

## The Attraction of *Tribolium castaneum* to Flour

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Very little is known about the factors governing the invasion of foods by species of *Tribolium*. The entire life-history of these insects is typically spent in their original environment, e.g. flour (Park 1934). Although the adults of *T. confusum* do not fly, they can spread throughout a building and eventually infest all available food (Good 1936). That *T. castaneum* Herbst, however, may spread by flight is shown by the report that it has been trapped in numbers in a rotary net at an elevation of about 3 feet (Barnes & Kaloostian 1940).

Good (1936) reported that flour beetles of the genus *Tribolium* feed in over 100 different foods, while *T. confusum* has been listed as feeding on at least 70 kinds of food (Hayhurst 1937). *T. confusum* and *T. castaneum* are reported to be the most abundant insects in flour mills (Cotton *et al.*, 1945), these insects being encountered at all stages of the manufacture and storage of cereal products.

Chapman (1918) stated that a coarse, flaky food was somewhat more "attractive" than fine or granular material, and that the dominant factor in the invasion of flour or cereal by *Tribolium* may be its coarseness. Although coarseness may be a factor in retaining beetles once they have invaded a material, particle size can hardly act as an attractant from a distance. Later Chapman (1931) stated that *T. confusum* seemed to exhibit relatively little olfactory selection of food.

DeCoursey (1931) found that adults of *Tribolium confusum* could be trapped between two pieces of wood separated by

tacks or in corrugated paper baited with wheat flour. Of several materials tested in the wood traps, including certain cereals and essential oils, he concluded that flour was the best attractant. However, this method of trapping does not permit the conclusion that the beetles made an olfactory selection of food. There is the possibility that beetles moving at random may have encountered the flour-baited traps and have been retained by the flour. DeCoursey also found that beetles congregated in large numbers in a flour-baited, corrugated-paper trap placed in a culture jar containing flour infested with these insects. This is an indication that it was not so much the flour used as bait which was important in the initial aggregation of the beetles in the trap, but rather the crevices in the corrugated paper since the flour was present outside the trap as well. In our laboratory, adults of *Tribolium* have been found to collect in large numbers in unbaited, corrugated-paper traps. Good (1936) noted that both larvae and adults collect or conceal themselves under any suitable object near their food.

Adults of *Tribolium castaneum* that were starved for 2 or more days were strongly attracted to flour (Fig. 1, D-F) as well as to moisture (Fig. 1, A-C). However, in studying the olfactory responses of *Tribolium*, with the aid of an olfactometer, the writers observed that beetles were slightly repelled by previously dried air after it had been passed over whole wheat flour as a source of odor. This repellency was apparently related to the moisture content of the flour (about 10 to

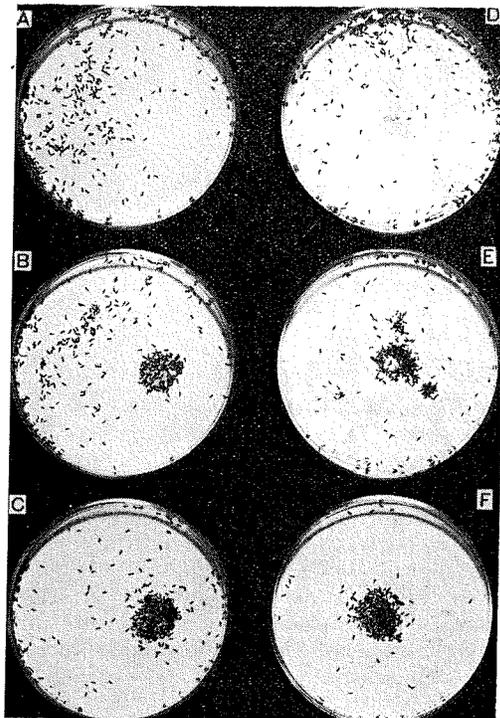


FIG. 1.—Attraction of *Tribolium castaneum* adults to whole wheat flour and to water. Tests performed in darkness in 15 cm. petri dishes lined with filter paper. Photographs A to C show attraction to water of beetles starved for 2 days: (A) distribution of beetles prior to introduction of drop of water; (B) 3 minutes after drop of water was placed on filter paper; (C) 6 minutes after introduction of water. Photographs D to F show attraction to flour of beetles starved for several days: (D) distribution of beetles immediately after introduction of flour; (E) 5 minutes later; (F) 20 minutes after introduction of flour.

12 per cent water by weight) for it was possible to eliminate the repellent effect of the flour by equalizing the humidities of the 2 air streams in the instrument. Further studies on the humidity responses of *Tribolium*, which will be reported elsewhere, substantiated this conclusion since it was found that unstarved, non-desiccated beetles preferred the drier of alternative humidities when given a choice between zero and 5 per cent and between zero and 15 per cent relative humidity. Using the olfactometer, attempts to show whether or not flour odor was a factor in attracting *Tribolium* were negative. The following experiments were performed to determine the effect of the moisture content of flour on its "attractiveness" to adult flour beetles.

APPARATUS AND METHODS.—The behavior of *Tribolium castaneum* adults towards flour and water was observed in a reaction chamber in which the insects were separated from the attractant by a wire screen (Fig. 2). In the arena the attractant operated from a distance, and retention of the beetles above the attractant did not result from contact with the material other than that which existed between olfactory stimuli or hygrostimuli and the receptors of these stimuli. The test method used with the arena was based on the relatively rapid, directed response of starved beetles toward water or flour.

The arena was a plastic cylinder 2.5 cm. high and 14.5 cm. inside diameter with a bottom of 40 mesh, brass-wire screen. During tests the arena was secured over the inverted top half of a petri dish 15 cm. in diameter by an encircling band of thin plastic. Containers for the attractants, 0.5 ounce, metal, salve boxes 3.5 cm. in diameter, were raised off the petri dish so that their rims nearly reached (but did not touch) the screen bottom of the arena. The top level of the attractant was approximately 0.5 cm. below the screen. The area of the arena was 165.13 square cm., and the area of each attractant container was 9.62 square cm. Thus when only one container was employed in a test, the ratio of attractant area to the total arena was 1:17.2; when 4 containers were used, the ratio was 1:4.29.

Twenty adult beetles of mixed sexes, 1 to 2 months after emergence, were used in each test; each test was repeated 5 or more times so that a total of 100 or more different insects were used in each experiment. To determine actual distribution of test insects over empty containers, control tests were run just before each group of experimental tests. When one container was used, it was placed in the center of the petri dish beneath the arena; when 2, 3, or 4 containers were employed, they were placed centrally about 1.5 cm. apart. In experiments in which more than 1 attractant was employed, the arrangement of the containers was changed for each test by rotating the containers successively through each position beneath the arena. During the experiments the only light (from an overhead source) measured less than 1 foot candle at the arena level.

Tests were conducted in the following

manner. After 20 beetles had been placed in the arena it was covered with a glass plate; this plate served to restrict the flight of the beetles and to screen the arena from external air currents and gross changes in humidity from the breath of the observer. The container of attractant was placed in the petri dish, and, following dispersal of the beetles over the screen, the arena was lowered over the petri dish. The numbers of insects that collected above the attractant on the area of screen outlined by the rim of the container were counted at intervals of 1 minute for a 5-minute period. From the totals of the 5 counts the response to the attractant was computed as a percentage of the total possible number of position records (100 position records in a 5-minute test using 20 insects).

#### BEHAVIOR TOWARD WATER AND FLOUR.

—The effect of starvation on the reaction of *Tribolium castaneum* toward water and whole wheat flour<sup>1</sup> was determined in the arena. Beetles were starved in petri dishes lined with filter paper either dry or wet with water. In the following tests the attractant, either water or flour, was confined in one container beneath the center of the arena. Since these beetles respond strongly to humidity, both flour odor and humidity might be involved in the response of the beetles to flour. To separate

the combined effects of odor and humidity on the responses of the beetles, both fresh and dry flours were used. Fresh flour was that which had been stored at room humidity and had a moisture content of 10 to 12 per cent by weight. Dry flour was either dried over concentrated sulfuric acid for 10 days (final moisture content not determined) or air-oven dried at 130° C. for 1 hour (A.O.A.C., 1940); this latter sample after storage over night above calcium chloride had a moisture content of 0.28 per cent.

The peak response to the attractant, of beetles starved longer than 1 day, occurred by the second or third minute of each experimental test. With unstarved beetles and those starved only 1 day the peak response occurred about the fifth minute. In no case was the percentage of position records above empty containers in the control tests as large as that which might have been expected from theoretical considerations. The results of these tests expressed as mean percentages of position records above the attractant are given in table 1.

The results show that the amount of moisture in the whole wheat flour was an important factor in attracting the beetles, dry flour eliciting equally low responses from both desiccated and non-desiccated

<sup>1</sup> *Wheatsworth*, National Biscuit Co.

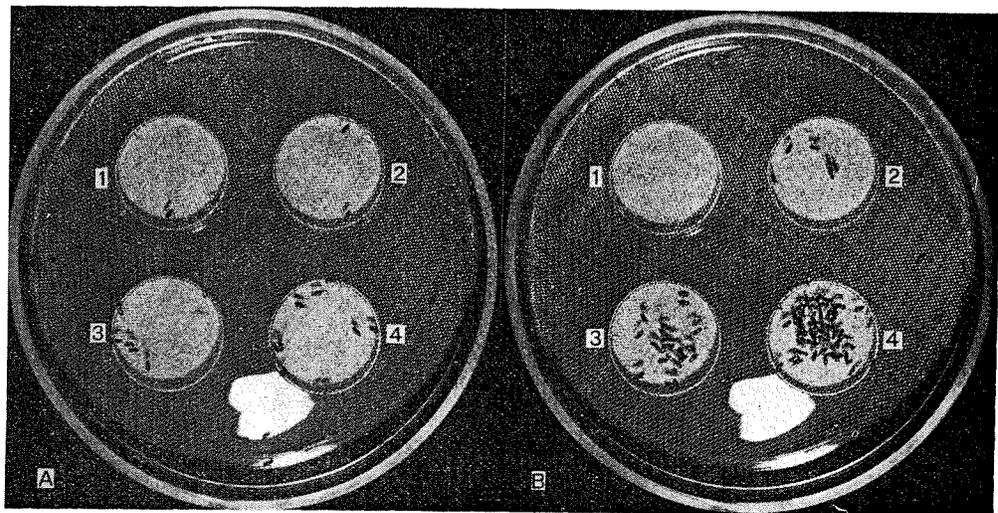


FIG. 2.—Arena in which beetles were separated from attractant by wire screen. Preference shown by 100 adults of *Tribolium castaneum*, starved 7 days without water prior to test, for samples of whole wheat flour containing different amounts of moisture. (A) distribution of insects a few seconds after start of test. (B) distribution of insects after 1 minute. Moisture content of flour: (1) 7, (2) 10, (3) 12, and (4) 15 per cent moisture by weight.

Table 1.—Effects of different conditions of starvation on the attraction of adults of *Tribolium castaneum* to water vapor or whole wheat flour.

ATTRACTANTS	CONDITIONS AND DAYS OF STARVATION	PERCENTAGES OF POSITION RECORDS ABOVE ATTRACTANTS (mean ± S.E.)		NUMBER OF TESTS
		Experimental	Control	
Water vapor <sup>1</sup>	Without water			
	0	3 ± 1.8	3.4 ± 1.54	5
	1	4 ± 0.9	2.0 ± 0.54	10
	3	50 ± 5.8	4.2 ± 0.58	5
	5	68 ± 1.4	3.0 ± 0.63	5
	7	81 ± 2.6	3.2 ± 0.37	5
Fresh flour <sup>2</sup>	Without water			
	0	21 ± 2.1	1.9 ± 0.48	10
	1	22 ± 2.9	1.1 ± 0.23	10
	3	37 ± 3.7	1.8 ± 0.39	10
	5	38 ± 2.4	2.3 ± 0.47	10
	7	55 ± 2.3	2.2 ± 0.34	10
Dry flour <sup>3</sup>	Without water			
7	5 ± 1.3	1.6 ± 0.68	5	
Dry flour <sup>4</sup>	7	8 ± 2.5	2.4 ± 0.36	5
Fresh flour <sup>2</sup>	With water			
	0	21 ± 2.1	1.9 ± 0.48	10
	1	14 ± 1.8	0.9 ± 0.28	10
	3	17 ± 3.9	1.0 ± 0.55	5
	7	8 ± 2.0	1.6 ± 0.68	5
Dry flour <sup>3</sup>	With water			
7	8 ± 2.3	1.0 ± 0.17	5	

<sup>1</sup> Vapor arising from an exposed water surface approximately 0.5 cm. below the screen of the arena; temperature of room 24°-26° C.

<sup>2</sup> Flour with a moisture content of 10 to 12%.

<sup>3</sup> Flour kept over concentrated sulfuric acid in a desiccator for 10 days.

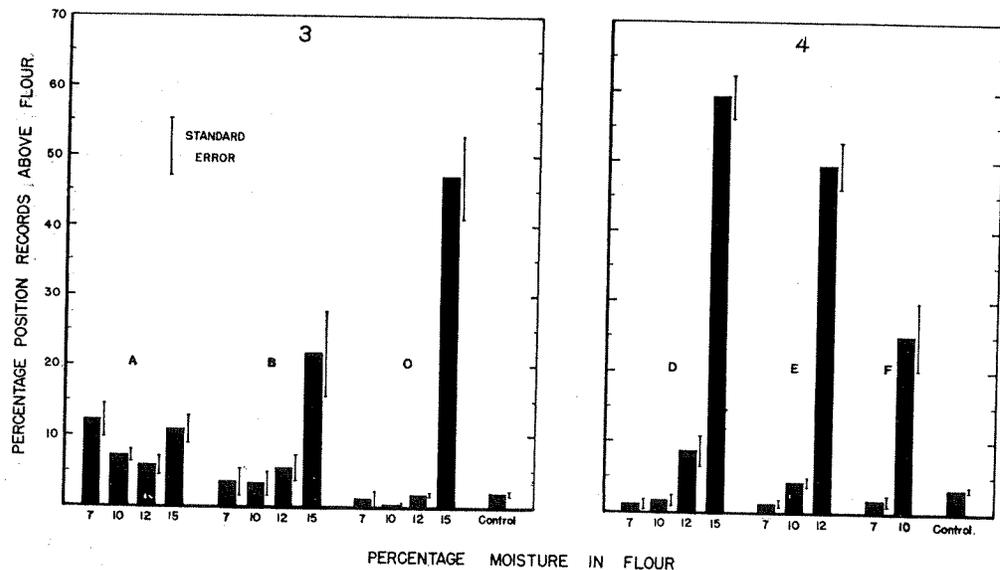
<sup>4</sup> Flour air-oven dried to a moisture content of 0.28%.

beetles that had been starved 7 days. The intensity of the attraction of desiccated beetles to water vapor or to flour containing 10 to 12 per cent moisture increased with the period of starvation. However, the intensity of the attraction of non-desiccated beetles to flour containing 10 to 12 per cent moisture decreased with the length of starvation.

**BEHAVIOR TOWARD SAMPLES OF FLOUR CONTAINING DIFFERENT AMOUNTS OF MOISTURE.**—The previous experiments demonstrated that the moisture content of whole wheat flour was an important factor in the movement, toward this food, of *Tribolium castaneum* adults that had been starved without water. The following experiments were performed to determine whether these beetles could discriminate among 2 or more samples of flour containing different amounts of moisture.

Samples of whole wheat flour were placed in desiccators above solutions of sulfuric acid and water adjusted to provide relative humidities of 20, 40, 60, and 80 per cent. After 16 days the moisture content of the flour was determined.<sup>1</sup> Rounded to the nearest whole number, the amounts of moisture in the whole

Indirect, air-oven method, A.O.A.C., 1940.



Figs. 3 and 4.—Attraction of adults of *Tribolium castaneum* to samples of whole wheat flour containing different amounts of moisture. Flour samples presented simultaneously in arena. Fig. 3. Conditions of beetles prior to test: (A) unstarved; (B)

starved 7 days in moist, moving air; (C) starved 7 days in dry, moving air. Fig. 4. All beetles starved 7 days in petri dishes on dry filter paper prior to test. Beetles given a choice of 4 (D), 3 (E), or 2 (F) samples of flour at one time.

wheat flour samples kept at the above humidities were 7, 10, 12, and 15 per cent by weight. Three series of 5 tests each were run in the arena using, simultaneously, samples of all 4 flours. Beetles were subjected to the following conditions of starvation prior to testing: (1) unstarved, (2) starved 7 days in moving air of 96 to 100 per cent R. H., and (3) starved 7 days in moving air of zero R. H. Control tests were run with 4 empty containers under the screen, and a mean percentage of position records above any one container for 10 tests was obtained ( $N=40$ ). Unstarved beetles, while exhibiting a significant response to all flour samples, showed little ability to discriminate between them. Beetles starved 7 days in either a dry or a moist atmosphere discriminated among samples of whole wheat flour differing in moisture content by 2 to 3 percentage points in the range 7 to 15 per cent moisture. However, beetles starved 7 days in a dry atmosphere were attracted more strongly to flour containing 15 per cent moisture than were beetles starved 7 days in moist air. Results of these tests are shown graphically in figure 3.

Since flour of 15 per cent moisture was more attractive to beetles starved 7 days than flour of lower moisture content, the following experiments were performed to determine the relative attractiveness of the other 3 flour samples. The beetles for these tests were all starved for 7 days in petri dishes on dry filter paper. In the successive experiments (5 tests each) the insects were given a choice of 4, 3, or 2 samples of flour containing different amounts of moisture. The control response was the mean percentage of position records above any one empty container in 15 tests ( $N=45$ ). The results are shown in figure 4. In each series of tests the beetles showed a marked preference for the flour of highest moisture content, even though the amount of water in the moistest sample was different in each series. However, both the response to the sample of highest moisture content and the total response to all flour samples decreased as the flours of 15 per cent and 12 per cent moisture were successively removed.

**DISCUSSION.**—The humidity responses of *Tribolium castaneum* in the arena are in fairly close agreement with those obtained in an olfactometer (Willis & Roth 1950). Unstarved, non-desiccated adult

flour beetles were not attracted by vapor from water exposed beneath the arena; beetles in a similar state of nutrition and moisture balance consistently preferred the lower humidity in the olfactometer. In both instruments starved, desiccated insects showed a preference for high humidities; thus the change in direction of response with desiccation was similar in both olfactometer and arena. However, in the arena unstarved, non-desiccated beetles were slightly attracted to flour containing moisture although in the olfactometer a similar response was not obtained; possibly flour odor enhanced this response in the arena.

Most non-desiccated insects have been found to aggregate in the drier microclimate, in what has been considered the more unfavorable humidity (Bentley 1944). However, despite this apparently unfavorable selection by the insect, it would seem that, through the medium of the dry reaction, insects of normal water balance might be enabled to migrate over a wider range of microclimatic conditions than would be available to them if they reacted preferentially only to optimal humidities. There is slight likelihood that insects showing a dry reaction would be trapped in an unfavorable humidity for, as Bentley (1944) has pointed out for *Ptinus* and as has been shown with *Tribolium*, the reaction of insects toward drier air disappears with desiccation and is replaced by a biologically advantageous reaction towards regions of higher humidity.

Integration of laboratory and field research has been emphasized with respect to the behavior of *Ptinus tectus*. This insect had consistently selected the drier end of a humidity gradient in the laboratory but was observed congregating in damp spots in a granary (Herford 1949). Subsequent study showed that after dry conditioning, *Ptinus* exhibited a preference for high humidity. The humidity behavior of *Tribolium castaneum* under field conditions may be predicted tentatively, although the hypothesis requires substantiation by field observations. Both the intensity and direction of the humidity reactions might be expected to depend on the state of water balance in the freely migrating insect. Adult beetles migrating from flour might be expected to wander, not strongly attracted to food,

until their humidity responses were reversed by desiccation. Under these circumstances the moisture content of available food could affect the intensity of beetle invasion, since desiccated beetles have been shown to have the ability to discriminate among flours containing different amounts of moisture. Thus damp storage conditions might tend to foster high initial infestations by attracting desiccated migratory beetles. Moist storage conditions in turn could contribute to a more rapid population increase since Holdaway (1932) has shown that the population per unit environment of *T. confusum* increased 58 per cent when humidity was raised from 25 to 75 per cent.

**SUMMARY.**—The reactions of adults of *T. castaneum* toward water and flour have been studied with the aid of an arena in which the attractant was placed beneath a screen floor. The amount of moisture in whole wheat flour was found to be an important factor in the attraction of the beetles toward this food. The ability of the beetles to discriminate among samples of flour differing in moisture content by 2 to 3 per cent over the range of 7 to 15 per cent moisture was a function of starvation; the flour of highest moisture content was the most attractive to beetles starved under either dry or moist conditions, those starved dry being attracted more intensely.

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