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ODORS AND INSECTS

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The following experiments were made at the suggestion of Dr. Frank E. Lutz during the month that I was a guest of the American Museum's Station for the Study of Insects near Tuxedo, N. Y. While not as complete as might be desired, they yielded apparently definite results so far as they went.

ODORS ATTRACTING ADULT *Drosophila melanogaster*

This "Pomace Fly" has become a standard laboratory insect. Twenty years ago Barrows (1907, Journal Exp. Zoology, IV, p. 515) reported on its reactions to odorous substances, particularly those chemicals that are found in fermenting bananas. More recently (See, for examples, Northrop, 1917, Journal Biol. Chemistry, XXX, p. 181, and Baumberger, 1919, Journal Exp. Zoölogy, XXVIII, p. 1) it has been found that *Drosophila melanogaster* larvæ feed on yeast rather than on the substratum (banana or the so-called "inorganic foods"). The fact that yeast, quite apart from its products, is a sufficient food for these larvæ was confirmed by Dr. Lutz at the Station this summer when he reared a large number of the flies on Fleishmann's yeast that was continuously washed in a filter with running water.

Last year Mr. Steele, also working at the American Museum's entomological field station, found that carrion beetles were attracted by the odor of fly-larvæ feeding upon carrion rather than by the odor of the carrion itself; likewise, that they eat the maggots rather than the "substratum" of the maggots, the carrion. The analogy of this case to that of the pomace fly is practically complete.

EXPERIMENT 1.—Apparatus: A wooden box $10 \times 15 \times 3\frac{1}{4}$ inches having a glass cover luted for tightness, fitted with an opening on each side for inlet and outlet. For this trial one opening in a 10-inch wall was used as a suction, the remaining three openings were each fitted with a piece of $\frac{3}{16}$ inch glass tubing used to introduce the odors. One tube supplied the odor from fermenting banana, another moist air, and through

the third tube air was drawn through a piece of cotton moistened with water, the cotton previously having been treated with a purified benzine extract of fermenting banana, and solvent evaporated. The odor imparted to the cotton was decidedly yeasty.

Suction was begun at the rate of approximately 500 cu. in. of air per hour during the first 30 minutes of the test. Through this period 10 flies entered the tube containing the banana pulp, none entering the tube which supplied the moist air or that through which the benzine extract residue was being aspirated. This benzine residue was then changed to a 50% alcoholic extraction of the benzine containing the extracts of the banana, and solvent evaporated. Suction was again begun at the same rate and at the expiration of 30 minutes there were 11 flies in the tube of the banana pulp, the tube containing the residue of the alcoholic washing of the benzine extract on the moistened cotton had not been visited. Suction was continued for 1½ hours during which time 3 flies visited the tube containing the extract residue. This experiment was then discontinued.

EXPERIMENT 2.—25 grams of soft banana, representing a cross-section including the peel, were extracted with 100 cc. of methanol (purified), the extract filtered and then evaporated to 10 grams. The approximate alcoholic strength of the strength of extract as filtered was 83%. Evaporation was carried on at a temperature under the boiling point of the liquid. The odor of the residual 10 grams was slightly acetic, mild, banana-like.

Three test tubes were prepared, one containing fermenting banana, the second a small plug of cotton moistened with water, the third tube containing the 10 grams of extract absorbed on cotton. The three tubes were placed in an observation box having 3 sides of fine mesh screening and 1 of glass. The one end was of wood, the other of glass.

A number of flies were introduced direct from the stock jar. Results of the visits are tabulated below:

TIME	WATER	BANANA	BANANA EXTRACT
1:55	0	0	2
2:00	0	1	6
2:05	1	7	1
2:15	1	10	2
2:30	0	3	2
	2	21	13

The flies showing no strong desire to feed, the banana and the banana extract were removed for 26 hours. When replaced, with an empty tube added to the equipment, the results were:

Time	Water	Banana	Banana Extract	Empty Tube
4:30	0	8	4	0
4:35	0	4	2	0
4:40	0	15	3	0
4:50	0	25	2	0
5:10	0	24	1	0
	—	—	—	—
	0	76	12	0

The results of these experiments indicate that the fermenting banana has greater attractiveness than the extract. The checks were blank.

Since fermenting banana possesses more attraction than fresh fruit and since yeast is the fermenting agent, possibly alcohol is the attractant (See also the paper by Barrows). With this in mind a mixture of glucose 1½ grams, molasses 2½ grams, sulphate of magnesia ⅒ gram, phosphate of magnesia ⅒ gram, citric acid ⅗ gram and water 100 cc. was inoculated by a stab into a soft banana and a stab at the broken section of the skin for cultural material.

While this was fermenting a box was prepared 16 inches square provided with a glass cover, the inner part of the box being divided into four triangular compartments, the partitions ending about 1½ inches from the center, leaving a three-inch gap. Each compartment was ventilated by providing a screen window 2½ × 8 inches, to permit a free flow of air currents. Three of the compartments were provided with Petri dishes. On one was soft banana, on one was a banana extract containing 5% of ethyl alcohol absorbed on cotton, and the third one was provided with the fermenting glucose and molasses mixture absorbed on cotton which had been permitted to stand at room temperature for 48 hours.

A number of flies were taken direct from the stock bottle and introduced into the vacant chamber. After 30 minutes of observation no feeding was observed although several flies visited the banana and the fermenting fluid, but none showed an inclination to eat. The glass cover was lifted to free the box of flies and then replaced. The Petri dishes were left in place for twenty-four hours, after which time a fresh batch of flies that had been kept without food for twelve hours was introduced and the following results observed:

Banana	Banana Extract	Fermenting Fluid
23	2	28

The flies were disturbed and the apparatus turned halfway around, and after an elapse of one hour they were again observed:

18	3	22

Observation half an hour later showed:

25	0	21

The cotton containing the banana extract, also the cotton containing the fermenting fluid was moistened with more of the fluid material, and after an elapse of twelve hours further observations were made:

Over 100	0	14

The materials were examined for eggs. Both of the prepared foods were apparently free from eggs while the banana showed deposits of eggs in several places. These were not counted.

This experiment was repeated with freshly prepared baits, banana extract being omitted, the fermenting fluid this time being glucose diluted with water containing magnesia sulphate and phosphate, inoculated with a stab of fermenting banana, and possessing a mildly vinous odor. The visits were as follows:

Banana	Water	Fermenting Fluid
2	0	7
4	0	6
4	0	6
5	0	8
8	0	10
2	0	5
—	—	—
25	0	32

The results indicating that either yeast or the products of fermentation possessed an attraction for the flies. Another experiment was carried on with fermenting soft banana, and a commercial yeast cake mixed with sufficient water to bring it to a stiff creamy consistency. These were placed in two compartments of the box, and into the third compartment was introduced a number of flies that had been kept without food for six hours. The following counts were made during the evening of July 20 and the morning of July 21:

YEAST	BANANA
7	9
6	9
6	15
10	15
3	15
23	63

On the morning of the 21st both the yeast and the banana were examined for eggs. On the yeast were found 9 eggs and 6 larvæ. On the banana 50 eggs and 40 larvæ.

These figures led us to attempt further trials, but with yeast culture from banana.

The box was prepared with 3 Petri dishes, 1 containing a piece of banana, the second moistened with a fluid composed of starch 6 grams, hydrochloric acid 2 grams, water 120 grams, boiled until clarified, then 1 gram of sulphate of magnesia, 1 gram of phosphate of magnesia and 1 gram of citric acid added after neutralizing the hydrochloric acid with ammonia. This solution when tested with Benedict solution showed no sugar and was fermenting for 11 days before being used. The third was fermenting Karo solution made by dissolving 20 grams of Karo syrup, 2 grams magnesium sulphate, 2 grams of potassium phosphate, 2 grams citric acid and 380 cc. of water. This was also permitted to stand for 10 days before being used. The odor of the fermenting starch was decidedly yeast-like. The odor of the fermenting Karo was vinous and the banana was typical.

A number of flies were introduced into the vacant compartment and the following results noted:

BANANA	STARCH	KARO
6	0	7
3	1	7
3	1	4
3	3	8
3	3	7
7	9	4
7	11	4
5	11	5
7	8	5
7	11	4
6	7	9
5	7	4
7	9	7
5	11	8
67	92	83

After 24 hours the contents of the dishes were examined for eggs. Eggs were observed on the banana and on the Karo fermentation product, while the starch showed none.

This experiment then led us to try the fermenting liquid as compared to yeast. The fermenting product of Karo was filtered and the yeast content on the filter paper was washed with water during 12 hours to completely eliminate all soluble matter. This filter paper containing the washed yeast was opened on the Petri dish, the filtrate was refiltered and a part absorbed on cotton. The filtrate possessed a vinous odor modified by a very slight yeast-like character. The material on the filter paper possessed a strong yeast odor.

A number of flies were liberated in the vacant chamber and the following count made:

Time	Filtered Liquid	Yeast
10:40	2	11
10:55	1	18
11:10	0	22
11:25	0	22

The position of the Petri dishes was then reversed and observations again made:

Time	Filtered Liquid	Yeast
11:45	0	25
12:	0	29

A test was made in a T tube, one arm provided with a plug of cotton containing yeast, the other arm a plug of cotton containing fermenting liquid. The stem was passed through a cork into a test tube containing a number of flies. Suction was applied to the test tube drawing the air through the prepared cotton plugs. The arm of the tube containing the yeast received 56 visits while that containing the fermenting fluid received 57 visits from flies.

Adult *Drosophila melanogaster* are clearly attracted by the odor of the yeast upon which their larvæ feed but this reflex appears to be at least somewhat "conditioned" by the odors usually associated with that yeast.

Training Bees to Odor

This experiment, based on the interesting work of Karl v. Frisch, was undertaken to analyze more completely the olfactory factors in flowers which are noted by the common honey-bee, *Apis*.

Upon a table-like surface 12 Petri dishes were each fitted with a 4-inch circle of filter paper upon which rested a watch glass. Four of the filter papers possessed no odor other than that of paper. The remaining eight were impregnated with a solution of paraffin and purified benzine to which was added an odor. 1% of the following odor bodies was used:

Anethol
Carvone
Citral
Synthetic oil of Lily of the Valley

The other four were scented with extracts of the following blooms:

Daisy
New Jersey Tea
Yarrow
Milk-weed

Eight grams of the bloom were extracted with sufficient purified benzine to make 50 cc. of extract. Into this was dissolved 3 grams of paraffin. The extracts expressed the odors of the respective flowers to a marked degree, and as yarrow presented no particular attractiveness to the bees it was decided to use this extracted odor to train the bee, which was done by putting into the watch glass, imposed upon the filter paper impregnated with the yarrow extract, some sugar and water and maintaining this supply continuously during the working hours of the bee. Each time the water or sugar in the watch glass was replenished the position of this unit on the board was changed.

During the first afternoon the bees hovered over the various dishes ultimately finding the sugar. The same conduct continued during the next morning, Friday, July 21st. During the afternoon of the 21st less time was spent in searching for the sugar dish. The bees apparently had begun to associate the odor of the yarrow with the sugar. The papers containing the extract were changed daily, this being done to maintain pungency.

On Sunday, July 24, 10 bees were marked for identification and at P.M. a new Petri dish and watch glass without sugar were provided with a freshly prepared disk of filter paper impregnated with the yarrow

extract. This unit replaced the yarrow unit that was used to support the watch glass containing the sugar. The position of the dish on the test board was changed and a count of the bees going to the board was taken:

Yarrow	43
Anethol	0
Citral	1
Daisy	1
Blank	1
Blank	1
N. J. Tea	0
Milk weed	0
Blank	0
Blank	0
Lily of Valley	0

The bees were driven from the units and the same rearranged when a 10 minute count was again made:

Yarrow	29
Anethol	0
Citral	12
Daisy	0
Blank	0
Blank	0
N. J. Tea	0
Milk weed	0
Blank	0
Carvone	1
Blank	2
Lily of Valley	0

Sugar water was again supplied to the yarrow unit and feeding continued during the preparation of a new agent, which was done as follows: 15 cc. of yarrow benzine extract was extracted with 10 cc. of 80% methanol, the alcoholic extract separated and evaporated on a disk of filter paper. The benzine residue was also evaporated on a disk of filter paper, and on July 26 the location of the feeding disk was again changed every 5 minutes for 20 changes, then the sugar water and yarrow unit was removed with 1 blank and two units containing respectively the benzine residue and alcoholic extraction evaporated on filter papers were substituted with a shift of positions. A count was taken during a period of 10 minutes with the following results: The alcoholic extract showed 46 visits; the benzine residue 4 visits; blanks 11 visits.

The alcoholic washing of the benzine extract was colorless and possessed the odor of the bloom to a marked degree, which diffused more rapidly than the benzine extraction, undoubtedly due to the absence of paraffin. The benzine residue from the alcoholic extraction contained the color and whatever wax and fixed oils that had been extracted from the flowers by the benzine, the odor having been yielded to the alcohol.

Unfortunately, it was necessary for me to leave the work at this point, but it is clear that the bees' mental association of sugar with extracted odors of yarrow concerns chiefly that substance or those substances which are removed from the benzine extract by alcohol.