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The Influence of Dowicide a on *Drosophila melanogaster*

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THE INFLUENCE OF DOWICIDE A ON DROSOPHILA MELANOGASTER

By

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-Introduction-

Drosophila melanogaster plays a far greater part in science than man at first realizes. A study of their heredity makes it possible to develop laws of human heredity and with this knowledge, thus provide for the betterment of mankind. Considerations which lead to the choice of the fruit fly, Drosophila melanogaster, in experimentation are briefly the following: (a) its small size which makes it convenient to keep; (b) its short life cycle; and (c) the ease with which it can be cultivated. Since fruit flies are so important to science, it follows that it is also important that a medium be developed wherein Drosophila melanogaster thrives readily. It is necessary not only that the food be suitable but also that mold be kept out.

For years experimentors have been seeking a suitable medium and they have found either the banana or the cornmeal formula to be very good. However, with the banana shortage brought on by the war, C.E. Myers (Lewis 1942) has seen the need for a new food medium and has accordingly acted upon it. He has introduced a substitute that is apparently superior to either the previously used banana or cornmeal media. He suggested that a tomato product might offer possibilities as a banana substitute. This suggestion was brought about as a result of his observations that throughout the fermentation of tomato pulp, numerous fruit flies were attracted to the pulp barrels.

"A two-month experimental period in the preparation

and use of tomato-paste medium has provided time in which to test proportions of ingredients of the formula, to observe the properties of the resultant medium and to note the size and vigor of the yields obtained. The formula recommended is as follows, with the customary drop of Fleischmann's yeast suspension to be added to each culture when the flies are introduced.

1000 cc water
100 gm tomato paste
100 gm white corn syrup
20 gm granulated agar-agar
1 gm Moldex"[1]

This medium is believed to be superior over others in that (a) it eliminates the uncertainty of obtaining bananas; (b) it is economical; (c) its red color makes a good background for observing the metamorphosis of Drosophila melanogaster; (d) it does not dry out readily; instead there is sufficient moisture in the medium to keep the culture over a period of three to four weeks. It keeps the absorbent paper amply moistened for pupation; and (e) its Moldex content makes it possible to keep unautoclaved medium at ordinary room temperature for two weeks or more in plugged bottles which are sterilized before filling.

Winchester in 1933 published an account in Science of a method of increasing the yield of Drosophila melanogaster. It was stated that the addition of about 2 grams of dried brewers' yeast to 100cc of the usual banana food media, gave rise to a 10-fold increase in yield of Drosophila melanogaster. The yeast was added immediately after the agar

1.Lewis, M.T., "A New Agar Medium for Drosophila Culture," Science, Vol. 96, No. 2490, Sept.18, 1942, p. 282.

had dissolved and was thus boiled with the food.

Sinnott and Dunn (1939), reviewing the work of Bridges (1937) and Lebedeff (1937), report that Moldex is extremely important in that it eliminates mold. However, in concentrations exceeding 0.1, it reduces the growth of yeast and flies. They also recommend the same concentration of Nipagin-T or Nipagin-M as a means of mold control.

Other scientists of the Texas College of Arts and Industries, however, prefer to control mold by means of a different formula. This particular formula, they maintain, is excellent because it stops growth on even banana medium, which, according to them is the medium which molds most readily of all. Mold usually gets started on banana food before the flies have time to produce larvae. As a result, the flies often do not breed well. In the laboratory in which the work was carried out, various species of mold were present abundantly. The chief offenders were species of Mucor, Rhizopus and Aspergillus. The mold was so abundant that contamination seemed to be from various sources. Consequently, more than one method of control was necessary. Hence, food was made accordingly and proved successful.

750 cc. of water and 75 cc. of white Karo syrup were mixed in a pyrex beaker. 20 grams of shredded agar were added. The mixture was boiled until the agar was liquefied. Two medium-sized bananas were crushed in a separate container and 25 cc. to 30 cc. of 95 per cent alcohol were

added. The bananas and the alcohol were stirred well and allowed to stand about twenty minutes and were then added to the water-syrup-agar mixture after that mixture had stopped boiling and had cooled to about ninety degrees Centigrade. Since alcohol had already been added, it was not necessary to spray the food with yeast as is done in some laboratories. And since no yeast was present, no carbon dioxide was formed. The food adhered well to the bottom of the bottles and the flies did not stick to it too readily. It was kept in the laboratory almost two weeks without having mold cover it. When the bottles were not autoclaved, however, mold appeared within four or five days.[2]

From this discussion it may be assumed that the immediate problem is to control mold on medium, and thus increase the Drosophila melanogaster population. Hence, it is the purpose of this experiment to determine whether or not Dowicide A added to the regular cornmeal formula (as recommended by Sinnott and Dunn (1939), in place of Moldex, is effective as a means of mold control, and whether or not it is advantageous or injurious to the flies.

"The Dowicides are a group of seventeen industrial germicides and fungicides which have been developed to meet the specific requirements of industry in eliminating manu-

2.cf. Cross, J.C., "Control of Mold in Food for Drosophilae," Science, Vol. 89, No. 2298, Jan.13, 1939, p. 40

facturing problems that are caused by bacteria and fungi."[3]

Dowicide A, the particular one used in this experiment, is substantially white, has the appearance of ground flakes, and has a faint, characteristic odor. Chemically it consists of Orthophenylphenol, Sodium Salt ($C_6H_4C_6H_4ONa \cdot 4H_2O$). The Dow company claims that it is effective in preventing decay, putrefaction, and mold in materials having a high nitrogen content.

3. _____, __, "Dowicides, Industrial Germicides and Fungicides", 1941, p. 3.

-Materials and Methods-

The fruit flies, Drosophila melanogaster, used in this experiment were procured from the original stock of the Biology Department of Marquette University. For purposes of experimentation, the flies were originally grown in sterilized half-pint bottles with a cotton gauze stopper and the usual cornmeal food media recommended by Sinnott and Dunn (1939). These same flies were later transferred to the experimental culture bottles. In preparing the first group of experimental culture bottles, the half-pint bottles were first cleaned. 0.5 gm. of Dowicide A was dissolved in 2.5 ml. of 95% alcohol. 7.5 gm. of agar were cut into 250 ml. of water and allowed to soak for 10 minutes. 55 gm. of cornmeal were soaked in 125 ml. of water. 62.5 ml. of molasses were measured out and the Dowicide A was added to the agar solution. The agar was boiled over a low flame until dissolved. The molasses was then added to the agar solution and was stirred constantly until it was brought to a boil. The cornmeal suspension was added while the solution was being stirred until it was brought to a boil. About 3/4 of an inch of the cornmeal media was then poured into the bottom of each half-pint bottle. A strip of absorbent paper-toweling was then placed in the bottom of each bottle and adjusted in order to make an incline for the metamorphic stages of the fruit fly. The necks of the bottles were cleaned of the media and a cotton-gauze stopper was adjusted to fit each bottle opening. The group of bottles -- six in

number -- were then sterilized in the autoclave. The bottles were permitted to stand for thirty-six hours before introducing the flies into them. In order to observe the possible influences upon the different types of flies, the various species were distributed as follows: The first bottle contained 3 male Taxi flies and 3 female Eight flies. The second bottle contained the reciprocal cross of 3 male Eight flies and 3 female Taxi flies. 3 female Eosin flies and 3 male taxi flies were placed in the third bottle, and its reciprocal cross consisting of 3 male Eosin flies and 3 female Taxi flies, was made in the fourth bottle. The fifth bottle contained 3 female Eight flies and 3 male Eosin flies, while the sixth bottle held the reciprocal -- 3 female Eosin flies and 3 male Taxi flies.

Experiment II was a repetition of experiment I with the exception that experiment II was again subdivided into two experiments (as it were.) There were sixteen bottles used in experiment II. Half of them contained food medium that was made with 0.5 gram of Dowicide A. The other half contained food medium that was made with 1.5 grams of Dowicide A. In this experiment four types of flies were used -- Eyeless, Plus, Sepia, and Vestigial. In the first half of the experiment -- that part wherein the bottles with the 0.5 gram of Dowicide A were used,-- there were two bottles, bottle A and bottle B, containing Eyeless flies, two bottles containing Plus flies, two bottles containing

Sepia flies, and two bottles containing Vestigial flies. In the second half of the experiment -- the half wherein the bottles with the 1.5 grams of Dowicide A were used -- the flies were distributed in the bottles in the same manner. Another way in which experiment II differed from experiment I was that the bottles and food used in experiment II were not sterilized.

-Observations-

As explained before in experiment I there was 0.5 gm. of Dowicide A while in experiment II there was 1.5 gm. of Dowicide A. These experiments were run over the same length of time in the life cycles of the various flies. The experiments ran from the beginning to twelve days after the first fly hatched. The quantity of flies per bottle is the basis for determining the results of this experimentation. Results showed that Dowicide A when used in amount of 0.5 gm. per half formula kept out the mold without injuring the flies. However, 1.5 gm. of Dowicide A proved to be too much for the flies and resulted either in their death or in their failure to reproduce or, if they did reproduce, they did so in fewer numbers.

Throughout the whole period of experimentation , which, when added together, amounts to almost six weeks, mold did not appear once.

More females hatched than did males. The flies in the 0.5 gm. of Dowicide A medium had a more regular life cycle than did the ones in the 1.5 gm. of Dowicide A.

Experiment I

0.5 gm. Dowicide A												
	Taxi-Eight				Eosin-Taxi				Eight-Eosin			
	3 m. Taxi – 3 ♀. Eight		3 ♀. Taxi 3 m Eight		3 ♀. Eosin 3 m. Taxi		3 m. Eosin 3 ♀. Taxi		3 ♀. Eight 3 m. Eosin		3 m. Eight 3 ♀. Eosin	
Start	3/5/43		3/5/43		3/5/43		3/5/43		3/5/43		3/5/43	
Larva	3/12/43		3/15/43		3/14/43		3/14/43		3/12/43		3/15/43	
Pupa	3/16/43		3/19/43		3/18/43		3/18/43		3/18/43		3/18/43	
Flies	3/22/43		3/22/43		3/22/43		3/22/43		3/22/43		3/22/43	
No. of Flies	m. 39	♀. 35	m. 23	♀. 25	m. 50	♀. 42	m. 21	♀. 24	m. 20	♀. 30	m. 36	♀. 44
Diff. in sex	m. + 4		♀. 2		m. 8		♀ 3		♀. 10		♀. 8	
Days-Larva	7		10		9		9		7		10	
Days-Pupae	11		14		13		13		13		13	
Days-Flies	17		17		17		17		17		17	

Experiment II

0.5 gm. Dowicide A								
	Eyeless		Plus		Sepia		Vestigial	
	A	B	A	B	A	B	A	B
Start	3/26/43	3/26/43	3/26/43	3/26/43	3/26/43	3/26/43	3/30/43	3/30/43
Larva	4/5/43	4/3/43	4/2/43	4/2/43	4/7/43	4/3/43	4/6/43	4/6/43
Pupa	4/9/43	4/9/43	4/6/43	4/6/43	4/12/43	4/9/43	4/12/43	4/12/43
Flies	4/12/43	4/16/43	4/12/43	4/12/43	4/16/43	4/12/43	4/16/43	4/16/43
No. of Flies	m 27 f 34	m 34 f 42	m 64 f 84	m 36 f 29	m 39 f 41	m 16 f 14	m 44 f 53	m 63 f 66
Diff. in sex	f 7	f 8	f 20	m 7	f 2	m 2	f 9	f 3
Days-Larva	10	8	7	7	12	8	10	10
Days-Pupae	14	14	11	11	17	14	16	16
Days-Flies	17	21	17	17	21	17	20	20

1.5 gm. Dowicide A								
	Eyeless		Plus		Sepia		Vestigial	
	A	B	A	B	A	B	A	B
Start	3/31/43	3/31/43	3/26/43	3/26/43	3/30/43	3/30/43	3/30/43	3/30/43
Larva	4/19/43	4/6/43		4/5/43				
Pupa	4/26/43	4/16/43		4/12/43				
Flies	5/1/43	4/26/43		4/16/43				
No. of Flies	m. 8 / f. 8	m. 5 / f. 9		m. 2 / f. 5				
Diff. in sex		f. 4		f. 3				
Days-Larva	19	6		10				
Days-pupae	26	16		17				
Days-Flies	31	26		21				

-Summary-

Different varieties of Drosophila melanogaster were grown on medium containing 750 cc. water; 15 gm. of agar, 125 cc. molasses, 110 gm. of cornmeal, 1 or 3 gm. of Dowicide A, and 5 cc. of 95% alcohol.

No mold grew at these concentrations of Dowicide A.

Medium containing 1.0 gm. Of Dowicide A was an asset to Drosophila melanogaster , while 3.0 gm. of Dowicide A added to the same amount of medium reduced the number of offspring produced.

The varieties of Drosophila melanogaster grown on these media showed different rates of reproduction. However, further studies should be made to show the significance of these results.

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