per cent. The results were recorded as in the previous experiments and are given in Tables 6 to 9.

Adults.—A negligible number of adult beetles died in 12 days exposure in flour to a temperature of 37.5° to 38.5° C. There was no apparent effect on the vitality of most of the individuals so exposed,

Table 7.—Mortality of Pupae Exposed at 46° C. (= 115° F.) and 30 Per Cent Relative Humidity; Start January 27, 1934

Length of exposure	Daily adult emergence									Per cent mortality
	Jan. 31	February			Groups				Total	
		1	2	3	A	B	C	D		
Control	19	148	33	..	50	50	50	50	200	....
40 mins.	15	105	74	1	48	50	48	49	195	2.5
50 "	10	82	104	..	50	49	47	50	196	2.0
60 "	..	71	109	4	47	46	46	45	184	8.0
70 "	5	69	87	5	42	40	39	45	166	17.0
80 "	2	49	86	8	38	38	30	39	145	27.5
90 "	..	33	79	6	31	28	25	34	118	41.0
100 "	..	27	58	8	16	24	23	30	93	53.5
110 "	..	11	26	5	7	13	11	11	42	79.0
120 "	..	8	21	7	8	13	8	7	36	82.0
130 "	..	7	14	9	11	8	6	5	30	85.0
140 "	..	3	9	8	5	4	4	7	20	90.0
160 "	..	..	3	3	..	3	..	3	6	97.0
180 "	..	..	2	2	1	..	2	1	4	98.0
200 "	..	..	..	..	..	..	..	..	..	100.0

Table 8.—Mortality of Pupae Exposed at 46° C. (= 115° F.) and 75 Per Cent Relative Humidity; Start December 17, 1933

Length of exposure	Daily adult emergence											Per cent mortality
	December						Groups				Total	
	19	20	21	22	23	25	A	B	C	D		
Control	4	5	49	141	..	..	50	50	49	50	199	....
30 mins.	..	5	38	95	53	..	46	45	50	50	191	4.5
40 "	3	5	28	89	65	2	49	49	47	47	192	4.0
50 "	..	7	32	91	62	..	48	49	47	48	192	4.0
60 "	..	4	16	77	68	3	45	44	38	41	168	16.0
80 "	..	4	2	44	61	22	37	32	33	31	133	33.5
100 "	..	8	5	20	57	18	27	28	25	28	108	46.0
120 "	..	2	4	7	10	11	14	5	9	6	34	83.0
140 "	..	2	2	5	2	5	2	4	1	9	16	92.0
150 "	..	4	4	5	..	..	2	4	3	4	13	93.5
160 "	..	2	2	4	..	2	1	4	4	1	10	95.0
180 "	..	1	3	1	..	..	2	1	1	1	5	97.5
200 "	..	1	2	1	..	..	2	2	..	..	2	98.0
220 "	..	..	..	2	..	..	2	..	..	..	2	99.0
240 "	..	..	..	..	..	..	..	..	..	..	..	100.0

Table 9.—Mortality of Pupae Exposed at 46° C. (= 115° F.) and 100 Per Cent Relative Humidity; Start November 9, 1933

Length of exposure	Daily adult emergence								Total	Per cent mortality
	November				Groups					
	13	14	15	16	A	B	C	D		
Control	15	170	15	..	50	50	50	50	200	....
30 mins.	7	91	82	7	43	49	48	47	187	6.5
40 "	2	67	76	22	47	43	37	40	167	16.5
50 "	..	32	71	38	36	33	36	36	141	29.5
60 "	1	20	27	33	14	22	15	30	81	59.5
70 "	..	2	13	15	9	5	8	8	30	85.0
80 "	..	1	5	12	5	3	5	5	18	91.0
90 "	..	6	2	3	2	3	2	4	11	94.5
100 "	..	5	..	1	2	1	..	2	6	97.0
110 "	..	3	..	..	1	..	1	1	3	98.5
120 "	..	1	1	..	1	..	..	1	2	99.0
130 "	..	1	1	..	1	..	1	..	2	99.0
140 "	..	2	1	1	..	2	1	1	4	98.0
150 "	..	..	..	..	..	..	..	..	..	100.0

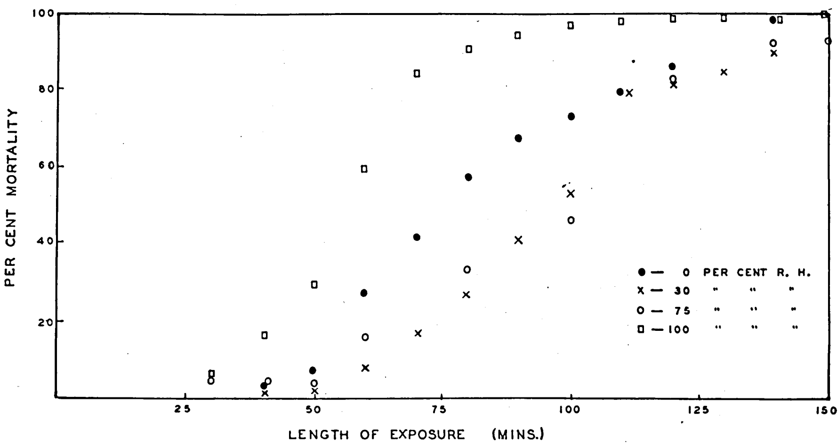

Fig. 6. The Mortality of Pupae of *Tribolium confusum* Exposed at 46° C. to Relative Humidities of 0, 30, 75, and 100 Per Cent

altho it is likely that their longevity would be much less at this temperature than at 32° C.

Two hundred adults exposed in flour at 40.5° to 41° C. for each of the periods, 4, 6, and 8 days, showed average mortalities of 48, 96, and 100 per cent, respectively.

Adults one month old were exposed in duplicate groups of 100 each for each exposure, at 44° C. and relative humidities of 0 and 75 per cent. After exposure they were placed at 32° C., and the percentage mortality was recorded after five days. The control kept at 32° C.

showed no mortality at the end of the experiment. The results are recorded in Table 10.

Table 10.—The Mortality of Adult Beetles Exposed at 44° C. (= 111° F.)

Length of exposure	Per cent mortality	
	Relative humidity 0 per cent	Relative humidity 75 per cent
3 hours	2.5	....
4 "	4.0	5.0
5 "	14.0	10.0
6 "	18.0	25.5
7 "	42.0	49.5
8 "	68.0	55.5
9 "	82.5	89.0
10 "	91.5	95.0
11 "	97.5	95.0
12 "	97.5	97.0
13 "	99.5	98.5
13.5 "	....	97.5
14 "	100.0	....
14.5 "	....	99.5
15 "	....	100.0

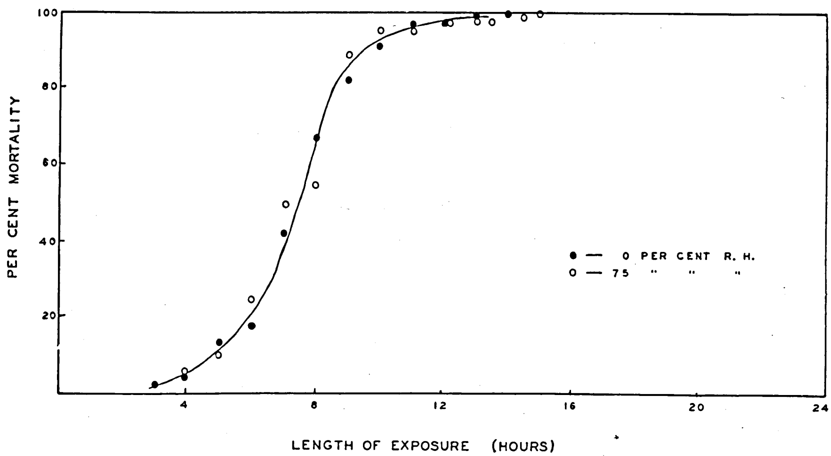

Fig. 7. The Mortality of Adults of *Tribolium confusum* Exposed at 44° C. to Relative Humidities of 0 and 75 Per Cent

Male and virgin female adults, one week old, separated into groups of 200 of either sex, were exposed at 44° C. and a relative humidity of 0 per cent. After exposure they were placed at 32° C. and the mortality recorded. The results are given in Table 11. The sexes apparently do not differ in resistance to these conditions.

Table 11.—The Mortality of Male and Virgin Female Adults Exposed at 44° C. (= 111° F.) and 0 Per Cent Relative Humidity. Mortalities of Mixed Adults Previously Recorded Are Added for Comparison

Length of exposure	Per cent mortality		
	Male	Female	Mixed
4 hours	3.0	3.5	4.0
6 "	9.5	7.5	18.0
8 "	84.0	69.5	68.0
10 "	95.0	74.5	91.5
12 "	99.5	99.0	97.5

Adult beetles, one month old, were exposed in four groups of 50 individuals each for each exposure at 46° C. and relative humidities of 0, 30, 75, and 100 per cent. The mortality was recorded after five days as in the previous experiments. The results are shown in Figure 8.

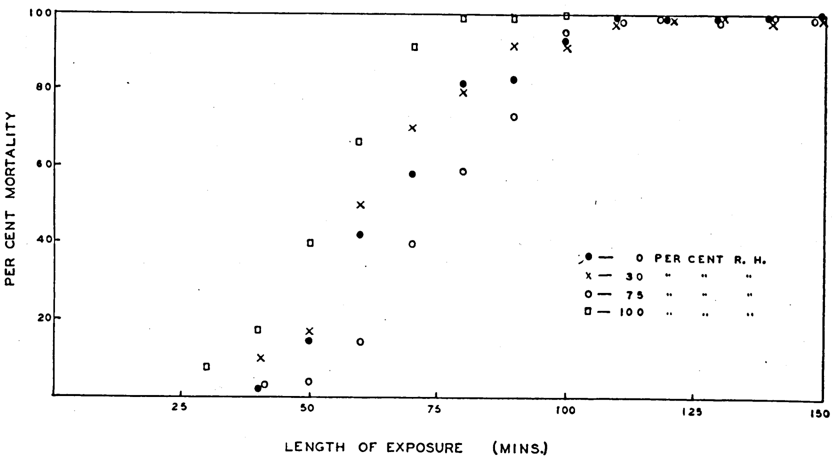

Fig. 8. The Mortality of Adults of *Tribolium confusum* Exposed at 46° C. to Relative Humidities of 0, 30, 75, and 100 Per Cent

It is of interest to note the exposures necessary to kill the average individual in the population. As an estimate of such values, one may take the exposures to kill 50 per cent of the population at each temperature investigated. These are given in Table 12 and plotted in Figure 9. The values for temperatures of 48° C. and above are taken from unpublished experimental data of the writer. In general the method of exposure was like that used at 44° and 46° C., but the relative humidity was not controlled.

Bodenheimer (1934) also figured a time-temperature curve from some of these data provided by the writer.

Table 12.—Exposures Required to Kill 50 Per Cent of the Adult Beetles at Different Temperatures

Temperature (° C.)	Length of exposure	Temperature (° C.)	Length of exposure
44	7.4 hours	54	137 seconds
46	64 minutes	56	112 "
48	29 "	58	92 "
50	290 seconds	60	59 "
52	175 "		

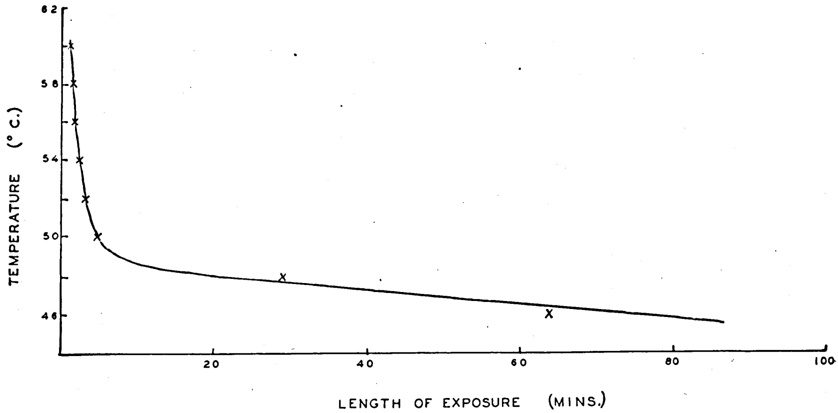

Fig. 9. The Median Mortality of Adults of *Tribolium confusum* Exposed at Various Temperatures

LETHAL EFFECT OF HIGH TEMPERATURE

Davey (1917) concluded that if the percentage of dead beetles of *Tribolium confusum* Duv. is plotted against the time which elapsed after their exposure to X-rays, the points follow a smooth curve which is the integral of a probability curve. "This is in agreement with the well known fact in toxicology that some individuals are especially susceptible to a given harmful agent, so that a very small dose causes death, while other individuals are especially resistant to the same agent so that they continue to live for a comparatively long time even when given large doses." A sigmoid curve, therefore, is obtained by plotting the percentage mortality of an organism against the length of exposure to the toxic agent. This is the type of curve secured when the method of measurement simply measures the presence or absence of a given effect in the individuals of a population (Clark, 1933, p. 142). Hence it is the type resulting from mortality data, in which the individuals are classified into the two categories, dead ones and live ones.

It has been shown (Tattersfield and Morris, 1924) that the dosages of two or more toxic agents to kill 50 per cent of the population are the best points statistically at which to make comparisons of the relative toxicity of those materials. The relative resistance of the different stages of *Tribolium confusum* is shown on this basis in Table 13. The results at 44° and 46° C. are for 75 per cent relative humidity, whereas the humidity was not controlled at the two higher temperatures. The eggs were three days old. The larvae were full grown.

Table 13.—The Relative Resistance of Different Stages of *Tribolium confusum* Exposed at Several Temperatures

Temperature		Median lethal exposure			
° C.	° F.	Eggs	Larvae	Pupae	Adults
44	111.2	14 hours	10 hours	20 hours	7 hours
46	114.8	1.2 "	1.0 "	1.5 "	1.2 "
48	118.4	......	8 mins.	12 mins.	26 mins.
50	122.0	......	4.7 "	4.5 "	4.9 "

The pupae are least affected, except at the higher temperatures at which differences between the stages are less marked. At 44° C. the adults are killed at shorter exposures than the other stages, but at 48° and above they appear to be somewhat more resistant.

At temperatures too low to kill within a reasonable time for practical purposes, it has been shown that the rate of development may be slower than at temperatures in the optimum range, the percentage of eggs hatching is reduced, larvae may not be able to complete their development, and lethal deformities of adults from exposed pupae may occur. At 38° C. only 64 per cent of the eggs hatched, as compared to 88 per cent at 32°. The mean length of the larval stage was increased from 17 days at 32° to 23 days at 38°. At 41° larvae were unable to complete their development. Many adults, developing from the shorter exposures at 44°, had deformed wings or pupa-like abdomens. They died after a few days. Other deformities of larvae exposed to this temperature will be described in another paper on the phenomenon of prothetely in *Tribolium confusum.*

The range of individual variation in resistance to high temperature within a given population of *T. confusum* is considerable, as shown by the mortality curves in Figures 1 to 8. At 44° C. the most susceptible adult beetles succumb in three or four hours, whereas some resistant individuals can stand 12 hours of exposure or a period three or four times as long.

Different species of stored-product insects differ in their resistance to high temperature. Dendy and Elkington (1920) observed that an exposure of 48.9° C. for three minutes was required to kill adults of *Sitophilus granarius,* whereas *Rhizopertha dominica* is rather resistant, requiring an exposure of five minutes at 62.8°.

Adults of *Tribolium ferrugineum* and *Dermestes coarctatus* were exposed to constant high temperatures in small cloth bags in an oven by Yokoyama (1927). His results may be summarized as follows:

Temperature	Exposure	Species	Per cent kill
50° C.	60 minutes	*D. coarctatus*	100
	70 minutes	*T. ferrugineum*	100
55° C.	10 minutes	*D. coarctatus*	88
	10 minutes	*T. ferrugineum*	40

T. ferrugineum seems to be a little more resistant than the other species. Grossman (1931) found groups of 30 adult *T. ferrugineum* to be completely killed in exposures of 5, 10, and 15 minutes to a temperature of 52° C.

Gortner (1913) exposed several hundred larvae of *Tenebrio molitor* and *T. obscurus* at 41.5° to 42° C. for 3½ hours. Ninety-one per cent of the *T. molitor* were either dead or barely alive, whereas only 16 per cent of the *T. obscurus* were so.

In Table 14 eight species of stored-product insects are named in the probable order of their resistance to high temperature, the first being the most difficult to kill. The references are given from which the necessary information was obtained.

Table 14.—The Order of Resistance of Some Stored-Product Insects to High Temperature

1. *Rhizopertha dominica* Fabr., Lesser grain borer	(Dendy & Elkington, 1920)
2. *Tribolium ferrugineum* Fabr., Rust-red flour beetle	(Grossman, 1931)
3. *T. confusum* Duv., Confused flour beetle	
4. *Tenebrio obscurus* Fabr., Dark mealworm	(Gortner, 1913)
5. *T. molitor* Linn., Yellow mealworm	(Gortner, 1913)
6. *Sitophilus granarius* Linn., Granary weevil	(Dendy & Elkington, 1920)
7. *S. oryzae* Linn., Rice weevil	(Grossman, 1931; Back & Cotton, 1924)
8. *Silvanus gemellatus* Duv., Square-necked grain beetle	(Grossman, 1931)

THE INFLUENCE OF MOISTURE ON THE LETHAL EFFECT OF HIGH TEMPERATURE

Some conflict in the views regarding the influence of relative humidity of the atmosphere on the resistance of insects to heat probably arises from the fact that different workers explored different ranges of temperature and used different species of insects. This is to be expected since recent work has shown that two species of insects may have entirely opposite reactions to dry and moist heat.

Egg.—Bellati and Quajat (1896) exposed eggs of *Bombyx mori* to dry hot air, while others were dipped in hot water, providing conditions more or less comparable to those in hot air saturated with moisture. Their data indicated that dry heat is distinctly more effective than hot water in killing the eggs. At a temperature of 48° C. they were all dead after 15 minutes in dry air, whereas 40 minutes in hot water were required to kill all of them.

Nuttall (1917), studying the resistance of louse eggs, found dry heat more effective than moist heat for control.

The mortality data for eggs of *Tribolium confusum* exposed at 44° C. show this stage to be more susceptible at 0 than at 75 per cent relative humidity. One hundred per cent are killed in 19 hours in dry air, whereas 24 hours are required at 75 per cent relative humidity.

Larva.—Headlee (1916) found the pea weevil to be more susceptible to dry heat than to moist heat.

Mellanby (1932) observed that larvae of *Lucilia* and *Xenopsylla* died at much lower temperatures in dry than in moist air, apparently because of desiccation. On the other hand, mealworms (*Tenebrio* larvae) die at a lower temperature in moist air than in dry. Mellanby believes this fact is associated with the ability of mealworms to conserve their water in excretion. Buxton (1931) found the same relationship of water conservation to dry heat in nymphs of the reduviid bug, *Rhodnius prolixus.*

Mortality data for mature larvae of *Tribolium confusum* exposed at 44° C. show that this stage is considerably more susceptible at 0 than at 75 per cent relative humidity. At higher temperatures the differences caused by variations in humidity are masked by the faster action of the heat. However, altho there is little difference in the reaction of mature larvae to a temperature of 46° at humidities of 0, 30, or 75 per cent, exposure at 100 per cent relative humidity kills them in a considerably shorter period.

Pupa.—The heat resistance of pupae, two to three days old, is affected by moisture in about the same way as that of mature larvae. At 44° the pupae are killed much more quickly at 0 than at 75 per cent relative humidity. At 46° they are killed in about the same time, whether the humidity be 0, 30, or 75 per cent, whereas they are killed sooner at 100 per cent relative humidity.

Adult beetle.—It is well known by persons accustomed to experimenting with flies in the laboratory that these insects soon die in a dry atmosphere. Rouboud (1909) estimated that tsetse flies kept at 33° C. live 12 times as long in air saturated with moisture as in dry air. The cotton-seed bug, *Oxycarenus hyalinipennis,* shows a greater resistance to heat at higher humidities, according to Kirkpatrick (1923). Mellanby (1932) reported that adults of *Pediculus humanus* die at a lower temperature in dry than in moist air.

Mortality data indicate that adults of *Tribolium confusum* are less affected by the moisture of the air at high temperatures than are the other stages. At 44° C. the adults die in the same time at 0 as at 75 per cent relative humidity. At 46° they die sooner at 100 per cent than at the other humidities tested, and somewhat sooner at 0 and 30 per cent than at 75 per cent.

General.—The results indicate, in general, that dry heat is more effective in killing *Tribolium confusum* than is rather moist hot air at 75 per cent relative humidity, altho a hot atmosphere saturated with moisture is apparently still more effective. Beattie (1928) found that air, either saturated with moisture or fairly dry, had the effect of lowering the thermal death point of *Calliphora erythrocephala.* Miller (1930) showed that, at 38.5° to 41.5° C., relative humidities below 60 and above 80 per cent were fatal to the Mexican bean beetle.

Experimentally, the exposure of insects to an atmosphere saturated with moisture is attended by some difficulty with the moisture which condenses on the insects upon cooling. This would be a reason for not using such a heavily moisture-laden air under practical conditions.

Speaking of some trouble he had in obtaining good control of insects with heat, Goodwin (1914) said, "The amount of heat required for a dry-atmosphere, high-temperature test was much less than for an extremely moist-atmosphere test, ... proving in a rough way that the leaks in the heating system and wet floors were responsible for the partial failures mentioned." Back (1920) recorded that a drop in relative humidity from 72 to 12 per cent may occur in a flour mill heated for about 24 hours. He concluded that insects die more readily in a dry than in a moist atmosphere.

Goodwin (1914) found much variation between different species of stored-product insects with respect to their susceptibility to moist and to dry heat. "The rice weevil seemed to be especially susceptible to heat in a moist atmosphere, repeated tests showing that 3° to 4° C. less are required to prove fatal than when using dry air. Several of the other species died at practically a uniform temperature, regardless of the presence or lack of moisture."

Uvarov (1931) stated: "There is a considerable amount of evidence to show that the same intensity of heat may affect an insect in a different way according to the relative humidity of the air, which in itself depends on the temperature." Evaporation, desiccation, and possibly water absorption are the important factors influencing the effect of humidity on the thermal death point of insects.

With respect to evaporation, Necheles (1924) stated that cockroaches are able to keep their bodies cool in dry air by evaporation and in that manner resist a much higher temperature than in moist air. "It is only possible for large insects to cool themselves by evaporation; small insects do not contain sufficient water in relation to the area of their body surface," according to Mellanby (1932). It is difficult to determine, as Buxton (1931) pointed out, whether evaporation into dry hot air cools the insect and thus allows it to survive higher temperatures than in moist air, or whether the evaporation kills the insect by causing undue loss of water.

Mellanby (1932) believed that, in insects killed by dry heat, death may be caused by desiccation, especially when rather long exposures are required.

Buxton (1932) showed that some insects can gain water from an atmosphere which is nearly saturated. This increase in body water may be a factor in causing death in a hot saturated atmosphere. According to Uvarov (1931): "Bachmetjew (1907) drew attention to the importance of the water-content of the insect body . . . . . and suggested that insects containing more water would die at a given high temperature more quickly than those with less water. This problem has, however, apparently not been studied, but it would be of considerable interest, since during droughts insects are subjected to a greater evaporation and obtain less water with their food than in normal conditions; at the same time it is during such periods that they are exposed to abnormally high temperatures."

HEAT TRANSFER IN FLOUR

A much longer time than the inexperienced person would expect must be allowed for even a small sack of flour to be heated through, when placed in a room or oven, even at such a moderate temperature as 120° F. The time required for the flour to come to equilibrium with its surroundings depends not only on the temperature of the room but also on the original temperature of the flour.

A one-quart jar, containing 150 grams of flour to a depth of four inches, was sunk in a small water bath to the level of the flour, i.e., four inches. The temperature was raised from room temperature at a fairly constant rate of five or six degrees Centigrade in 15 minutes until the water reached a temperature of 51° C. In Figure 10 is shown the increase in temperature both at the surface of the flour and in the center at a depth of two inches. Naturally the surface of the flour in the center of the jar, where the temperature was recorded, was the coolest spot in the flour and did not reach the temperature of the surrounding water bath. The center of the flour had reached a temperature two or three degrees below that of the surrounding bath about 2½ hours after the bath reached 51° C.

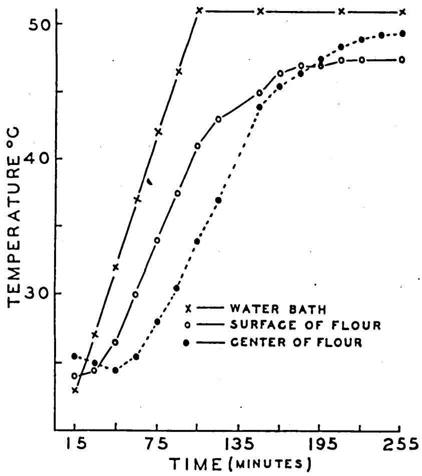

Fig. 10. Rise in Temperature of Flour Four Inches Deep in a Quart Jar Suspended in Water Which Is Gradually Heated to 51° C. (124° F.)

According to Goodwin (1922), "Flour in sacks will require 4 or more days continuous exposure to the high temperature—130 to 140 degrees F.—before it will be heated through. This means that the cooler flour left in the mill will act as a refuge trap for every insect that comes near it before a killing temperature is reached."

THE CAUSE OF DEATH BY HEAT

Death by heat is usually ascribed to the coagulation of proteins of the living cell in the same manner that egg albumin is coagulated when heated in a test tube. Chick and Martin (1910, 1912) showed that heat coagulation of egg albumin proceeded in two stages, viz., a chemical process of denaturation followed by a physical process of agglutination. Heilbrunn (1924) stated that the first effect of heat on an animal is to produce anesthesia, the animal failing to respond to stimulation, and its various activities ceasing. Longer exposures or slight increases in temperature are followed by death, accompanied by protoplasmic coagulation. If, after a light coagulation, *Cumingia* and *Arabacia* eggs are returned to a lower temperature, recovery takes place, showing that heat coagulation of protoplasm is reversible. Heat coagulation of proteins, however, is irreversible. This has been used as an argument against a direct protein effect as a cause of heat death. It is still an open question, as Heilbrunn (1928) pointed out, whether the direct effect of heat on proteins is the cause of death, since heat coagulation might set in after the cell is already killed. Ordinarily, proteins are not coagulated by such temperatures as 35° to 38° C., which normally cause death in many animals.

Fat solvents, such as ether, chloroform, and chloral hydrate, cause a coagulation of protoplasm when these are present in sufficient concentration. A dilute solution of ether produces a decrease in viscosity, whereas with an increased concentration a sudden coagulation results. Heilbrunn assumed that heat has an effect similar to that of ether in that high temperatures tend both to dissolve and to liquefy fats. According to him, these fat solvents hasten the process of heat coagulation of protoplasm, thus showing that coagulation is really initiated by a solution or liquefaction of lipoids. He found that when he added one to one and a half per cent of ether to *Arbacia* eggs exposed at 32° C., he could decrease the time of coagulation from 24 to 12 minutes. He also showed that distilled water causes a solution of protoplasmic lipoids in sea urchin eggs, and concluded: "If heat coagulation of protoplasm depends primarily on an alteration of cell fats, it is easy to understand why an increase in water content would favor heat death and a decrease retard it. The higher the percentage of water in the cell, the more readily would fats tend to dissolve." Animals suffering from heat at relative low temperatures must, therefore, have fats of low melting points in their cells. Leathes and Raper (1925) believed that the higher the temperature, the higher the boiling point of the fats that are formed. Heilbrunn (1928) stated that "if we assume that heat death depends on the liquefaction of fats, we have an excellent interpretation of an acclimatization of living organisms to higher temperatures."

Winterstein (1905) presented the view that heat causes death by asphyxiation, i.e., by the production of an excess of carbon dioxide. Mayer (1917) claimed that some toxic substance, possibly carbon dioxide, accumulates under the influence of excessive heat, the rate of its formation being proportional to the metabolism of the tissues. He stated that animals of the same class having a high rate of metabolism are more sensitive to heat and carbon dioxide than those with a low rate of metabolism.

Harvey (1911), working on *Medusa cassiopa,* believed some enzyme essential to nerve conduction in animals may be destroyed by heat, thus causing the rate of nerve conduction to decline. Gortner (1929) stated that "adsorptive reactions are, in general, characterized by a positive heat of adsorption. The amount of heat liberated in some instances is large and indicates the general affinity existing between the surface of the adsorbent and the substance being adsorbed." It is, therefore, quite possible that any external heat will interfere with or inhibit the process of adsorption. The mechanism of enzyme action seems to be through an oriented adsorption of the substrate followed by a chemical reaction. Heat, therefore, may be expected to retard enzymatic adsorption and accelerate the chemical reaction which follows.

Larvae of *Tribolium confusum* living for a long time after exposure to heat show a darkening of the digestive tract. This darkening may be present as a small anterior black spot or it may extend the length of the body. Histological sections of such larvae, fixed in Bouin's fluid and stained with Delafield's hemotoxylin and eosin, showed the presence of a brown spot in the anterior part of the mid-intestine. The brownish color is present in the intestinal contents, extending in some instances into the epithelial cells. It is possible that the production or subsequent action of digestive enzymes making the food available had been interfered with or inhibited. This interference of enzymatic activity may not cause immediate death, but the inability of the usual physical and chemical processes to take place, a continued inhibition of these processes, would finally cause death.

Mellanby (1932) suggested that death in insects is caused by some internal reaction, the products of which cannot be removed rapidly enough at high temperatures. In *Lucilia* adults and *Tenebrio* larvae killed at 45° C., the amount of lactic acid was increased.

PART II. THE INFLUENCE OF HIGH TEMPERATURE ON THE FERTILITY OF THE CONFUSED FLOUR BEETLE

INTRODUCTION

Moderately high temperature may be a controlling factor in insect abundance by affecting fertility. Field observations on this subject are scanty and inexact. As Uvarov (1931) has expressed it, "heat may either affect the rate of development of the gonads or may influence the mechanism by which mature eggs are deposited." The former possibility may appear the more important, probably because of insufficient attention to an analysis of the physiological effects of temperature on fertility. The following factors influencing the fertility of *Ephestia kühniella* (Mediterranean flour moth) are given by Norris (1933): (1) failure to pair; (2) failure of the male to introduce a spermatophore; (3) reduction in fecundity; (4) non-fertilization of the egg and failure to oviposit, and (5) abortion of the egg.

A knowledge of the effects of high temperature on egg production and fertility of stored-product pests is of practical value. Sub-lethal temperatures might be effective in producing either a partial or complete sterility of adults from exposed larvae and pupae, or influence the egg production and fertility of the exposed adults themselves.

SEX RATIO AND PREOVIPOSITION PERIOD IN THE CONFUSED FLOUR BEETLE

In adult flour beetles there seems to be no external morphological difference between the sexes, but the male and female pupae may be separated by distinct differences in the genitalia. The pupae of normal cultures were examined at different times during 1934 and the sex ratio determined by the Graham (1929) method of dividing the number of females in a given group by the total number of individuals. The data are given in Table 15.

Table 15.—Sex Ratio in the Confused Flour Beetle

Date	Total	Females	Sex ratio
1934			
March 26	1,045	494	.473
May 8	2,003	1,160	.579
" 16	1,925	906	.471
" 19	3,693	1,576	.427
July 9	1,233	697	.565
" 15	1,499	777	.518
Sept. 5	588	326	.554
Total	11,986	5,936	.495

A possible reason for the fact that in some cultures one sex is considerably in excess may be that at the time of sex separation not all the larvae in the cultures had pupated. The total shows the sexes to be present in almost even numbers. Holdaway and Smith (1933) made observations and gave data concerning the sex ratio in *T. confusum*. By averaging their control experiments, one obtains a sex ratio of 0.484 for 763 individuals kept under uniform experimental conditions.

Regarding a preoviposition period in *Tribolium confusum*, Brindley (1930) says, "The beetles mate soon after emergence and begin to lay almost at once. Out of twenty pairs mated eleven laid fertile eggs the following day and the remainder were all laying by the end of the fifth day." In order to obtain somewhat more definite figures regarding this period, the following experiments were made. Fifteen pairs, one to six hours old, having emerged at 32° C., were separated in one-half ounce pill boxes containing whole wheat flour for each of the constant temperatures, 27°, 32°, and 37.5° C. At 27° ten pairs started laying on the eighth day, followed by the rest on the ninth day; at 32° seven pairs were laying on the sixth day, the rest on the seventh day; at 37.5° six, nine, twelve, and fifteen pairs were laying on the seventh, eighth, ninth, and tenth days, respectively. The preoviposition period, therefore, varies from eight to nine days at 27°, from six to seven days at 32°, and from seven to ten days at 37.5° C. Further experiments should be made at a lower temperature and with adults reared at temperatures below 32° C., the optimum temperature for rate of development but one that may have a deleterious effect on some other vital activities of the insect.

Guyénot (1913), Adolph (1920), and Hanson (1928) obtained data showing that mating is a stimulus to egg laying in *Drosophila*. Guyénot thought it merely a mechanical stimulus since the first eggs deposited are frequently sterile. Hunter and Hinds (1904) and Smith (1921) state that normal oviposition evidently will not take place until fertilization has been accomplished in the Mexican bean beetle. Graf (1917) observed that fertilized potato tuber moths oviposit within 24 to 48 hours after emergence, with an egg production of 114 to 209 eggs. The average preoviposition period for virgin females was 4.4 days, with an average egg production of 22.6 eggs. Glaser (1923) found that association with the male sex stimulates egg production in *Musca domestica* and *Stomoxys calcitrans*.

Five virgin adult *Tribolium confusum* were separated into pill boxes at 32°. Only 21 eggs were laid in seven days. None of them hatched. On the eighth day a male was added to each box and 25, 35, 49, and 61 eggs, respectively, were laid by the mated females on the four succeeding days. Therefore it appears that mating is a stimulus with respect to both the time and the number of eggs laid.

According to Norris (1933), the number of eggs laid by unfertilized *Ephestia kühniella* females is much smaller than in normally mated females, a large number of eggs usually being retained in the ovaries. Park (1933) found that for *T. confusum* the egg-laying rate for fecundated females is approximately 20 times that for virgin females.

EXPERIMENTAL RESULTS

The mortality of *T. confusum* exposed to high temperatures has been given in Part I of this bulletin. Surviving individuals were reared to determine the effect of those exposures on their ability to reproduce further generations.

It appears appropriate to define certain terms used in the following paragraphs. *Fecundity* refers to the number of eggs laid by a female. The *fertility* of a female is stated in terms of the percentage of the deposited eggs which hatch, whereas in the male it is in terms of the ability to fertilize virgin females. *Completely sterile* females are those that produce eggs, none of which hatch. *Fertile* or *viable* eggs are those which hatch.

Exposed Larvae (One to Six Hours Old)

Larvae, one to six hours after hatching at 32° C., were kept at 37.5° to 38.5° to complete their development. After the adults emerged, the separated sexes were placed at 32° for eight to ten days. Then the females were placed in two boxes containing 25 individuals each. To each box were added 25 normal males. The total egg production from both sexes, i.e., from 50 females, and the percentage hatch are given in Table 16.

Table 16.—Production and Fertility of Eggs Produced by 50 Females Developing from Larvae Reared at 37.5° to 38.5° C.

Date	Total daily production	Percentage hatch
August 14	372	3.5
" 15	361	4.7
" 16	463	3.6
" 17	313	3.2
Sept. 7	258	3.8
" 8	212	5.0
" 9	227	3.4

When the females were two months old, they were isolated and the individual egg production and fertility recorded. There was an average hatch of 3.3 per cent. Thirty-two out of 35 females were completely sterile, the number of eggs produced per female varying from 5 to 48, with an average of 28. The three remaining females produced 32, 36, and 48 eggs, respectively, and the numbers of larvae emerging were 3, 5, and 27, indicating varying degrees of sterility in these three females. During the course of the experiment, 15 females and two males died.

Forty males reared at 37.8° to 38.5° C. were mated to that number of normal virgin females. Only eight pairs failed to produce fertile eggs after four days. The female reproductive system is evidently more susceptible to heat than that of the male.

Exposed Larvae (Mature)

Mature larvae were exposed for 24, 48, and 120 hours at 37.5° to 38.5° C., then returned to 32° C. to complete their development. In one series, the individuals that had pupated at the higher temperature were kept there for 14 more days, so they spent a total of 17 days at about 38° C. When the females were 9 days old those from each exposure were separated into two groups of 20 each, normal males added, and the daily egg production and percentage of fertile eggs recorded after the fourth day. Only one group of 20 females was obtained in the case of the exposure for 17 days. The data are presented in Table 17.

Table 17.—Production and Fertility of Eggs Produced by Females Developing from Mature Larvae Exposed at 37.5° to 38.5° C.

Length of exposure	July, 1934							
	13th		14th		15th		16th	
	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch
Control	390	79.2	437	81.4	440	83.2	445	81.5
1 day	367	80.9	471	82.1	453	78.1	463	81.2
2 "	320	78.7	351	75.2	360	79.7	364	79.1
5 "	337	75.0	368	73.3	335	80.3	346	89.3
17 "	117	37.6	187	45.4	193	59.0	201	67.1

The influence on the fertility of females developing from mature larvae exposed for 24, 48, and 120 hours appears to be negligible. Apparently, exposures of 48 hours and longer cause a reduction in the number produced. In the case of the 17-day exposure, there is a pronounced reduction in fertility. About five weeks after the data in Table 17 were recorded, it was found that eggs laid by these individuals were from 75 to 85 per cent fertile. Individual records were made from subsequent isolations in flour for four days. Only one female was found to be completely sterile, the remaining number showing various degrees of partial sterility. Four females and no males had died during the experiment.

Males developing from mature larvae exposed at 37.5° to 38.5° C., when placed with normal virgin females, showed no signs of sterility.

Mature larvae exposed at 40.5° to 41° C. and a relative humidity of 20 to 25 per cent for 12, 24, 48, and 60 hours were returned to 32° C. to complete their development. When the virgin females were one week old, they were separated into duplicate series of 25 each for each exposure, normal males were added, and the egg production and fertility recorded, starting from the fifth day. The data are given in Table 18.

Table 18.—Total Daily Egg Production and Percentage Egg Fertility in Females Developing from Larvae Exposed at 40.5° to 41° C., Fifty Individuals for Each Exposure

Length of exposure	August, 1934								Average per cent hatch
	8th		9th		10th		11th		
	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	
Control	443	83.2	421	81.5	451	80.4	477	83.7	82.23
12 hours	459	81.9	453	80.8	479	83.7	455	80.0	81.68
24 "	427	71.7	395	73.6	367	76.5	321	74.8	73.32
36 "	463	22.2	334	24.5	362	22.6	301	23.8	23.16
48 "	466	7.7	418	9.3	485	9.5	405	10.6	9.23
60 "	424	0.0	381	0.0	474	0.0	409	0.0	0.00

An exposure of 12 hours to this temperature had no apparent influence on female fertility, whereas increasing lengths of exposure reduced more and more the percentage of eggs hatching, until a complete sterility was produced with an exposure of 60 hours. The egg production, on the other hand, remained fairly constant, regardless of the length of exposure up to and including 60 hours. The total egg production and the percentage egg fertility remained fairly constant for each lot for at least four weeks.

Mature larvae that had been exposed at 44° C. and a relative humidity of 0 per cent for 4, 6, 8, and 10 hours, were kept at 32° to complete their development. When the females were two weeks old, they were placed in pill boxes in duplicate series of 20 each for each exposure, and the daily egg production and percentage fertility (hatch) at 32° recorded after the fourth day. Because of the high larval mortality at exposures of 8 and 10 hours, only one group of 20 females was obtained at either of those exposures. The data are given in Table 19.

The reduction in the fertility of eggs produced by females from larvae exposed to this temperature is very marked, even in the case of an exposure of only four hours. It becomes more pronounced as the length of exposure is increased, until practically complete sterility is produced after a 10-hour exposure. In a repetition of this experiment two weeks later, females developing from larvae exposed for eight hours were completely sterile.

Table 19.—Total Daily Egg Production and Percentage Egg Fertility in Females Developing from Larvae Exposed at 44° C., Fifty Individuals for Each Exposure

Length of exposure	June, 1934								Average per cent hatch
	10th		11th		12th		13th		
	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	
Control......	358	79.5	354	81.4	415	83.5	356	81.2	81.48
4 hours.....	338	55.8	304	52.6	380	52.1	337	54.6	54.49
6 "	304	13.1	278	11.1	303	13.2	319	12.2	12.42
8 "	176	2.2	182	4.9	186	3.7	163	3.6	3.90
10 "	163	0.6	125	0.0	149	0.0	132	0.0	0.17

There is also a reduction in the daily production of eggs which is more pronounced the longer the exposure. Records were kept over a considerable period, about six weeks in some cases, and the egg production and egg fertility were fairly constant for each set of conditions.

Isolations of the females developing from larvae exposed for 4, 6, and 8 hours showed only partial sterility for the 4-hour group. The 6- and 8-hour exposures produced complete sterility of 14 out of 38 and 12 out of 15 females, respectively.

Males, two weeks old, developing from larvae that had survived exposure to a temperature of 44° C., were isolated in pill boxes with single normal virgin females. The results after three days indicated that the fertility of the 25 males from larvae exposed for six hours was not affected. In the case of the 8- and 10-hour exposures, only 3 out of 20 males at either exposure failed to fertilize the females. This number was reduced to one at the 8-hour and to two at the 10-hour exposure three days later.

Exposed Pupae

Pupae were exposed in flour at 37.5° to 38.5° C. and a relative humidity of 20 to 25 per cent for 24, 48, and 72 hours, then returned to a temperature of 32° C. until emergence of the adults. In one set of experiments, the pupae that had become adults after three days were kept at this temperature for nine more days; that is, they were kept a total of 12 days at 37.5° to 38.5° C. After the females had reached the age of six days, they were divided into duplicate series of 25 each for each exposure, normal males were added, and the total egg production and percentage fertility recorded after the fourth day at 32° C. The results are given in Table 20.

Table 20.—Total Daily Egg Production and Percentage Egg Fertility in Females Developing from Pupae Exposed at 37.5° to 38.5° C., Fifty Individuals for Each Exposure

Length of exposure	July, 1934						Average per cent hatch
	3d		4th		5th		
	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	
Control	488	86.0	402	91.5	459	88.2	89.13
1 day	456	83.2	425	85.6	493	85.5	84.80
2 "	397	81.3	424	82.7	498	84.2	82.84
3 "	425	81.4	380	82.6	468	83.5	82.53
12 "	403	77.2	414	74.1	441	78.2	76.53

The results indicate that females developing from pupae exposed for different periods to this temperature are not much affected. There was a slightly lower percentage fertility when the adults were left at the higher temperature for nine additional days. Males from pupae exposed to this temperature showed no indication of sterility when mated to normal virgin females.

Female pupae were exposed at 40.5° to 41° C. and a relative humidity of 20 to 25 per cent for periods of 24, 36, 48, 60, and 72 hours, then returned to 32° C. to complete their development. When the adults were 10 days old, they were separated into two groups of 25 each for each exposure, normal males were added in equal numbers, and the egg production and percentage fertility recorded after the fourth day. Because of the high pupal mortality, only one group of 35 females was obtained from pupae exposed for 48 hours. The data are given in Table 21.

Isolations of the individual females for four days about a month after procuring the data shown in Table 21 indicated that no completely sterile females develop from pupae exposed at 40.5° to 41° C. for 36 or for 48 hours. At an exposure of 60 hours 34 out of 43 of the females were completely sterile.

Table 21.—Total Daily Egg Production and Percentage Fertility in Females Developing from Pupae Exposed at 40.5° to 41° C., Fifty Individuals for Each Exposure, Except the 48-Hour Exposure, in Which Case There Were 35

Length of exposure	July, 1934								Average per cent hatch
	28th		29th		30th		31st		
	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	
24 hours	446	81.1	455	80.9	438	81.5	437	81.8	81.32
36 "	461	77.5	428	74.7	390	72.5	390	65.6	72.83
48 "	298	46.9	281	52.6	211	49.3	233	53.6	50.49
60 "	427	4.0	435	3.9	362	6.3	362	4.7	4.66
72 "	137	0.0	136	0.0	104	0.0	114	0.0	0.00

Female pupae were exposed at 44° and relative humidities of 0 and 75 per cent, then returned to 32° to complete their development. When the emerging adults were one week old, they were separated into two groups of 25 each for each exposure, and 25 normal males were added to each group. Because of the high pupal mortality, only one group of 22 females was obtained for the 12-hour exposure to dry heat. The figures given in Table 22 are averages of the data for the sixth to ninth days in the case of 0 per cent and for the second to fifth days in that of the 75 per cent relative humidity.

Table 22.—Average Daily Egg Production and Percentage Fertility in Females Developing from Pupae Exposed at 44° C., Fifty Individuals for Each Exposure

Length of exposure	Relative humidity			
	0 per cent		75 per cent	
	Average daily egg production	Average per cent hatch	Average daily egg production	Average per cent hatch
Control	250	83.75	376	79.06
4 hours	225	49.00	388	79.23
6 "	222	1.10	...	....
8 "	176	0.00	382	1.33
12 "	...	0 00	390	1.29

The females from pupae surviving exposures of eight hours or longer at 75 per cent relative humidity were made partially or completely sterile. The daily egg production and percentage fertility remained fairly constant for each exposure as long as five months after they were first recorded.

In the group of females from pupae exposed to dry heat, kept for a period of about three months, there was a gradual increase in egg production for a week or two after fertilization, even in the case of those exposures producing complete sterility. Partially sterile females gradually produced not only more eggs but a greater percentage of fertile ones, the egg production and fertility becoming fairly constant at about the same time. Females from the four-hour exposure finally produced from 70 to 75 per cent, those from the six-hour exposure produced from 8 to 10 per cent fertile eggs.

Adult males from pupae exposed at the same time as the previous lot were separated into groups and mated with equal numbers of normal virgin females. Because of the high pupal mortality, only one group of 13 males was obtained for the 12-hour exposure. Data relative to egg production and fertility are given in Table 23.

That the male reproductive functions were retarded when male pupae were exposed is substantiated by the fact that the egg production of

Table 23.—Total Daily Egg Production and Percentage Fertility in Females Mated to Males Developing from Pupae Exposed at 44° C. and a Relative Humidity of 0 Per Cent, Fifty Individuals for Each Exposure

Length of exposure	May, 1934									
	8th		13th		14th		15th		20th	
	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch	No. of eggs	Per cent hatch
Control....	273	88.2	300	88.6	293	83.3	...	...	...	...
4 hours...	107	18.6	91	16.4	179	59.7	220	72.7	386	87.8
6 " ...	61	0.0	68	1.5	109	51.3	194	70.1	335	84.4
8 " ...	75	2.7	96	4.1	122	36.8	177	61.5	350	77.6
12 " ...	19	0.0	17	0.0	14	0.0	28	17.3	46	71.7

groups containing normal virgin females and treated males remained fairly low at first. From previous observations it appears that mating is a stimulus to egg production, so it is likely that copulation in the groups exposed for six and eight hours did not take place until after six days when the egg production for each exposure was approximately doubled with a high degree of egg viability. After this date there was an increase both in egg production and in the percentage of fertile eggs until the latter had become normal in a few days. The increase in egg production and in egg fertility started after seven days for the exposure of 12 hours. The increase in the percentage of fertile eggs deposited was, however, slower than for the two shorter exposures. The results for an exposure of four hours show almost the same trends as those at six and eight hours, altho a number of viable eggs were deposited from the first day of observation.

Isolations were made of the males that had developed from pupae exposed for 8 and 10 hours, and a normal virgin female mated with each one. Only three out of 44 pairs for the 8-hour exposure and none out of 15 pairs for the 10-hour exposure failed to produce fertile eggs after three days at 32° C. During the course of the experiment, 6 males in the former lot and 7 in the latter had died, indicating the effect of exposure of the pupae to high temperature on the longevity of the resulting adult males.

In order to determine whether longer exposures of pupae would induce permanent sterility in the males developing therefrom, the following experiment was performed. Adult males, two weeks old, that had developed from pupae exposed for 14 and 16 hours, were isolated with normal virgin females at 32° C. and the number of fertile eggs laid per pair recorded after every three days. At the en dof the first period of three days, 10 out of 50 pairs at the former and 30 out of 39 pairs at the latter exposure failed to produce fertile eggs. Six days later only

Altho in general the experiments at different humidities were fairly comparable, not all conditions were the same. At the time of exposure to dry heat the females were one week old, but those exposed to moist heat were a month old. The treated females were mated two days after exposure in the first case and one week after exposure in the second. One hundred females that had been exposed to moist heat were paired for a fertility test, but only 50 that were exposed to dry heat, the daily egg productions of the larger series being divided by 2 for comparison with those of the shorter one.

The figures indicate that recovery was nearly complete in two to four weeks. There was much more injury to fertility of the females at 0 than at 75 per cent relative humidity.

The question arises as to whether this temperature would have the same effect on the fertility of paired individuals. Three days after pairs were exposed at 44° and 0 per cent relative humidity, they were separated into groups of 50 individuals each. It was impossible to know exactly how evenly the sexes were distributed in each individual group. Comparison of the results with those for virgin females showed no apparent difference in effect of the temperature on mated and unmated females.

DISCUSSION OF THE RESULTS

High temperature may affect an insect species in many ways. All have some practical importance, depending upon the problem at hand. A given temperature may affect longevity of the adult but little, yet be high enough so that, in a generation or two, the insects will become sterile. Northrop (1920) studied the effect of raising *Drosophila* at high temperatures. Individuals raised at 30° C. and kept there permanently were unable to produce eggs capable of development at that temperature. When removed to 20° C. for 24 hours or longer within a week after emergence, the adults were able to produce eggs capable of development at 30° C. If, however, they were left longer at the higher temperature, the injury became permanent. Plough and Strauss (1923), studying the toleration of high temperature by *Drosophila,* showed that ordinary stocks may breed up to 31° C., but mutant stocks fail to tolerate this temperature for more than one generation. Kühn and Henke (1929) observed that *Ephestia kühniella,* kept continuously at 25° C., had fewer progeny. Normal productivity did not return immediately when they were transferred to a lower temperature, but only in the course of several generations.

The average hatch of eggs of *Tribolium confusum,* laid at 32° C. and kept at that temperature, was 88.5 per cent, whereas for eggs from the same lot kept at 37.5° to 38.5° C., it was only 64.0 per cent. When adults were kept at 37.5° to 38.5° C., the percentage of eggs hatching was reduced until for eggs laid on the twelfth day, it was 40.1 per cent. Whether adults would be completely sterile if kept at this temperature permanently, was not determined.

Larvae can develop at 37.5° to 38.5° C., altho the developmental rate is definitely slower than at 32° C. Nearly all the female adults developing from them, however, were found completely sterile, whereas, with few exceptions, the fertility of the males was unaffected.

Various comparisons of the sexes with respect to the relative effects of high temperature in sterilizing them show that the female sex is the more susceptible one, whether the insects are exposed as newly hatched larvae, mature larvae, pupae, or adult beetles. That the male reproductive functions are retarded is shown in Table 39, altho comparison with the data in Table 38 shows the female sex to be much more affected. This is in striking contrast to the results of Young and Plough (1926) for *Drosophila.* They report that in a culture kept at 31° C. most of the males were sterile, while most of the females were fertile. The males remained sterile only as long as this temperature was maintained, provided the exposure was not too extended. They recovered normal fertility if returned to the optimum temperature of 24° C. The number of males permanently sterilized at 31° C. was increased at longer exposures.

The rate of oviposition may be affected adversely by high temperature. In recording the number of births for each viviparous individual of *Aphis avenae,* Ewing (1916) observed the number to be 6.1 at 26.6° C., but only 0.25 at 32.2° C. Schulze (1926) studied the egg production of *Trichogramma evanescens* at different temperatures. The maximum number of eggs (43) was laid at 27.8° and 29.3° C. At 31.1° C. the number of eggs per female was only 22, at 32.3° C. it was four, and at 33.3° C. no eggs were laid. In extensive studies of the oviposition rate of the grape leaf-hopper, Bliss (1927) observed that temperature may affect egg production, first, by affecting the mechanism of egg production directly, and by altering the rate of egg formation in the ovary. A correlation between the rate of egg laying and the temperature of the day on which the eggs are laid might be attributed to either factor, whereas a correlation between rate and temperature of the preceding day can only be attributed to the second factor. In Table 24 it is shown that females of *Tribolium confusum* lay from 45 to 50 per cent fewer eggs at 37.5° to 38.5° C. than at 32° C., at least when the latter is their accustomed temperature.

In Figure 11 the percentage fertility of females developing from exposed mature larvae is compared with that of females from exposed pupae. The temperature to which the larvae and pupae were exposed was 40.5° to 41° C. Apparently the larvae are more affected in this respect than the pupae.

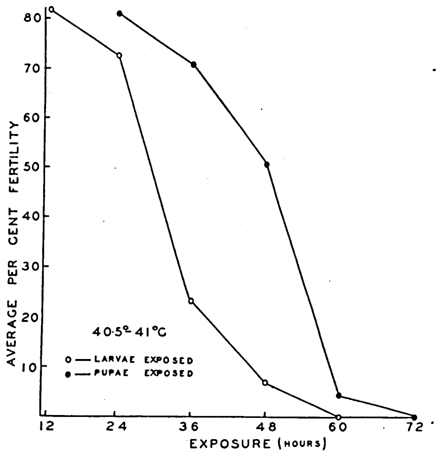

Fig. 11. The Average Percentage of Fertility of Eggs Laid by Females Developing From Larvae and Pupae Exposed to a Temperature of 40.5° to 41° C.

Females of *T. confusum* that had developed from larvae and pupae exposed to high temperatures showed either a partial or complete sterility, depending on the temperature and the length of exposure. The daily percentage of fertile eggs produced by the partly sterilized females varied within narrow limits for any one exposure. Occasionally only one to several fertile eggs were produced. The results suggest the possibility that exposures of larvae and pupae produce females in which an abnormal maturation division occurs. Whether these results are due to chromosomal fragmentations or cytoplasmic changes is unknown from present observations. Abnormal maturation divisions frequently result in an unbalanced chromatin condition which proves to be lethal to the zygote. There is also the possibility that the spermatozoa were unable to accomplish fertilization of the ova. The fertility of males that had developed from exposed larvae and pupae seems to be but slightly influenced. A possible retardation in spermatogenesis has been discussed. The results on this species, therefore, do not agree with those of Young and Plough (1926) and Norris (1933). The former authors observed that the males of a culture of *Drosophila* begin to be sterilized by heat before there is any effect on the females. Norris, speaking of *Ephestia kühniella,* states: "The results indicate clearly that the responsibility for the unsuccessful pairings lies with the male, and, moreover, that the importance of the male is in some cases overcome in old age. . . . . At 30° C. nearly 100 per cent of the males are sterile."

Plough and Strauss (1923) examined sections made from gonads of newly emerged *Drosophila* adults at 31° C. and found both mature eggs and mature sperm. The gonads of exposed individuals appeared to be similar to those of the control except for their smaller size. Cytological examinations aided Young and Plough (1926) but little in their experiments on the influence of heat on the same species. They state: "In none of the species is it possible to discover any difference in the cytological picture between normal ovaries and ovaries of females from sterile cultures, except that the latter appear to be smaller. . . . . In other sections from sterile males degeneration is also suggested for the sperms are decidedly reduced in number, have lost their orderly arrangement in masses in the testes, and seem shrunken in compact, irregular aggregations."

Exposures of female adults of *T. confusum* to heat reduce the egg production during and after the exposure. This suggests a retardation in the mechanism of egg formation in the ovaries. A reduced percentage of fertile eggs laid during and just after exposures, with a recovery in time, suggests a destruction of the matured egg cells, primary and secondary oocytes, while the oogonia are affected adversely but little. It is extremely difficult to determine to what extent this injury occurs in the ovarian tubules. Sterility induced by exposing female adults is only temporary.

Complete sterility of females induced by heat is of considerable economic importance in the control of the species, since apart from an inability to produce any progeny, the adult longevity is also reduced. Sterility induced by heat in males of *Tribolium confusum* is of no apparent economic importance, since, apart from being much less affected than the females, one male has been shown to be capable of fertilizing 36 virgin females in three days.

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APPENDIX A

The Effect of Temperature on the Confused Flour Beetle

Temperature, ° C.	Summary of data
−18	Average time to kill adults, 9 minutes
−12	" " " " " 14 "
− 6	" " " " " 8.4 hours
+ 7	" " " " " 14 days; maximum, 22 days
+12	No eggs are laid
17	Eggs are laid and hatch, but larvae die before completing development
22	Life cycle completed in 93 days
	(Maximum longevity at ordinary temperatures, probably between 20° and 25° C., 3 years 9 months, recorded for 1 male.)
27	Life cycle completed in 37 days
32	" " " " 27 "
38	" " " " about 38 to 40 days; egg viability reduced about 25 per cent
41	Larvae unable to complete their development
44	Average time to kill adults, 7.5 hours; maximum, 14.5 hours
46	" " " " " 66 minutes
48	" " " " " 26.5 "
50	" " " " " 7 "

APPENDIX B

Temperature Conversion Table

(For calculating intermediate values, 1° C. = 1.8° F.)

° C	° F.	° C	° F.
−20	−4.0	25	77.0
−15	+5.0	30	86.0
−10	14.0	35	95.0
− 5	23.0	40	104.0
0	32.0	45	113.0
+ 5	41.0	50	122.0
10	50.0	55	131.0
15	59.0	60	140.0
20	68.0		