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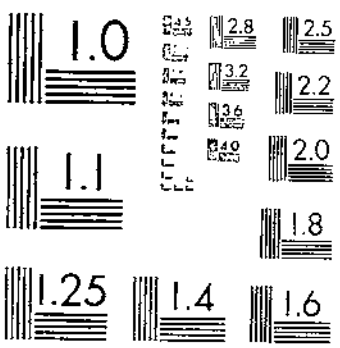

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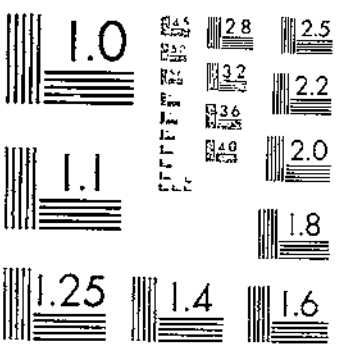

MICROCOPY RESOLUTION TEST CHART
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STUDIES ON THE MEXICAN FRUIT FLY, ANASTREPHA LUDENS (LOEW)

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INTRODUCTION

During the period when the authors were stationed at the laboratory in Mexico City maintained by the Bureau of Entomology of the United States Department of Agriculture in cooperation with the Mexican Department of Agriculture, they carried on a number of investigations on the Mexican fruit fly, *Anastrepha ludens* (Loew). These studies covered a variety of subjects on the biology of the fly, its parasites, and its responses under various conditions to various materials. The results of the studies on the effect of high and low temperatures on this fruit fly have already been published (*6*).[3] The results of studies on five other general subjects appear in the present bulletin.

HYDROGEN-ION CONCENTRATION OF SOIL IN RELATION TO THE PUPATION OF ANASTREPHA

In making field collections of pupae of *Anastrepha striata* which had migrated as larvae from fallen guavas, the authors were struck by the fact that the pupae were to be found lying practically equidistant from the center of each fruit, at a depth which varied slightly with the moisture of the soil. Very rarely was a pupa to be found directly underneath a fruit. Determinations of the hydrogen-ion concentration of the soil under the fruit and out to the pupae gave a gradient starting with pH values as low as 3.6 underneath the

[1] Resigned June 30, 1931. This manuscript is based on work done while the author was connected with the old Division of Tropical, Subtropical, and Ornamental Plant Insects of the Bureau of Entomology and in charge of the laboratory at Mexico City.

[2] Resigned June 30, 1931.

[3] Italic numbers in parentheses refer to Literature Cited, p. 19.

fruit and ending with 7.2 in the neighborhood of the pupae. In other words, the acids of the fruit had soaked out into the previously alkaline soil and produced a pH gradient. Examples of the pH values found are given in table 1.

TABLE 1.—*Hydrogen-ion concentrations of fallen guavas and surrounding soil*

Fruit (pH)	pH of soil (at stated distances in centimeters from center of fruit)				
	0	1.5	3.0	4.5	10.0
	pH	*pH*	*pH*	*pH*	*pH*
3.8	4.2	4.4	6.2	7.2	7.3
3.6	4.4	4.4	6.4	7.2	7.4
4.2	6.0	7.0	7.2	7.2	7.4

From the above observations two lines of experimentation were drafted: (1) On the extent of the acid changes in fruits; and (2), on the sensitivity of Anastrepha larvae to the hydrogen-ion concentration.

The original observations were made underneath guavas, which was fortunate, as they showed the most marked acidity changes of any fruit studied. The guava when ripe gives a reaction of pH 3.8 to 4.2. As the fruit lies on the ground and begins to rot, the pH drops to about 3.0. The larvae leave during this change, especially if there is much moisture in the fruit. The fruit maintains this low point (pH 3.0) for a few days, and then the pH starts to climb. It finally reaches equilibrium in the vicinity of pH 6.0. The mango does not undergo any striking changes in pH, but may reach a low figure of pH 3.0. The sweet lime has an original pH of 5.8, and rarely, while decomposing, reaches pH 4.0. As larvae do not migrate from all of these fruits at the same pH, it is likely that the type of acid also plays a part. The pH of guavas indicates when the larvae may be expected to leave. It must be remembered that in this case we are dealing with *A. striata* Schiner, which shows a greater sensitivity to acids than *A. ludens*.

The sensitivity of larvae to the hydrogen-ion concentration was investigated in the following experiments. Full-grown larvae of *A. ludens* were distributed as evenly as possible on petri dishes of soil (pH 7.8) which had been acidified in the center to pH 2.6 with hydrochloric acid. When the pupae were collected subsequently, they were all found in the periphery of the dish, where the soil had a pH of 7.8. To offset any chance of the above results being due to some tropistic response to light or to the glass surface, the experiment was repeated in reverse. In this case the same number of larvae were placed in dishes acidified peripherally to about pH 2.6. They all pupated in the center. Acetic acid produced substantially similar results. It was clear from the above that larvae migrate away from areas of high acidity in order to pupate. This behavior is in direct agreement with the previous studies on rotting fruit in the field. The writers have also observed a similar response to acids by larvae of *Drosophila melanogaster*.

The influence of the hydrogen-ion concentration was studied also by keeping fully grown larvae of *A. ludens* in Petri dishes on moist

paper of definite reaction. In this method the time taken for puparium formation under such conditions was used as the criterion of the effect of the medium. The results are given in table 2. Lowering the pH to 4.5 did not increase the death rate of either larvae or pupae above the normal expectancy, but the time taken to pupate was three times what it was at pH 8.7. Lowering the pH to 3.2 or 2.0 showed no further effect on the length of time necessary for all the larvae to pupate, but it had two other results. They either pupated at once, to emerge as normal flies, or they took a long time to pupate, and the longer the time the greater the mortality within the puparium.

TABLE 2.—*The effect of the hydrogen-ion concentration on the time necessary for puparium formation in Anastrepha ludens*

pH	Maximum days	Larvae	Dead larvae	pH	Maximum days	Larvae	Dead larvae
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
8.7	5	383	3	4.5	17	136	5
6.2	11	209	6	3.2	17	302	11
5.0	14	190	5	2.0	17	273	24
4.8	14	180	7				

With the foregoing data in mind, it is not strange that the sour lime, with a pH of about 2.0, is never infested. Such is also the case with the sidra, another citrus fruit which has a thick inner rind somewhat like a grapefruit and a small juicy center with a pH of about 2.0. Information on the effect that the hydrogen-ion concentration has on the immature stages of the fly suggests that this factor affects the distribution and relative abundance of the fly in Mexico both as to location and host fruit. In many of the regions of infestation visited by Elizabeth Skwarra and the authors, soil reactions were almost all alkaline or on the border line of neutrality. In a few sections where no infestations were found, however, the soil reactions were slightly acid. The means by which soil reaction affects infestation would seem to be indirect. The authors have, however, observed larvae moving considerable distances over areas of unfavorable soil in search of more suitable locations for pupation, and the longer the distance traversed the greater the chance of destruction by natural enemies. The reduction in numbers surviving would in time affect the distribution of the fly.

THE LENGTH OF LIFE OF ANASTREPHA LUDENS

The length of life of *Anastrepha ludens* is a subject of economic importance to the citrus growers of the Rio Grande Valley for the following reason: One of the earliest methods adopted of controlling the fly population (and which is still in use) was to remove completely all fruit from the trees for a certain period each year. In this way adult flies would have no place to deposit eggs and thus produce a new generation, and would themselves die off before the advent of the new crop. The question then arose as to whether adult flies could possibly live over this period and oviposit successfully in the new crop. The answer to this question is given in detail below.

The situation in Mexico itself is totally different. There is no large area in the fly-infested zones given over to a single fruit crop, such as oranges or grapefruit. There are side by side several host fruits of the fly, such as sweet limes, mangoes, oranges, and guavas, all growing in one orchard. This furnishes the fly with a continuous supply of fruits, both for food and oviposition. Even a single mango tree may be in continuous bloom for several months, first on one side and then on the other. The length of life of the fly is therefore of little importance to Mexico under the present conditions of fruit culture.

Previous to these studies very little information was available as to the length of life of the adults. It was the general opinion, however, that they did not live more than 6 months. If such had been the case, a host-free period of 7 months, as established in the Rio Grande Valley, would in itself have been a very effective means of eradication.

There is no way of determining the length of life of an adult fly in the field. The best that can be done is to determine the longest possible survival under laboratory conditions, and consider that as at least a possible value. Many thousands of *Anastrepha ludens* have been maintained in the laboratory for various lengths of time. The standard technic developed consisted in keeping the flies in cages of various types with pieces of cut ripe orange as food and a separate water supply in saturated absorbent cotton. The fruit was changed every second or third day, depending upon the rate of drying and the growth of molds on the fruit. Fresh water was added daily. The room in which the flies were kept had a temperature of 20° to 22° C. (68° to 71.6° F.), rarely falling below 18° (64.4° F.) and rarely rising above 25° (77° F.). The humidity was much more variable, in the dry months ranging from 20 to 35 percent saturation, in the rainy months from 50 to 70 percent. However, the flies were at no time left without a supply of water in the cages. For this reason the humidity in the cages tended to be higher than that in the room generally. This was especially true in the case of "closed" cages, where only one side permitted the passage of air, and this through closely woven muslin. In these the humidity was probably much more constant, as well as higher, than that of the room.

Table 3 is a compilation of the ages over 5 months attained by all flies of which any record was kept. It is by no means complete, and it does not represent the last part of the age distribution in its entirety. It does, however, show that some flies can survive for these periods of time, and that males in general live longer than females.

TABLE 3.—*The numbers of adults of Anastrepha ludens that attained various ages over 5 months under laboratory conditions*

Sex	Number of insects of indicated age in months								
	6	7	8	9	10	11	12	13	14
Females	25	20	15	10	7	3			
Males	143	120	[1] 104	[2] 45	18	7	2	2	1

[1] 54 of these were destroyed deliberately.
[2] 10 of these were destroyed deliberately.

The age at death, as distributed over a whole population of flies, was determined by detailed records kept on relatively small numbers of individuals. The experiments recorded in Figures 1, 2, and 3 were made in wooden boxes painted white, inside capacity 7½ by 1½ by 4½ inches, with the top of glass and one side of heavy muslin. Food and water supply were as previously described, which was the standard for these studies.

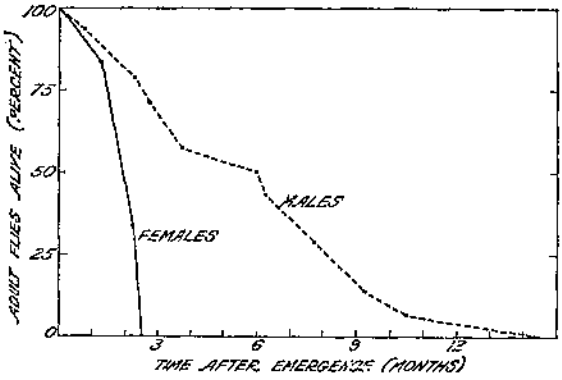

FIGURE 1.—Length of life in a group of 12 female and 14 male adults of *Anastrepha ludens*.

Figure 4 represents the dying off of a much larger group of flies (250 females and 220 males) kept in a much larger cage (1 by 1 by 2 feet). The detailed record in this case was not started until the flies were 2 months old. In general, the graph is similar to the others; and all show that males live longer than females. This is true both for the extreme cases and for the bulk of the population.

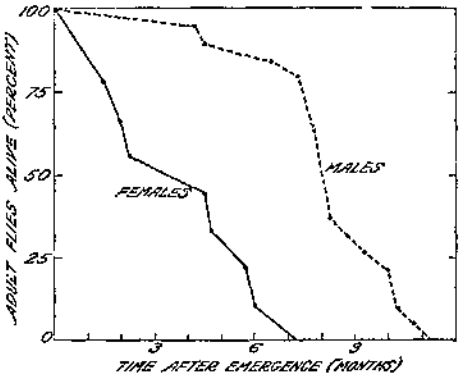

FIGURE 2.—Length of life in a group of 9 female and 19 male adults of *Anastrepha ludens*.

A reason which suggests itself for the early death of females under cage conditions is the twofold relationship between females and fruit, as compared with the single relationship between males and fruit. In the case of males, fruit is used for nutrition only. In the case of females it is also a medium in which to oviposit. Caged flies mate freely, and the females frequently oviposit in pieces of

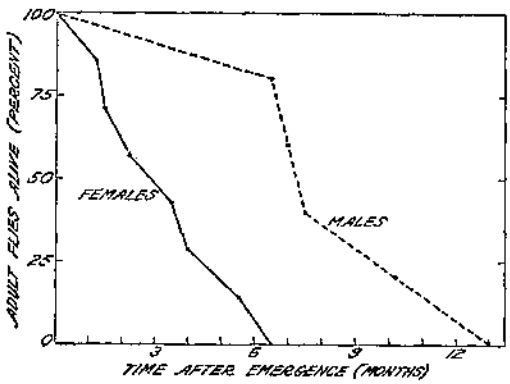

FIGURE 3.—Length of life in a group of 7 female and 5 male adults of *Anastrepha ludens*.

orange [4] (with rind), as well as in crevices or on the sides of the cage. It is entirely possible that there is some injury to females ovipositing in ripe orange, although fruit in this form is entirely suitable as food; or the withholding of their eggs may fundamentally affect their health. On this hypothesis, females might be expected to live even longer in the field than in the laboratory, without, of course, taking into account the presence or absence of natural enemies.

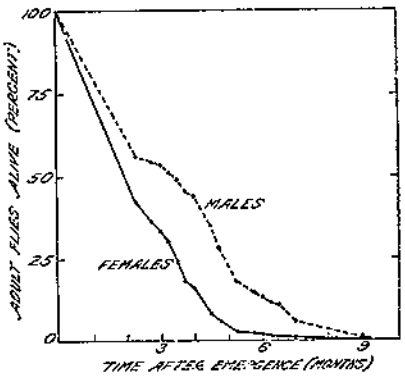

FIGURE 4.—Length of life in a group of 250 female and 220 male adults of *Anastrepha ludens*.

Since adult females are known to live as long as 11 months, and males still longer, it is clear that a host-free period between annual crops cannot in itself be effective in eradicating the fly. However, depending on the length of the fruit-free period, there is a decrease of population due to this method of attack. Just what percentage, may be guessed at by reference to figure 4. But if to the length of life of the adult fly there is added the time passed in egg, larval, and pupal development, it will be seen that a considerable proportion of the total number of flies can be expected to survive. Pupal development in this species can take as long as 100 days, at a temperature of 11.9° C. (53.4° F.), without entirely eliminating normal emergence of adults. This consideration alone adds another 3 months to the possible life of the organism. The possible length of the life cycle of *A. ludens* under laboratory conditions at 21° C (69.8° F.) is given in graphic form in figure 5 and totals, roughly, 13 months.

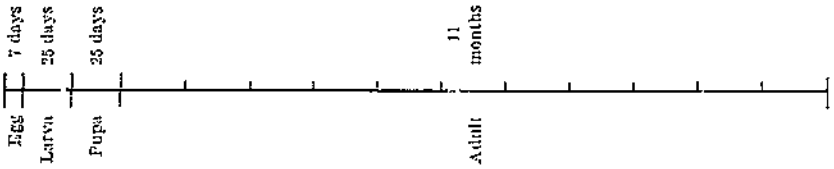

FIGURE 5.—Possible length of life of *Anastrepha ludens* by stages

Of course, length of life as related to fruit infestation has to be considered in connection with fertility. Viable eggs have been laid by a female 7 months old, caged with a male of the same age; while spermatozoa in all stages of development have been observed in sections of a testis taken from a male 9 months old. These, again, are not maximum figures for behavior in the field; and as long as a fly lives, there is at least the possibility of its being fertile.

Another supposed role of the host-free period is that of starving the fly. It has been tacitly assumed that the fly can feed only on

[4] Though the oviposition of the females in the pieces of orange was heavy at times, it would have been instructive to compare it with that of the normal free female. This was impossible owing to the nature of the observations necessary. It must suffice to say, however, that many hundreds of eggs were laid in the cages.

fruit, and that, in the absence of the various hosts, there will be no food supply. Direct observation in two directions has furnished data on the possibility of the fly's survival on foods other than fruit. (1) In the field *Anastrepha ludens* has been observed many times feeding on the outside of unbroken mangoes and guavas, on which there seemed to be no exudate whatever. It was deduced that the flies were feeding on some external growth on the fruit. Similarly, it has been noticed in the laboratory that the flies when feeding frequently pay no attention to freshly cut surfaces of fruit, until the outside has been completely explored. (2) Dissection of the crop of the insect has shown that it contains yeast cells.

If the two foregoing observations are relevant, a nutrient medium suitable for the fly and seeded with yeast should be an adequate food. Such a medium (*2*) was made up of corn meal and Knop's solution and inoculated with a yeast culture. On this a group of flies lived for over 3 months. The difficulty with the food lay in the fact that the flies frequently got stuck in the medium while feeding or buzzing about. This mechanical fault contributed largely to their early death. The above experiments and the field observations on the flies present evidence for the possibility of the fly feeding on any source of yeast that might be available in nature apart from that found on its host fruits.

Another experiment was run in which the flies were given only a lump of sugar and a separate water supply. No attempt was made to keep either the sugar or the water free of bacteria or mold. At the end of 4 months only 30 percent of the total population had died. This figure represents a somewhat better survival than that obtained for flies living on pieces of orange (figs. 1, 2, 3, and 4). In the field honeydew is always present, and yeasts and molds are universally to be found in it and in all plant exudates. The experiments of Delcourt and Guyenot (*7*), Baumberger (*1*), and others have shown that the fruit fly *Drosophila* (*ampelophila*) = *melanogaster* can live on a diet consisting entirely of yeast, either growing or dried. In view of the above facts, the possibility of eliminating a species of fly of this type solely by a host-free period is very remote.

The following facts regarding the adult longevity of *Anastrepha ludens* may be stressed as having an important bearing on the possibility of control through a host-free period.

An adult male and female have been kept alive for 14½ and 11 months, respectively. Under laboratory conditions females die sooner than males. Both males and females have been found fertile after a length of time greater than the host-free period which was established in the Rio Grande Valley. A host-free period between annual crops, depending on its length, can eliminate a varying proportion of the adult population of *A. ludens*. It cannot be expected to effect a complete elimination. The fly can live for long periods on yeasts or sugars whose availability in the field is independent of the presence of host fruits.

THE TOXICITY OF COPPER TO ANASTREPHA LUDENS

In a recent paper (*6*) the writers mentioned the fact that copper chloride, used to keep down the growth of molds in dishes in which pupae were kept, proved to be highly toxic to newly emerged flies if

they were not removed from the dish at once. The quickness of their death and their appearance led to an investigation of the underlying mechanism. The use of copper as an insecticide is well known, and its application in fruit-fly control has been reviewed recently by Miller and McBride (*8*).

In a series of experiments a comparison was made of the toxicity of various compounds of copper, made up in a spray mixture of the following composition:

Granulated sugar	grams	25
Maple sirup	cubic centimeters	50
Copper compound	gram	1
Distilled water	cubic centimeters	1,000

This mixture was fed on saturated absorbent cotton in 2½-inch Petri dishes. It was given fresh every second or third day, with intermediate additions of distilled water to the cotton when necessary. No other (copper-free) food or water was supplied. The temperature of the laboratory was kept within a few degrees of 22° C. (71° F.).

The results (fig. 6) completely confirm the findings of Miller and McBride as to the relative toxicity of the different salts. The toxicity is directly parallel to the concentration of copper ions in each solution. The above authors have shown that the toxicity of copper carbonate increases with acidity. This is simply another way of expressing the concentration of ionic copper. The lower the pH the greater the dissociation of copper carbonate. For instance, at pH3 this salt would be completely dissociated and the carbonate anion decomposed.

Curve *A*, *a* (fig. 6) shows the effect of allowing the solution to age. In what manner it changed the authors cannot say, but when tested against a new copper chloride solution the older one gave a slightly less toxic effect than the freshly made one. The possibility of a reaction between the food materials and the salts would bear investigation.

In another series of experiments the efficacy of copper was studied, different degrees of obligatory feeding being used. Copper chloride was employed, because it supplies the active agent in its most available form. In figure 7 are plotted the lethal curves in several fly populations which had been fed in the following manners, the technic being identical with that described for the previous series: (1) Food and water both poisoned. (2) Water supply, given on cotton as above, poisoned with copper chloride, 1 g per liter. Food consisted of lump sugar, given dry. (3) Water poisoned as in 2. Food consisted of sections of cut orange which, being moist, reduced the necessity for additional water consumption. (4) Controls.

From the curves in figure 7 it is clear that a spray of the type employed by Miller and McBride, to be effective in the field, must contain as much of the toxic agent as possible, to offset the dilution produced by alternative sources of food and water.

The reason for the shape of these curves is unknown. The presence of other fluids (orange juice) would account for the low initial death rate in curve *C'*; but why there should be a double inflection in curves *B* and *C'* is entirely obscure.

The main interest, however, lay in the mechanism by which the copper produced its effects. *Anastrepha ludens* in its normal habitat feeds largely if not exclusively on yeasts. The flies can be seen feeding on the skins of fruits where there is no rupture or exudation of juice whatever. Under cage conditions, they usually exhaust the possibilities of the skin of freshly cut fruit before examining the cut surface. Furthermore, the writers have kept flies alive and in normal condition (as judged by their subsequent reproduction) for 3 months with a yeast-growing medium as their sole source of nutri-

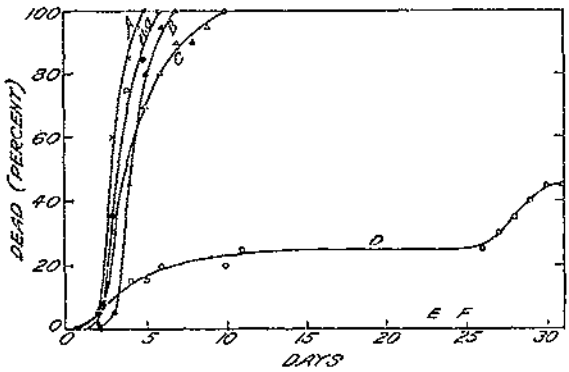

FIGURE 6.—Toxicity of various compounds of copper, fed in a spray mixture, to *Anastrepha ludens*: *A*, Copper chloride (freshly prepared spray); *a*, the same 1 month old; *B*, copper nitrate; *C*, copper sulphate; *D*, copper carbonate; *E*, copper oxide; *F*, control.

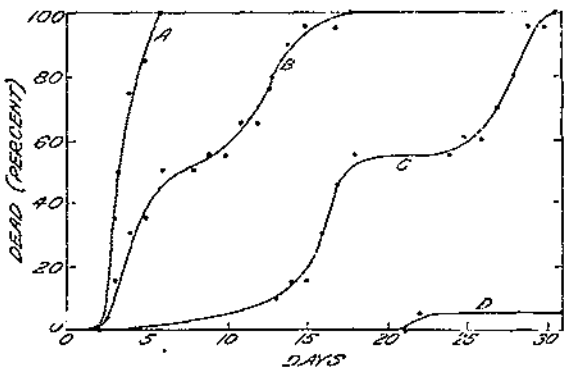

FIGURE 7.—Toxicity of copper chloride to *Anastrepha ludens*: *A*, Food and water poisoned; *B*, water alone poisoned, food dry; *C*, water poisoned, moist food unpoisoned; *D*, control.

ment. *Drosophilia melanogaster*, the vinegar fly, lives on yeasts, dead or alive. It was entirely to be expected that on dissecting out the alimentary tracts of several normal adults living on pieces of cut orange large numbers of yeast cells would be found. In the case of copper-killed flies, the number of yeast cells was greatly diminished, and those present appeared to be dead. In line with this is the observation by Miller and McBride (*8*) that Mediterranean fruit flies fed with copper died off at a rate similar to flies dying of starvation.

All of the above facts suggested the hypothesis that the effect of copper on fruit flies is connected with its toxicity for molds. Further evidence for this idea was obtained by inoculating with mold the spray mixtures used in the series of experiments illustrated in figure 6. The only solutions which showed any growth were the ones which were nontoxic—that is, the copper oxide mixture and the copper-free control. This concept fits in completely with the researches of Cleveland on termites (*4*), which showed that any agency which destroyed the protozoan fauna of the intestine produced death of the termite by starvation.

In this study the following copper compounds were found to be toxic to adults of *Anastrepha ludens* and are listed in order of toxicity: Copper chloride, copper nitrate, copper sulphate, and copper carbonate. The action of these compounds is apparently indirect, depending on their toxicity to yeasts and molds.

TEMPERATURE IN RELATION TO HOST-PARASITE EQUILIBRIUM

The biological control of pests by their parasites has been deemed sufficiently important to warrant large expenditures for that purpose. The fundamentals underlying the biological relations of pest to parasite have, however, quite frequently been overlooked. That the parasite successful in one environment will be equally effective as a control in another, is always open to question. A study was made stressing the importance of temperature in the establishment of a parasite in a new environment. The results of this study suggest that a parasite of little use in one region might be very valuable in another, under different climatic conditions. In this section the authors deal entirely with the difference in the reactions to temperature displayed by a host and its parasite.

The organisms used in the experiments to be described were *Anastrepha ludens* and its hymenopterous parasite *Opius crawfordi* (Vier.) that occurs frequently in larvae obtained from mangoes in the State of Morelos. The details of development of *Opius* have not been described, but the following facts are available. *Opius* lays its eggs in the larva of *Anastrepha* while the latter is still within its host fruit. The *Anastrepha* larva grows to full size, migrates from fruit to soil, and forms a normal puparium. On inspection through the wall of the puparium under suitable conditions, no distinction between normal and parasitized individuals can be detected until almost the end of the last larval instar. At this stage the shiny, annulated surface of the half-grown *Opius* larva can be distinguished, lying alongside of the relatively structureless remains of the *Anastrepha*. By the time pupation proper would have occurred the parasitized *Anastrepha* has completely disappeared, and the *Opius* larva can be seen moving about within the otherwise empty puparial shell. Within a few days the *Opius* pupates, and the fully differentiated imaginal structures can be clearly seen. The pearly white pupa gradually develops the characteristic red and black pigment of the adult, and the imago bites its way through the puparium at more or less the same time that its host would have emerged under the same conditions.

Anastrepha ludens is very abundant at certain times of the year, so it was possible to obtain from the field thousands of mature larvae, a varying proportion of which proved to be parasitized. The numbers of *A. ludens* used were so large (400 to 500 per temperature class) that a significant number of cases for the data on *Opius* was obtained.

Mature larvae were collected from mangoes and allowed to pupate on moist soil. The pupae were collected at intervals, and all those which formed in the course of 24 hours (midnight to midnight) were given the date of that day. As soon as collected the pupae were covered with a centimeter or less of sterile soil in covered Petri dishes. They were incubated at constant temperature (5) (see table 5 for range of temperatures used) until emergence took place. With this technic, 95 percent emergence was obtained regularly for *A. ludens* at nonlethal temperatures.

The means by which temperature may affect the host-parasite equilibrium differ. First, the limits of tolerance of the two organisms may be quite different at both high and low temperatures. *Opius* failed to emerge at 30° C. (86° F.), whereas a good emergence (85 percent) was obtained for *Anastrepha* at this temperature. The upper limit for *Anastrepha* was found to be considerably higher; an emergence of 87.4 percent was obtained at about 30.45° C. (86.8° F.), and a few emerged even at 31.4° C. (88.5° F.). *Anastrepha*, therefore, can develop and emerge at a temperature about 2 centigrade degrees higher than *Opius*. The lower extreme produced similar results. At 12.1° C. (53.8° F.) no *Opius* emerged, while 83.6 percent of *Anastrepha* emerged at this temperature. At 14.5° (58.1° F.) only 52 percent of *Opius* emerged, as against 96 percent of *Anastrepha*. The percentage of emergence over a series of temperatures is given in table 4, and graphically in figure 8.

TABLE 4.—*Emergence of Opius crawfordi and Anastrepha ludens at various temperatures*

Temperature		*Opius crawfordi*			*A. ludens* emerged	Temperature		*Opius crawfordi*			*A. ludens* emerged
		Dead	Emerged					Dead	Emerged		
°C.	°F.	*Number*	*Number*	*Percent*	*Percent*	°C.	°F.	*Number*	*Number*	*Percent*	*Percent*
10.0	50.0	.....	0	0	0	25.05	77.1	.....	.....	.....	96.0
11.0	53.4	10	0	0	27.2	26.95	80.5	136	235	63.3	90.5
12.1	53.8	109	0	0	83.6	28.0	82.4	40	42	47.7	97.0
14.5	58.1	56	61	52.1	95.8	29.1	84.4	6	3	33.3	99.1
16.0	60.8	155	135	46.6	96.6	29.95	85.9	27	0	0	84.6
18.0	64.4	32	47	59.5	97.3	30.45	86.8	123	0	0	87.4
19.75	67.5	53	132	87.9	97.3	31.0	87.8	87	0	0	40.6
22.0	71.6	18	60	76.9	97.3	31.4	88.5	112	0	0	.3
24.0	75.2	5	10	70.2	91.0						

The points obtained for *Opius* with *A. ludens* as host were supplemented and supported with data obtained from *A. striata*, another host. The latter points are plotted as black dots in figure 8.

The limits of the range of temperature toleration have been stressed in the above discussion and *Anastrepha* has been found to have a wider range than *Opius*. Attention focused exclusively on the ends, however, gives an incomplete impression of the relation-

ships. If the entire picture, as shown in table 4 and figure 8, is examined there will be found a gradually increasing lethal effect of temperature on *Opius* long before it begins to operate on *Anastrepha*. It would seem from the figures given in table 4 that there is a wide range of temperature (14.5° to 28° C. or 58.1° to 82.4° F.) that is perfectly satisfactory for the development of *A. ludens*. On the other hand, *Opius* passes through a sharp optimum at about 25° C. (77° F.), with a sharp slope on either side. The practical result is that although total mortality of the parasite does not occur at these nonlethal temperatures, a considerable diminution of its numbers takes place. Furthermore, if there is a high death rate it is logical to assume that even some of the survivors are injured in such a way that early death will ensue. This assumption is in line with the findings of Osterhout (*9*, *p. 18*).

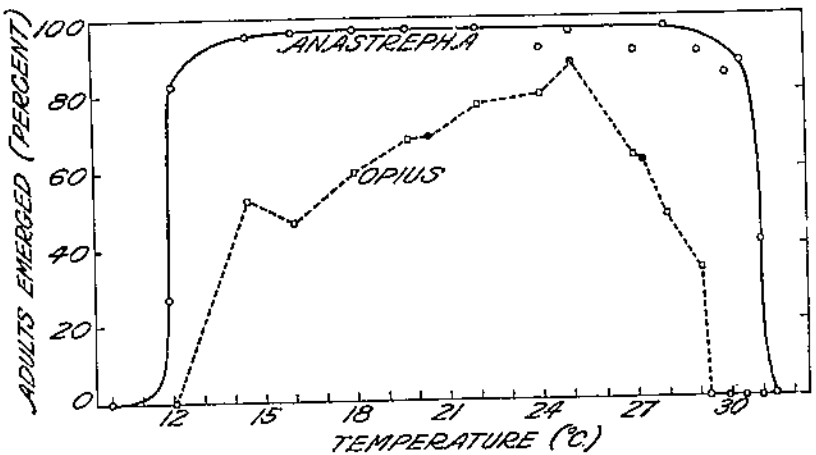

FIGURE 8.—Percentage of emergence of *Anastrepha ludens* and *Opius crawfordi* at different temperatures. The circles indicate emergence of *A. ludens*; the black round dots, emergence of *O. crawfordi* from *A. striata* as host; the squares, emergence from *A. ludens*.

A point which might be raised in connection with figure 8 is the disregard, in the curve for *Anastrepha*, of the points corresponding to 24°, 26.95°, 29°, and 29.95° C. All these points lie below the curve as drawn and are not balanced by other points lying above it. The curve was drawn deliberately above the four points for two reasons: (1) The emergence of the organism in this case is more significant than failure to emerge; that is, whereas there is a greater emergence at 30.45° than at 29.95° C., temperature cannot have been the detrimental factor causing the low emergence at the lower temperature; some other factor must be responsible; (2) this detrimental factor is known, since these experiments were the first run, and the technic of handling the pupae had not been perfected. In the light of this information the writers consider it justifiable to plot the points, but draw the curve above them.

Another wholly different effect of temperature is to be found in the data on the length of time spent within the puparium by the two organisms under consideration. These data are presented in table 5 and figure 9.

TABLE 5.—*The period in puparium of Opius crawfordi and Anastrepha ludens at various temperatures*

Temperature		*Opius* (male)				*Opius* (female)				*Anastrepha*			
		Cases	Period in puparium			Cases	Period in puparium			Cases	Period in puparium		
			Minimum	Maximum	Average		Minimum	Maximum	Average		Minimum	Maximum	Average
° *C.*	° *F.*	*Number*	*Days*	*Days*	*Days*	*Number*	*Days*	*Days*	*Days*	*Number*	*Days*	*Days*	*Days*
11.9 ±0.15	53.4±0.3	(1)	(2)	(2)		(1)	(2)	(2)		171	99	107	102.68
12.1 ± .3	53.8± .6	(1)	(2)	(2)		(1)	(2)	(2)		286	81	94	85.46
14.5 ±1.0	58.1±1.8	11	59	62	59.6	6	65	75	69.0	637	53	65	57.74
16.0 ± .2	60.8± .4	31	39	46	43.6	27	45	52	48.5	570	42	50	45.25
18.0 ± .15	64.4± .3	16	33	37	34.6	15	37	44	38.3	671	33	41	35.62
19.75± .2	67.5± .4	35	22	31	27.2	50	28	34	30.3	874	20	37	28.14
22.0 ± .1	71.6± .2	20	19	22	20.4	34	22	26	23.7	428	20	25	22.02
23.45± .05	74.2± .1	7	19	21	19.7	8	21	24	22.4	750	18	27	20.08
24.0 ± .1	75.2± .2	10	17	19	18.2	9	19	22	20.1	802	17	24	18.33
24.5 ± .1	76.1± .2	15	17	20	18.2	18	19	21	20.1	442	17	22	18.09
25.05± .1	77.1± .2	0				2	19	20	19.5	457	15	21	16.54
25.05± .1	78.7± .2	1	16	16	16.0	0				451	15	20	15.92
26.95± .05	80.5± .1	100	13	18	15.4	103	16	21	17.3	477	14	17	15.01
28.0 ± .05	82.4± .1	20	14	16	15.3	23	15	18	16.5	803	13	16	14.14
29.1 ± .1	84.4± .2	2	15	15	15.0	0				511	12	15	13.55
29.95± .05	85.9± .1	(1)	(2)	(2)		(1)	(2)	(2)		630	12	17	13.17
30.45± .05	86.8± .1	(1)	(2)	(2)		(1)	(2)	(2)		684	12	15	13.17
31.0 ± .1	87.8± .2	(1)	(2)	(2)		(1)	(2)	(2)		[3] 204	12	14	13.10
31.4 ± .1	88.5± .2	(1)	(2)	(2)		(1)	(2)	(2)		[4] 2	13	13	13.00

[1] See table 4 for number of *Opius* dead.
[2] No emergence.
[3] 590 pupae were incubated in this experiment. See table 4 for percent emerged.
[4] 664 pupae were incubated in this experiment. See table 4 for percent emerged.

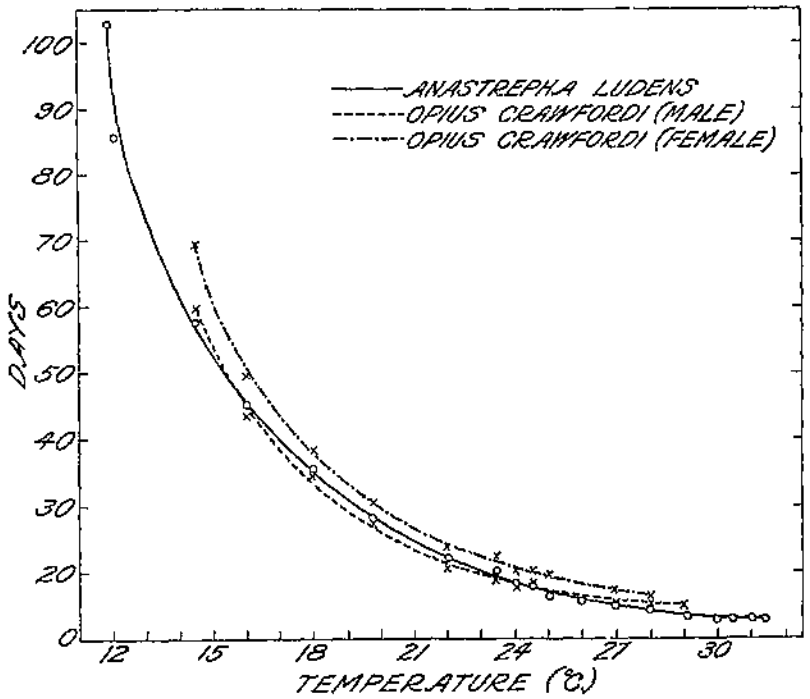

FIGURE 9.—Average length of the period in the puparium of *Anastrepha ludens* and *Opius crawfordi* at various temperatures.

The length of the pupal period of *A. ludens* was recorded in whole days, since the spread in emergence was so wide as to make closer observations insignificant. Pupae formed within 15 minutes of each other and and kept at constant temperature (26.95° ± 0.05° C., or 80.5° ± 0.1° F.) may be as much as 48 hours apart at emergence.

The figures for males and females have been averaged separately in the case of *Opius*, while for *Anastrepha* a single series of figures is given. This was done because the difference between the sex averages, while invariably present, is very small in the case of *A ludens* (0.4 day at most), and would not show in the graph if plotted on the same scale.

That part of the developmental process under comparison is not strictly equivalent in the two species in a morphological sense. In *Anastrepha* it is the measure of the length of the last larval instar, plus the pupal stage proper. On the other hand, in the case of *Opius* it is known that within the host puparium is passed an undetermined fraction—conceivably all—of the larval life, plus the pupal stage. However, it is a comparison of periods each of which always begins at the same developmental point, so differences in rate of development after that point can properly be attributed to differences in temperature.

A few individual cases have been omitted from the calculations on account of their anomalous behavior. Among parasitized pupae which had failed to produce any adults at the expected time, there was occasionally found a living *Opius* which was still in the larval condition, although all of its contemporaries had completed their development. These delayed larvae, if the puparium was not broken, would stay in the same condition for long periods, sometimes many months, eventually to pupate and emerge as perfectly ordinary looking *Opius* adults. Although not immobile, they seemed to be in a quiescent state of suspended development—presumably diapause. The phenomenon was observed in *Opius* reared at several temperatures. It seemed to occur more frequently in dishes which had become somewhat dry; and although the cases were too few and far between to control, the onset of pupation seemed to be hastened by a moist environment, after which development apparently proceeded at the usual rate. These individuals were so widely aberrant from the general population (one *Opius* emerged 7 months after pupation, its fellows in about 25 days) that they have been omitted entirely from the calculations relating to the length of the pupal period.

It will be seen in figure 9 that although the curves for host and parasite are in a general way similar, the *Opius* curves diverge from the *Anastrepha* curve as they approach their limits of tolerance. That is, near the ends of the range *Opius* is accelerated less or retarded more by the same rise or fall, respectively, of temperature. Its generation time is consequently lengthened more than that of the host, and its effectiveness as a control thereby impaired.

It has been found, then, that *A. ludens* has a wider range of emergence (11°–31.5° C. or 51.8°–88.7° F.) than *O. crawfordi* (12°–29° C. or 53.6°–84.2° F.). *A. ludens* has a wide range of temperatures at which normal development takes place. *Opius*, in contrast, has a peak of optimum development with marked lethal effects on either side. The development of these two organisms is accelerated to a different degree by the same increase in temperature.

From the foregoing data it may be seen that temperature can play a distinct role in the success of a parasite. That other controlling factors exist will be made clear in the next section of this bulletin.

HUMIDITY IN RELATION TO THE SURVIVAL OF OPIUS CRAWFORDI

Anastrepha ludens, in the State of Morelos, is parasitized in its larval stage by *Opius crawfordi*. The possibility of using this organism in the control of *A. ludens* led to a study of its biology. Some of the results, especially the vital role of humidity, are presented here. The contention and evidence of Buxton (*3*) on the role of climatic humidity are further supported by these findings.

At almost all times of the year during the period from 1928 to 1931, inclusive, there was an abundance of mangoes and, therefore, of *Anastrepha* larvae, in the city of Cuernavaca, Mexico, and from these the *Opius* were recovered. It is significant, however, that from the small amount of citrus in this section which is also infested by *A. ludens* no recovery of *Opius* was made. But it must be borne in mind that in the field there is always a great abundance of infested mangoes and guavas, favored fruits, which more than supply the needs of the parasite.

A few general remarks on the behavior of this parasite might help in the breeding of similar organisms. *Opius crawfordi* adults cannot be kept in very small cages or containers—they tear one another to pieces. At the beginning of these studies parasitized pupae were reared and allowed to emerge in Petri dishes, so that they might be handled easily. However, if two or more newly emerged adults were allowed to stay in these dishes for half an hour or so, at least half would be dead and the others seriously mutilated. The system used was to keep the Petri dishes covered (and very moist) until the insects were almost ready to emerge. The dishes were then uncovered and placed in a large cage, where there was plenty of space for the adults to fly about. By simply shifting the dish to a new cage at convenient intervals, the emergences could be easily recorded, and loss by injury was avoided entirely.

To facilitate the separation of the parasitized pupae from the normal fly pupae a study was made of the puparia. It was found that some puparia were markedly darker than others, and that these dark-colored puparia were generally parasitized. An attempt was made to sort out the parasites before emergence, using this criterion. But a few pale puparia yielded parasites, and a few dark ones yielded flies, so the method did not hold absolutely. The foregoing observations were all made on pupae formed and reared in slightly moist soil, under which conditions the puparium is opaque. If, however, the pupae, after formation in moist soil, are washed and placed on wet absorbent cotton in covered Petri dishes, they stay somewhat lighter in color and are quite transparent. The developing insect can be readily observed through the puparium with the aid of a low-powered binocular. On the fifth day of incubation at 25° C. (77° F.), parasites and flies can be identified with complete certainty, as at this stage the *Anastrepha* has become a proper pupa, or the *Opius* larva can be seen moving around inside

the puparium, any remnants of the host larva having completely disappeared.

Adults emerging over a period of a few days were placed in cages and individual records kept of the date of death. The cages in which they were kept had a wooden floor and frame, the top and front of glass, and the other sides covered with muslin. They consequently permitted relatively little air circulation, a point which will be discussed more fully later. The dimensions of the cages were 12 by 12 by 8 inches, and not more than 24 individuals were kept in a cage at one time.

In an attempt to rear *Opius crawfordi* for breeding purposes, difficulty had been experienced in finding a suitable food that would keep the adults alive for more than a few days. Various fruits which are subject to infestation by *Anastrepha ludens*, the host of *Opius*, were supplied as food, with always the same result of death within a few days. Finally, however, the use of ordinary cane lump sugar, with a dish of wet absorbent cotton, met with much better success; and detailed records were started on the length of life of each *Opius*.

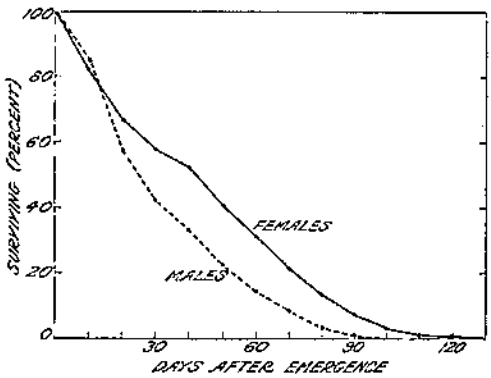

FIGURE 10.—Length of adult life of males and females of *Opius crawfordi*. The records are from 238 females and 133 males.

Figure 10 shows the length of adult life of the entire population of *Opius crawfordi* considered as a whole, with the one exception that males and females are plotted separately. This shows that *Opius* females live longer than the males, both the extreme cases and the bulk of the population.

An analysis was then made of the data of each separate experiment. It was seen that with each experiment the life span of the insect became progressively longer, beginning with the group started in February. To state it another way, the life span of those *Opius* that emerged in February was shorter than that of those which emerged in March and much shorter than that of those emerging in May. As there had been no known change in technic in the course of the observations, the temperature and humidity records for the room in which the insects were kept were examined for a possible explanation. The temperature had been fairly constant, averaging 21° C. (69.8° F.), and without significant fluctuations[5] (fig. 11). The relative humidity, however, had undergone a sharp increase with the advent of the first rains in April and had maintained a consistently higher level since that time. The *Opius* records were therefore divided up into small groups; (1) those which lived only

[5] That the temperature variation (18° to 26° C.) is of little importance is suggested by earlier findings, namely, that good emergence was obtained for *O. crawfordi* when incubated at these temperatures.

during the dry season, (2) those which emerged about a month preceding the rains, and (3) those which emerged after the rains began. Figure 10 shows the dying off of each of these three groups, considered in relation to their dates of emergence. In the same graph are included the mean daily temperature and humidity for the period under consideration.

The data in figure 11 are for females only, since there were relatively few males, and the large sex difference has already been commented on. Group 1 comprised 21 cases, emerged February 10 to 18; average, February 15. Group 2 comprised 40 cases, emerged March 18 to 31, average, March 25. Group 3 comprised 50 cases, emerged April 16 to 26; average, April 21.

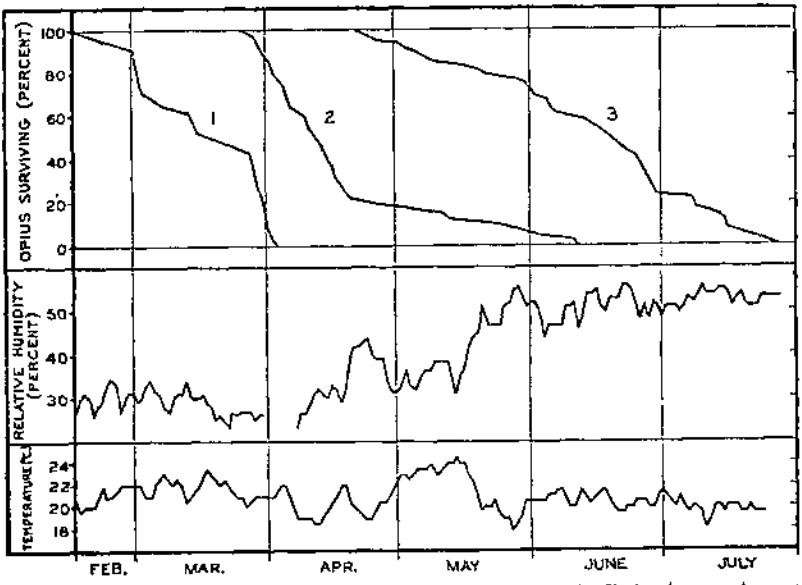

FIGURE 11.—Relation of the length of life of *Opius crawfordi* to temperature and humidity. The insects represented by curve 1 lived entirely in the dry season, those in curve 2 emerged about 1 month preceding the rains, and those in curve 3 lived in the rainy season.

It will be seen that the slopes of the curves for group 1 and for the first part of group 2 are quite similar and that they correspond with a consistently low relative humidity—30 percent on the average. Coincident with the first rainfall, April 19, there is a sharp rise in humidity and a break in curve 2. Subsequent to this date curve 2 is much more nearly parallel to curve 3, in spite of the fact that group 2 was nearly a month older than group 3. The steep drop in curve 3 near the end of June was coincident with the accidental drying up of the dishes of water in the cages, a circumstance which serves to emphasize the importance of moisture to this organism.

As mentioned above, the cages permitted very little air circulation. For this reason, with a constant supply of water in each cage, the humidity inside them was probably somewhat higher than that out in the room. From the humidity record of the room alone, there seems to be a critical point in the neighborhood of 30 percent, below

which *Opius* dies off much more rapidly than above that point. But the accident mentioned above would indicate that a humidity as low as 50 percent can cause considerable damage, since in the absence of an additional source of moisture the cage humidity would become the same as that prevailing in the room. On this occasion the cage and room humidity had every reason to be identical. It is therefore possible that the cage humidity was normally about 20 points higher than the record, and that the marked improvement in viability corresponds with an increase from 50 percent up, rather than from 30 percent. In any case, in setting up conditions suitable for the survival of this insect, it would be much safer to aim at a humidity higher than 50 percent.

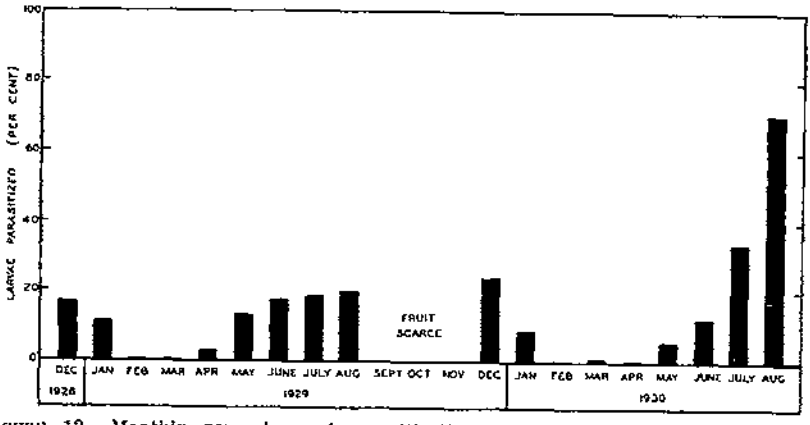

FIGURE 12.—Monthly percentage of parasitization by *Opius crawfordi* in larvae of *Anastrepha ludens* collected from mangoes, Cuernavaca, Morelos, Mexico, December 1928 to August 1930, inclusive.

In connection with the effect of humidity on the survival of *Opius*, the percentage of parasitization in field-collected larvae of *A. ludens* supplies a verification of laboratory findings. *Anastrepha* larvae were taken from mangoes, collected in Cuernavaca twice monthly. These larvae were reared, and the percentage of parasitization for the month was determined. In figure 12 these data from December 1928 to August 1930, inclusive, are placed in graphic form.

The repetition of the history of infestation from one year to the next is clearly shown, even though the magnitude differs somewhat. Unfortunately the humidity and rainfall records for this entire period cannot be obtained. However, the rains generally begin in April and last until October, with a peak in June and July. From November through March there is very little or no rain. There is a steady decline in the parasitization to almost zero, as the dry season advances, followed by an increase with the advent of the rains. A graph of the average rainfall for Cuernavaca, based on observations taken over 9 years, is given in figure 13.

It might possibly be thought that the abundance of parasites is controlled by the number of *Anastrepha* larvae, and this in turn by the quantity of fruit. Such, however, is not the case. There are in Cuernavaca a number of off-season mango trees, which bear during

January, February, and March, and furnish an adequate supply of larvae to be parasitized; but the number of adult *Opius* during those months is so small that the percentage of parasitization is very low. One other confirmation is that of direct observation. In the dry months it is almost impossible to find an *Opius* adult in the field, but in the rainy season they can be observed on trees, on the ground, and on mangoes in all locations.

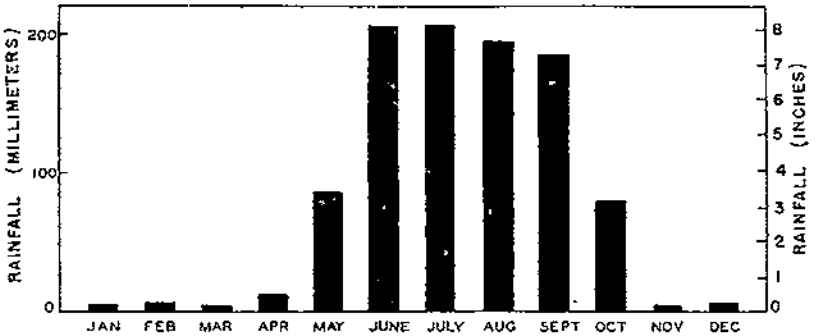

FIGURE 13.—Average monthly rainfall in Cuernavaca, Mexico, over 9 years

SUMMARY

Studies on the Mexican fruit fly, *Anastrepha ludens* (Loew), have shown that the larvae on leaving the fruit avoid as far as possible an acid soil for pupation. Almost all districts found infested had alkaline or nearly neutral soils.

Both males and females have been found fertile after an adult life longer than the host-free period that was established in the Rio Grande Valley. The possible life cycle at 21° C. (70° F.) is estimated to be about 15 months. The adults are not dependent on the host fruits for food.

Copper chloride, copper nitrate, copper sulphate, and copper carbonate are toxic to the adults of *A. ludens*, the toxic action being apparently associated with the destruction of the yeasts and molds that may be a necessary part of their diet.

A. ludens can develop under a wide range of temperatures, whereas its parasite *Opius crawfordi* has a short range of optimum development which limits its effectiveness to favorable climates.

The parasite *O. crawfordi* is very decidedly affected by variations in atmospheric humidity. A humidity higher than 50 percent seems necessary for optimum development.

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