

HUMIDITY REACTIONS OF TRIBOLIUM CASTANEUM (HERBST)

EDWIN R. WILLIS AND LOUIS M. ROTH

Quartermaster General Laboratories, Philadelphia, Pennsylvania

TWO FIGURES

INTRODUCTION

It has been found that the magnitude of the attraction of the red flour beetle *Tribolium castaneum* to whole wheat flour is closely related to the moisture content of the flour and to the nutritional state of the insect (Willis and Roth, '51). Discrimination among samples of flour which differed only in moisture content was governed by the ability of the insect to respond to differences in humidity conditions above the flour and to sense these differences from a distance. It was also found, with the insect olfactometer described below, that unstarved, non-desiccated adults of *T. castaneum* avoided air that had been passed over undried whole wheat flour but not that passed over dried flour. This avoiding response was later eliminated by equalizing the humidities of both air streams in the olfactometer. Hence, since humidity has been found to influence the orientation of *T. castaneum* to food, the following survey of the humidity reactions of this insect has been made as a preliminary to other chemotactic studies.

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APPARATUS, METHODS, AND MATERIAL

The olfactometer. The insect olfactometer described below was developed for studying the behavior of adult flour beetles toward odors under controlled conditions. With minor modifications the instrument served equally well for studying the reactions of these insects toward alternative humidities.

The apparatus shown in figure 1 is a modification of the so-called Venturi-type olfactometer (see Dethier, '47). The main differences between this instrument and earlier Venturi olfactometers (see Wieting and Hoskins, '39; Willis, '47) are (1) the position of the influent ports, (2) the shape and size of the insect cage, and (3) the use of non-Venturi flowmeters (rotameters). In this olfactometer the influent ports, mouths of the funnels O (letters refer to fig. 1), were placed above the insect cage S. The air streams flowed straight down through the cage to the exhaust port beneath, without changing direction within the cage. This is in contrast to olfactometers in which the air streams flowed from laterally placed ports in through the side of the cage and out through the bottom leaving portions of the cage in which insects were not necessarily subjected to the odor gradient.

In contrast to early model cages, which were rectangular parallelepipeds considerably larger than the influent ports, the insect cage used in this olfactometer was reduced in size with respect to the ports, and its shape was altered to a right cylinder. The wall of the cage, an aluminum strip 2 cm high, conformed closely to the shape of the ports (see fig. 2). The areas of the top and bottom of the cage (bases of the cylinder) were congruent with the influent ports (combined areas of both ports about 29 cm²) including the central, double-triangular space between the ports (area about 5.7 cm²). The bottom of the cage was 40-mesh, brass-wire screen; screen was omitted from the top of the cage since *T. castaneum* showed no tendency to fly during the tests. This arrangement, of two air streams flowing vertically down through a cylindrical cage that conformed to the air streams, resulted in high insect participa-

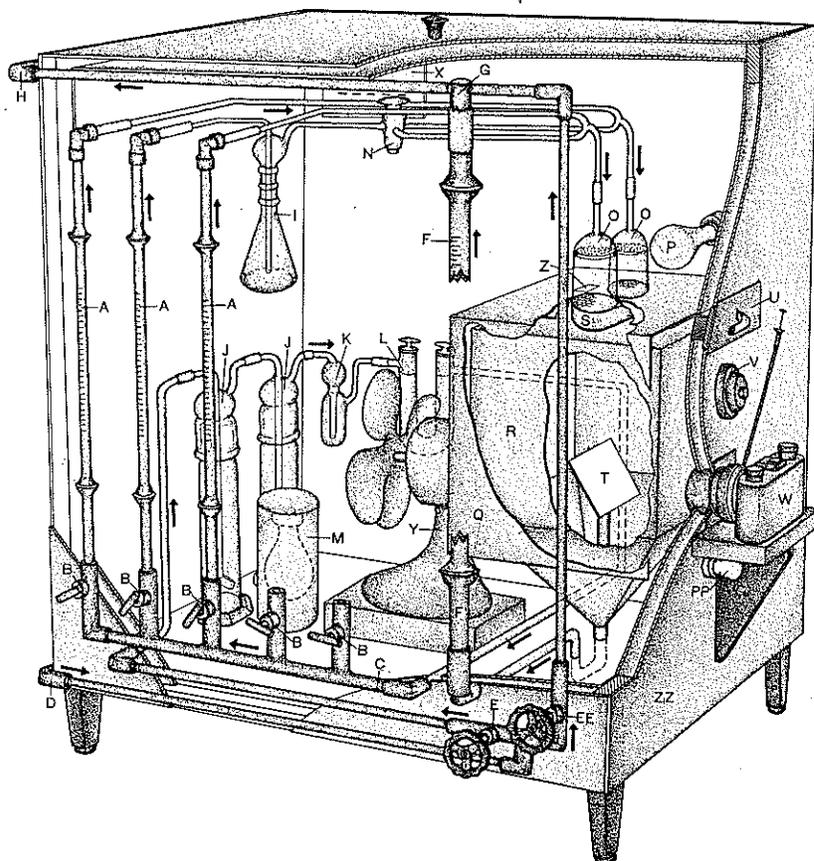


Fig. 1 Cutaway diagram of the olfactometer as it was used in tests with flour. Modifications for humidity studies are described in the text. Key: (A) influent flowmeters; (B) needle valves; (C) manifold; (D) compressed air lines; (E) valve controlling influent air line; (EE) valve controlling air to aspirator; (F) effluent flowmeter; (G) Venturi aspirator; (H) exhaust line to hood; (I) odor saturator; (J) humidifiers; (K) trap; (L) tube containing activated charcoal; (M) heating element in opaque shield; (N) 3-way valve; (O) glass Buchner funnels; (P) light bulb; (PP) pilot light; (Q) shield; (R) exhaust box; (S) insect cage; (T) mirror; (U) cage carrier; (V) switch controlling lights; (W) recording camera; (X) electrical outlet; (Y) fan; (Z) window for code number to identify test; (ZZ) double-walled, asbestos-board box: 25 inches long, 20 inches wide, and 26 inches high. Arrows indicate the direction of air flow through the olfactometer.

tion in each test without the use of auxiliary attractants (cf. Hoskins and Craig, '34).

The two glass Buchner funnels, O, inverted over the cage to form the influent ports, measured 4.3 cm inside diameter and 5 cm from rim to perforated plate; each plate was pierced by 37 small holes. Tests with ammonium chloride smoke demonstrated that funnels of this type would deliver collimated air streams to the cage; there was little visible mixing of the two streams across the common boundary within the cage. An aluminum shield, Q, protected the cage from air-currents set up by the fan, Y.

The exhaust port in the top of box R was of the same size and shape as the horizontal cross section of the interior of the cage; this port was about 2 mm below the bottom of the cage. Negative pressure was developed within the exhaust system by action of a Venturi aspirator, G, (Peterson, '47) which was connected through a flowmeter, F, to the inverted pyramidal base of box R. The aspirator was operated by compressed air which in turn blew the exhaust gases into a chemical hood.

Insects in the cage were silhouetted by light from two symmetrically placed 100-watt incandescent lamps, P. Images of the insects were reflected by a mirror, T, to an observation port where their distribution was observed visually or recorded by a camera, W. The lights, controlled by switch V, were synchronized manually with the operation of the camera cable release. All tests were conducted in total darkness except for the brief periods when the lights were flashed on in order to record insect distribution.

Air for ultimate delivery through the insect cage was humidified by passing the air stream through sulfuric acid solutions (Wilson, '21) in gas-washing bottles, J, which were equipped with fritted-glass dispersing discs. Extraneous odors were removed by passing the air through activated charcoal. Air stream temperatures were controlled at $27^{\circ} \pm 0.5^{\circ}\text{C}$. by a mercury thermoregulator. Very close agreement existed between the temperatures of the two air streams measured at the cage by means of mercury in glass thermometers.

Needle valves in a manifold, C, regulated delivery of air to the individual flowmeters, A. In experiments with odor either of the main air streams could be mixed with odor-saturated air by means of a three-way stopcock, N. Additional stopcocks, not shown in the diagram, made it possible to switch the main air streams from one port to the other.

The above description delineates the olfactometer as it was used in preliminary tests with flour. For studying the humidity behavior of *T. castaneum* the instrument was modified as follows. Air, first filtered through charcoal, was divided into two streams by the manifold. Each stream was humidified separately on passing through a gas-washing bottle containing sulfuric acid solution of requisite density; adjustment of the density of the acid solutions was made, when necessary, after each series of 5 tests. It was found desirable to humidify the air after metering to prevent condensation in the flowmeter tubes. Glasswool traps were employed on the effluent side of the wash bottles to remove possible acid spray from each air stream. Following this modification the flowmeters were recalibrated to compensate for the resistance of the acid solutions, dispersing discs, and traps to the passage of air.

The relative humidities (R.H.) of the air streams as delivered at the cage were checked against theoretical values by means of an electric hygrometer, a commercial model of that devised by Dunmore ('39) which has an accuracy of $\pm 1.5\%$ R.H. During humidity measurements two sensing elements of the hygrometer were mounted in the position to be occupied by the insect cage during an actual test. Each sensing element was centrally positioned beneath an influent port. Measured humidities were found to be in close agreement with theoretical values.

In all tests the rate of flow of each air stream was 50 liters an hour (a velocity of about 1 cm a second as the air flowed from the port into the cage). However, the velocity of the air through the triangular portions of the central zone between the ports was probably not identical with that directly beneath the ports. Since the air beneath each port was continuously

replaced from above, there was little opportunity for the streams to mix within the cage except in the narrow central zone between the ports. Thus the transition from one humidity to the other at the center of the cage was abrupt, and the relative humidity in each half of the cage remained constant once a steady flow was established. The exhaust was regulated at 100 liters an hour since smoke tests had demonstrated that the removal of effluent air at the same rate as the sum of the rates of flow through the two influent ports was as effective in this instrument as exhausting at a higher rate.

Test method. A typical test was conducted as follows. Twenty insects were distributed as evenly as possible across the bottom of the cage which was then placed in the olfactometer. At the end of the first minute after insertion of the cage, and at successive one-minute intervals thereafter, the distribution of the insects was recorded. After 10 observations had been made, the two air streams were reversed so that each flowed through the opposite half of the cage. A period of 5 minutes was allowed for the establishment of the reversed odor or humidity conditions within the cage, though less time would suffice to reverse the insects' reaction (see fig. 2). The responses of the insects were then recorded for a second 10-minute period. Switching the air streams half-way through each test tended to cancel bias resulting from uncontrollable differences between the two air streams. The development of the humidity reaction was very rapid, usually increasing to about the mean value within one minute, except in tests with 95 and 100% R.H. In all tests with pairs of humidities, except those with 95 and 100% R.H., the reaction remained relatively constant for the duration of each test.

Using 20 insects per test, accurate counts of their distribution could be made visually; under conditions of equal distribution of insects in both halves of the cage, the use of the camera facilitated taking simultaneous records of the insects beneath each port. A photographic record of a typical test showing the reaction of 20 unstarved beetles when given a choice between 15 and 75% R.H. is shown in figure 2. Posi-

tion records for minutes 1-10 and 16-25 (numerals in fig. 2) constitute one 20-minute test for which the index of reaction (see below) toward the lower humidity was 70%. Records of beetle distribution at minutes 11-15 would not ordinarily be made but are included in the figure to show the rapidity with which the insects shifted position when the humidity conditions within the cage were reversed.

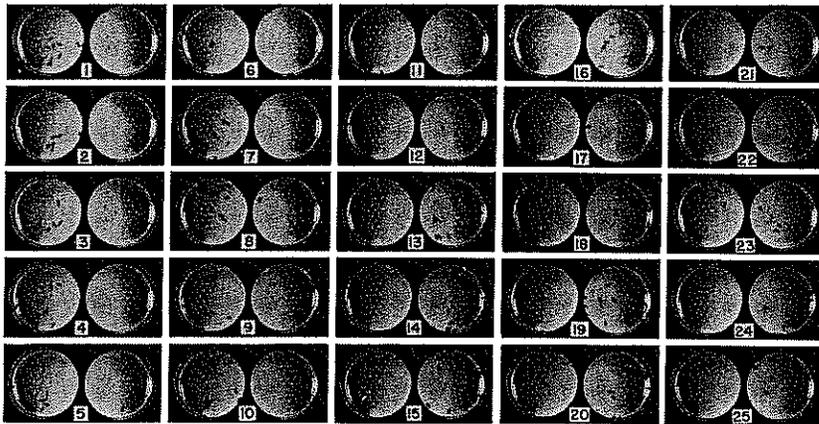


Fig. 2 Photographic record of the reactions of 20 unstarved adults of *Tribolium castaneum* when given a choice between 15 and 75% R.H. in the olfactometer. Numerals indicate minutes from the start of the test. During minutes 1 to 10 air issuing from the left hand port was adjusted to 15% R.H. while that from the right hand port was 75% R.H. Between the 10th and 11th minutes the humidity conditions were reversed. Notice that the curved ends of the cage conform to the outer semicircles of the ports.

As a measure of the intensity of the humidity reaction, an index was calculated from the formula $100(D - W)/N$, in which D was the sum of the successive positions occupied by the insects in the drier humidity, W the sum of the positions in the wetter humidity, and N was the total possible number of position records per test, that is the number of insects times the number of observations (cf. Bentley, '44). It was assumed that a test based on 20 observations reflected the reaction of one group of insects more accurately than a single observa-

tion. The index of reaction for a single test was of the same relative magnitude when calculated from 20 observations as when calculated from a single observation. The use of the sums of the 20 successive positions of the same group of insects did not artificially increase the population since the test was the population unit rather than the individual insect or the single observation. The index of reaction is an excess percentage of position records in one humidity over the records in the other. The index values extend from +100 to -100; 0% indicates no reaction, equal numbers of insects having been recorded beneath each port; a positive index indicates a preference for the drier humidity, and a negative value indicates a preference for the wetter humidity.

Because of the shape of the central zone between the ports, the boundary between the air streams was not sharply defined across the entire width of the cage. The orientation of the insects in this zone (average about 12% per test) could not be definitely ascribed to either humidity. Since there was unrestricted movement throughout the cage, these insects were not necessarily the same individuals in successive observations, yet some may have occupied the central zone by choice. Hence, since all insects in the cage were exposed to the humidity choice, a triple choice (one or the other air stream, or the central zone) was assumed to be available. Cognizance was taken of this triple choice by employing N in the denominator of the formula rather than $(D + W)$, the sum of the responses toward the alternative humidities alone. Indexes calculated using the above formula were about 12% less than those that would be obtained by substituting $(D + W)$ for N in the formula. As the value of $(D + W)$ approaches the limit N , the discrepancy between the respective indexes approaches zero. It would be possible to ensure the identity of $(D + W)$ with N in all tests by modifying the influent ports to eliminate the triangular areas between them. If the ports had a sharply defined common boundary extending straight across the width of the cage, only a double choice, one or the other air stream,

would be available to the insects, there being no neutral zone either in the center or elsewhere in the cage.

Insects. Mixed groups of adults of both sexes of *T. castaneum* were used in all tests. The insects at the time of testing ranged in age from one to two months after emergence. By this age the normal reproductive processes were well under way, and since this species may live as an adult for a year or more, possible effects of senility on the reactions were avoided.

Both larvae and adults were reared in 100% whole wheat flour. Recently-emerged adults were removed daily from groups of isolated pupae and were set up in new flour to provide insects of known age. When necessary, these adults were transferred to fresh flour to prevent admixture with younger age groups developing within the cultures.

The cultures were maintained at a temperature of about 27°C. in a room of uncontrolled humidity; the relative humidity of this room varied from 20% to 60% depending on the season. Uninfested flour kept at these humidities varied in moisture content from 7% (at 20% R.H.) to 12% (at 60% R.H.). Although the equilibrium moisture content of flour is a function of the relative humidity (see Bailey, '25), samples of flour from active cultures contained 10-12% moisture by weight. If moisture content of the food affected the water content of the beetles, some influence on their humidity reactions might also be expected. It seems unlikely that such a relationship held here since both larvae and pupae of the closely related species, *T. confusum*, have been found to contain identical amounts of water when reared in flour kept at both 20% and 70% R.H. (Fraenkel and Blewett, '44).

HUMIDITY REACTIONS

Choice between humidities. In surveying the behavior of *T. castaneum* when given a choice between humidities in the olfactometer, different pairs of alternative humidities were presented to the insects in the manner described previously. The humidity of each air stream was adjusted by passing the

air through sulfuric acid solutions of requisite density. For simplicity theoretical values of relative humidity are used throughout the following discussion since they were closely approximated by actual values. Since unstarved, non-desiccated beetles had been found to give a strong dry reaction (preference for lower humidity) following removal from flour, beetles in this "normal" state of water balance were used in all tests reported below. Until a few minutes prior to each test the insects were maintained in whole wheat flour at 27°C. A different group of insects was used for each test.

For the first series of tests, pairs of humidities were selected in which the components differed from each other by 15 percentage points (except in two pairs which differed by 10% and 100% respectively) over the range from 0 to 100% R.H. The alternative humidities used and the excess percentages of position records in the lower humidity of each pair are given in table 1. While the indexes of reaction were all positive, indicating preference of the beetles for the lower humidity of each pair over the entire range, the highest values were obtained at the wet end of the range. However, the lowest excess percentage was not found at the extreme dry end of the range but at pair 30 and 45% R.H. It is interesting to note that the index of reaction between 0 and 100% R.H. is very similar to that obtained between 90 and 100% R.H. despite the difference in the humidity range. The mean index of reaction of 26 control tests, both air streams 0% R.H., was $0.2 \pm 1.5\%$ (standard error of the mean). The mean index of reaction of 5 control tests, both air streams 100% R.H., was $3 \pm 2.9\%$.

In the second series of tests 15% R.H. was paired with alternative humidities from 0 to 90% R.H. in successive steps of 15 percentage points. The results are also given in table 1. The magnitude of the dry reaction increased with the value of the alternative humidity, being highest at the wet end of the range.

For the third series of tests 75% R.H. was paired with alternative humidities from 0 to 90% R.H. in successive steps of 15 percentage points. The results are given in table 1. A range of 30% R.H. was required to elicit the most intense dry

reaction when 75% R.H. was the higher alternative. Lowering the alternative humidity, that was paired with 75% R.H., below 45% R.H. did not result in further significant change in the intensity of the reaction.

TABLE 1

Reactions of unstarved adults of Tribolium castaneum when given a choice between alternative humidities

ALTERNATIVE RELATIVE HUMIDITIES	INDEX OF REACTION ¹ ± STANDARD ERROR	NO. OF TESTS
0 and 15	24 ± 2.2	5
15 and 30	14 ± 3.0	5
30 and 45	12 ± 1.8	5
45 and 60	42 ± 3.1	5
60 and 75	41 ± 4.3	5
75 and 90	54 ± 3.2	5
90 and 100	79 ± 1.8	5
0 and 100	80 ± 1.0	5
15 and 0	24 ± 2.2	5
15 and 15	4 ± 2.5	5
15 and 30	14 ± 3.0	5
15 and 45	28 ± 2.4	5
15 and 60	57 ± 3.8	5
15 and 75	69 ± 3.1	13
15 and 90	74 ± 2.4	5
75 and 0	62 ± 2.8	5
75 and 15	69 ± 3.1	13
75 and 30	57 ± 3.3	5
75 and 45	65 ± 4.9	5
75 and 60	41 ± 4.3	5
75 and 75	2 ± 3.1	15
75 and 90	54 ± 3.2	5

¹ Mean excess percentage of responses in lower humidity.

Since the reactions of *T. castaneum* were strong in all pairs of humidities in which the two components differed by 15 percentage points or more, a survey was made of the reactions of beetles in pairs of less widely separated humidities. Alternative humidities differing by 5 percentage points were presented to unstarved beetles at the low, mid, and high portions of the

relative humidity range. Five groups of 20 insects each were tested with each pair of humidities.

An index of reaction of $9 \pm 1.4\%$ toward the lower humidity was obtained when the beetles were given a choice between 0 and 5% R.H. The reduction in the intensity of reaction from that obtained between 0 and 15% R.H. indicates that the beetles showed less ability to discriminate between humidities as the value of the higher humidity was reduced.

In mid-range, indexes of reaction of $4 \pm 3.2\%$, $-3 \pm 2.2\%$, and $-2 \pm 3.7\%$ were obtained toward the lower of the alternatives of the pairs of humidities 40 and 45%, 45 and 50%, and 50 and 55% R.H. respectively. It is not surprising that beetles did not discriminate between humidities differing by only 5 percentage points in this portion of the range since the lowest index of reaction (12%) in the series in which the alternative humidities differed by 15 percentage points was obtained with pair 30 and 45% R.H.

At the moist end of the range an index of reaction of $58 \pm 3.9\%$ was obtained toward the lower humidity of the pair 95 and 100% R.H. A higher response might have been expected because of the large excess percentage (79%) of position records in 90% R.H. when 90 and 100% R.H. were paired. However, it was apparent while recording the insect distribution between 95 and 100% R.H. that the beetles discriminated between the two humidities more intensely as the test progressed. When the positions of the humidities in the cage were reversed at the end of the first 10 minutes of each test, the process of increasing reaction, minute by minute, was repeated for the second half-test. Mean excess percentage values per minute for each of 10 minutes (both halves of each test combined) of the 5 tests with 95 and 100% R.H. were 26, 40, 46, 54, 65, 62, 68, 66, 78, and 73%. This clearly indicates that with this particular pair of humidities the development of the intensity of the reaction was a function of the period of exposure of beetles to the choice. A similar delayed reaction was not observed in any of the other humidity combinations that were tested. Pursuing the matter further, one group of

20 beetles was tested for one hour without changing the positions of the 95 and 100% R.H. air streams in the cage during the test period. Observations were made every minute, and the position records were grouped in six 10-minute intervals. Mean excess percentages of position records in 95% R.H. for each of the successive 10-minute periods were 28, 79, 89, 87, 90, and 92%, again an indication of the dependence of reaction intensity on time. Although this kind of reaction was not observed among the other pairs of humidities, it is not implied that differences in the intensity of reaction might not be exhibited in tests run for longer periods. No data are available other than the above covering periods longer than 10 consecutive minutes without reversal of the relative positions of the humidities in the test cage.

Effect of starvation and desiccation. The effects of both starvation and desiccation on the humidity reactions of *T. castaneum* were determined by presenting insects that had been starved under dry or moist conditions with a choice between 15 and 75% R.H. A different group of insects was used for each test.

In the first two series of tests the beetles were held in Petri dishes lined with filter paper. For the "dry" series the beetles were starved in an atmosphere of 20-30% R.H. For the "wet" series the filter paper was kept wet with distilled water, the beetles thus being starved in an atmosphere of high humidity with water to drink. Results of these tests, expressed as excess percentages of position records in the lower humidity for different periods of starvation, are given in table 2.

In both the dry and the wet series strong initial aggregations of unstarved beetles were observed in the lower humidity. By the third day of starvation the reaction of the dry series beetles toward the dry air stream had ceased; by the 5th day the direction of the response of this series had reversed, becoming a strong wet reaction. In contrast the responses of beetles in the wet series toward dry air dropped steadily for the first week, but even after two weeks of starvation beetles in this series did not develop a significant wet reaction. Thus

it would appear that while desiccation contributed to a sharp reversal of response from low to high humidity, starvation even without desiccation also reduced the magnitude of the dry reaction.

In the second two series of tests a wider range was provided between the humidities in which the beetles were starved prior to testing. Beetles were held in filter paper lined vials in bottles conditioned by either a dry or a moist stream of moving air at 27°C. The "dry" bottles contained 30-50 ml of concentrated sulfuric acid, the "wet" bottles contained a similar quantity of distilled water. Tightly fitting two-hole rubber stoppers closed the bottles. Through one hole of each stopper compressed air was piped at a rate of 3 liters an hour, a separate flowmeter being provided for each bottle. The influent air was discharged low in the bottle forcing the effluent out through the other hole in the stopper. Air for the dry bottles was first passed through concentrated sulfuric acid while that for the wet series was passed through distilled water. Under these conditions the relative humidities in the bottles, measured with the electric hygrometer, were less than 5% R.H. for the dry series and over 96% R.H. for the wet series. The humidity responses of beetles starved for various periods in dry or moist, moving air are summarized in table 2.

In general the results of these later tests agree with those of the earlier series. The shift in response from 15% R.H. toward 75% R.H. came after a shorter period of starvation in the second dry series; by the third day there was an excess percentage of position records in the drier air stream of — 29% compared with — 3% in the earlier dry series tests. This increased wet reaction possibly resulted from a more rapid loss of water by beetles starved at 0 to 5% R.H. compared with the water loss of those starved at 20-30% R.H. The series of tests with beetles starved in moist, moving air showed a gradual increase in the excess percentage of response of the beetles toward the lower humidity until after the 5th day of starvation. From the 5th day to the 13th day the effect of starvation

on reduction of the intensity of the reaction among beetles held in moist air was again noted.

In the final series of tests (5 tests for each period of starvation) beetles that had been starved in moving air of from 0-5% R.H. were placed on wet filter paper for about an hour before testing. The positive indexes of reaction were $66 \pm$

TABLE 2

Effects of starvation and desiccation on the humidity reactions of adults of Tribolium castaneum given a choice between 15% R.H. and 75% R.H.

DAYS STARVED	BEETLES STARVED IN PETRI DISHES		BEETLES STARVED IN MOVING AIR	
	Index of reaction ¹ \pm standard error	No. of tests	Index of reaction ¹ \pm standard error	No. of tests
Dry conditions during starvation				
0	69 ± 3.1	13
1	42 ± 2.4	5	54 ± 2.1	5
2	58 ± 3.9	5
3	-3 ± 8.8	5	-29 ± 5.6	5
5	-65 ± 4.3	5	-58 ± 5.1	5
7	-83 ± 1.8	5	-82 ± 1.8	5
8	-81 ± 3.2	5
9	-88 ± 2.2	5
Moist conditions during starvation				
0	68 ± 6.5	5
1	78 ± 2.4	5	62 ± 2.4	5
2	68 ± 3.6	5
3	45 ± 6.2	5	79 ± 4.8	5
5	19 ± 9.7	5	81 ± 3.4	5
7	-5 ± 2.8	5	36 ± 4.7	5
9	-4 ± 4.1	5	24 ± 7.6	5
11	16 ± 3.9	5	29 ± 3.3	5
13	21 ± 7.3	5	24 ± 5.1	5

¹ Mean excess percentage of responses in 15% R.H.

6.8%, $69 \pm 2.7\%$, $50 \pm 9.3\%$, and $6 \pm 4.8\%$ for 3, 5, 7, and 9 days starved respectively. It is readily apparent from a comparison of these data with table 2 that beetles which have been permitted to drink water following periods of desiccation behaved much like beetles starved in a moist atmosphere; they again showed a preference for the lower humidity, and

the direction of the response was similar to that of beetles starved under wet conditions.

EFFECT OF STARVATION ON WATER CONTENT

In the foregoing experiments it was presumed that the behavior of desiccated insects given a choice of alternative humidities was related to their water balance. On the other hand beetles starved under very moist conditions were assumed able to maintain their water content at a near constant level. Yet these latter insects, as well as those starved dry, exhibited a decreased intensity of reaction toward dry air with increased starvation. The following experiments were performed to determine the effect of starvation on the water content of beetles kept under both dry and moist conditions. Two groups of beetles (each group half males and half females) were starved until death ensued under the conditions of dry or moist, moving air described previously. The dry atmosphere was maintained at close to 0% R.H. and the moist air at 96-100% R.H., both at 27°C. Air appropriately adjusted for these humidities was blown continuously through each humidified chamber, in which the insects were starved, at a rate of 3 liters per hour.

The times of starting individual beetles on starvation were staggered so as to provide weight data for days starved that otherwise might not have been obtained because of the intervention of week-end periods. Time of death of individual beetles was never known exactly; if death occurred during the week, it was arbitrarily assigned at the half day between two successive daily weighings. Over the week end the time of death was even more uncertain; starvation periods terminated by death of an insect on a week end were given the mean of two possible values: (1) the number of days starved through Friday plus one-half day, and (2) the number of days starved through Sunday plus one-half day. The mean age of the dry-series insects at the start of the test was 36.6 ± 0.33 days. The mean age of the wet-series insects at the start of the test was 36.7 ± 0.31 days.

Beetles, brushed free of adhering flour particles, were weighed individually and placed separately in small vials. A disc of wire gauze in the bottom of each vial provided the insect with a foothold. Each beetle was weighed daily except on week ends until death, all weighings being made on a torsion balance sensitive to 0.02 mg. The mean, initial live weight of the 14 males of the series starved in dry air was 1.74 ± 0.05 mg, and that of the 14 females was 1.76 ± 0.05 mg. The mean, initial live weight of the 15 males starved in moist air was 1.66 ± 0.03 mg, and that of the 15 females was 1.75 ± 0.04 mg. The differences between the mean weights of the two sexes of both series are not significant ($P > .05$). The mean initial live weight of the 28 dry-series insects was 1.75 ± 0.04 mg, and that of the 30 wet-series insects was 1.70 ± 0.03 mg.

Following death each beetle was air-oven dried at 130°C . to constant weight. The mean dry weights of the dry-series beetles were 0.56 ± 0.01 mg for the males and 0.57 ± 0.02 mg for the females. Mean dry weights of the wet-series beetles were 0.44 ± 0.01 mg for the males and 0.46 ± 0.01 mg for the females. The differences between the mean dry weights of the sexes of both dry- and wet-series beetles are not significant ($P > .1$); the means of the combined dry weights were 0.56 ± 0.01 mg for the 28 dry-series insects, and 0.45 ± 0.008 mg for the 30 wet-series insects.

For comparison with the starved beetles 290 adults of the same age (mixed sexes) were weighed in three groups immediately after removal from flour, then air-oven dried to constant weight. The mean live weight per beetle was 1.70 mg and the mean dry weight was 0.79 mg. The mean water content of the three groups of unstarved beetles was $53.4 \pm 0.10\%$ of the live weight.

Water content of each starved beetle was obtained for the day on which it was last weighed prior to death by subtracting the dry weight of the beetle from final live weight. The water content was computed as a percentage both of the initial live weight and of the final live weight. The former percentage is perhaps more nearly comparable with the water content of

unstarved beetles since total solids undiminished by starvation or excretion are included in both figures. However, since the beetles decreased in dry weight with starvation, the water content based on the final live weight was greater in proportion to solids than was that based on initial weight, even though water may have been lost during starvation.

Daily weights expressed as mean percentages of the initial weights and the mean percentage water content for each day of starvation are given for the dry-series beetles in table 3 and for the wet-series beetles in table 4. The differences be-

TABLE 3

Effect of starvation on daily weight and water content of adults of Tribolium castaneum held in an atmosphere of 0-5% relative humidity

DAYS STARVED	MEAN DAILY WEIGHT		MEAN WATER CONTENT		
	% of initial weight ± standard error	N ¹	% of initial weight ± standard error	% of final live wt. ± standard error	N ²
0	100	28	53.4 ± 0.10	53.4 ± 0.10	290 ³
1	89.8 ± 0.98	24	49.0 ± 9.55	57.9 ± 5.45	2
2	83.2 ± 1.05	18	43.8 ± 1.46	57.0 ± 1.00	3
3	79.5 ± 1.33	16	45.7 ± 1.09	59.5 ± 0.92	6
4	73.9 ± 1.14	14	36.2 ± 1.73	52.0 ± 1.32	6
5	70.6 ± 1.16	11	33.8 ± 1.45	52.0 ± 2.00	2
6	65.4 ± 1.67	9	30.9 ± 1.94	48.7 ± 1.58	6
7	62.6 ± 0.52	3	30.9 ± 0.38	49.3 ± 0.34	3

¹ N = number of surviving insects yielding weight data.

² N = number of dead insects yielding data on water content.

³ These insects weighed in three groups.

tween daily weight losses of males and females were not significant except on the first day of starvation for the wet-series beetles and on the second day for the beetles starved in dry air ($P < .05$ for both days); hence data from both sexes were combined in the tables. Starting with the first day of starvation, the differences being the mean daily weights of the dry-series and wet-series beetles are highly significant ($P < .001$).

The onset of mortality was rapid in the dry series, all beetles having died by the end of the 7th day of starvation. From a value of about 53% of the weight of unstarved beetles,

the water content of beetles in the dry series dropped to a low of 31% of their initial weights after 6-7 days of desiccation, the mean value for the 7-day period being $37.9 \pm 1.46\%$ of the initial weight. It is interesting that, even among beetles

TABLE 4

Effect of starvation on daily weight and water content of adults of Tribolium castaneum held in an atmosphere of 96-100% relative humidity

DAYS STARVED	MEAN DAILY WEIGHT		MEAN WATER CONTENT		
	% of initial weight \pm standard error	N ¹	% of initial weight \pm standard error	% of final live wt. \pm standard error	N ²
0	100	30	53.4 \pm 0.10	53.4 \pm 0.10	290 ³
1	94.6 \pm 0.52	24
2	92.1 \pm 0.75	18
3	89.7 \pm 0.58	18
4	89.0 \pm 0.66	18
5	88.1 \pm 1.00	18
6	85.6 \pm 0.81	24
7	84.4 \pm 0.70	30
8	83.8 \pm 0.81	24	43.1	60.8	1
9	82.6 \pm 0.80	18	53.6 \pm 1.93	65.7 \pm 0.70	3
10	78.8 \pm 1.49	17	47.5 \pm 1.67	64.1 \pm 1.18	7
11	81.0 \pm 1.14	15
12	79.5 \pm 1.43	13	50.6 \pm 0.00	67.5 \pm 0.50	2
13	78.1 \pm 1.58	11	46.2 \pm 0.83	63.2 \pm 0.48	4
14	79.1 \pm 1.09	13	48.2 \pm 1.10	65.0 \pm 1.00	2
15	77.8 \pm 1.47	11	46.6 \pm 3.51	64.3 \pm 2.67	3
16	77.3 \pm 2.06	6	49.5 \pm 3.85	65.5 \pm 1.41	2
17	76.2 \pm 3.39	4	44.1	61.4	1
18	75.9 \pm 2.72	5	46.5 \pm 0.65	65.0 \pm 1.00	2
19	74.3 \pm 4.20	2	48.8	69.8	1
20	77.2	1
21	76.6	1
22	78.7 \pm 4.10	2	50.2 \pm 1.40	64.0 \pm 2.00	2

¹ N = number of surviving insects yielding weight data.

² N = number of dead insects yielding data on water content.

³ These insects weighed in three groups.

starved in a dry atmosphere, the beetles lost solids more rapidly than they lost water. The per cent of body water at death was higher in the 11 dry-series beetles dying during the first three days of starvation than in unstarved beetles; the mean

water content for all dry-series beetles for the 7 days was $53.5 \pm 0.99\%$ of the final weight.

Since no beetles in the wet series died before the 8th day, the water content for each of the first 7 days was not determined. During the first week the water content of the beetles starved in the moist atmosphere dropped from about 53% of the weight of unstarved beetles to $48 \pm 0.71\%$ of the initial live weight (mean value for all 30 insects); the difference is significant ($P < .05$). From the 8th day to the 22nd day the mean water content of beetles dying during this period was maintained without further significant drop at about 48% of the initial weight. However, based on final live weight, the mean water content of this series for the second and third weeks of starvation was $64.6 \pm 0.50\%$.

Initial weights of beetles that have just been removed from flour include food in the gut some of which later is excreted. The weight of feces excreted by 30 individual beetles was determined during a period of starvation terminated by death of the insects. All feces excreted prior to death amounted to 4-5% of the initial weight of the live beetles. If the initial weights of the beetles in the above experiments were to be corrected for the weight of feces, the values of the water contents as percentages of initial weight would be increased by about 2 percentage points.

DISCUSSION

In comparing the humidity reactions of insects, it should be noted that the various data have been secured by different experimental methods. In contrast to the olfactometer used in this work, modifications of the alternative chamber (Gunn and Kennedy, '36) have been employed most frequently in studying the humidity behavior of insects. The main difference between these two techniques is the method of presenting alternative humidities to the insects. In contrast to relatively static microclimatic conditions in the alternative chamber, microclimatic conditions in the olfactometer were maintained by the continuous flow of two streams of air. The alternative chamber

provides a short range humidity gradient whereas the olfactometer provided a choice between two differentiated columns of humidified air; the only humidity gradient in the olfactometer was confined to the very narrow area between the ports. Although the velocity of the air flowing through the cage of the olfactometer was about 1 cm a second, this steady current did not affect the reactions of the insects adversely either in control tests or in tests with alternative humidities. The different effects that these two methods might have on the humidity reactions of specific insects cannot be evaluated in the absence of comparative data.

No attempt was made to analyze experimentally the mechanism of the reaction by which *T. castaneum* aggregated in the preferred air stream in the olfactometer. Using the alternative chamber technique, Gunn and Pielou ('40) showed that ortho-kinesis and klino-kinesis¹ were involved in the orientation of *Tenebrio* toward humidity. In the present work the manner in which the humidities were presented in the olfactometer suggests that a major factor in the aggregation of the beetles was a kinesis (undirected movement) rather than a taxis (directed movement) since the insects in one of the alternative humidities (a uniform microclimate) presumably could not sense the other humidity from a distance. However, klino-taxis¹ might have been a factor in the reaction of the insects near the center of the cage where a gradient was produced by the mixing of the two air streams. Experiments and observations that indicate that klino-taxis is involved in the directed responses of desiccated adults of *T. castaneum* toward water will be described in a later paper (Roth and Willis, to be published).

As was shown in the experimental section, *T. castaneum* displayed a preference for the drier of alternative humidities

¹ Fraenkel and Gunn ('40) define these terms as follows: ortho-kinesis = speed or frequency of locomotion dependent on intensity of stimulation; klino-kinesis = frequency or amount of turning per unit time dependent on intensity of stimulation; klino-taxis = attainment of orientation indirect, by interruption of regularly alternating lateral deviations of part or whole of body, by comparison of intensities of stimulation which are successive in time.

if the insect was in a state of normal water balance or had been conditioned with water. This dry reaction is very similar to that found with most non-desiccated insects thus far investigated, e.g. the roach *Blatta orientalis* (Gunn and Cosway, '38); females of the mosquito *Culex fatigans* (Thomson, '38); the mealworm beetle *Tenebrio molitor* (Pielou and Gunn, '40); the louse *Pediculus humanus corporis* (Wigglesworth, '41); the spider beetle *Ptinus tectus* (Bentley, '44); the house fly *Musca domestica* (Dakshinamurty, '48); the flour beetles *Tribolium confusum* and *T. destructor* (Roth and Willis, to be published); and also the tick *Ixodes ricinus* (Lees, '48). After a period of desiccation *T. castaneum* showed a preference for air of higher humidity. This wet reaction following desiccation is similar to that reported for *Anopheles gambiae* and *A. funestus* (De Meillon, '37); *Blatta* (Gunn and Cosway, '38); *Ptinus* (Bentley, '44); males of *Drosophila melanogaster* (Begg and Hogben, '46); and *Ixodes* (Lees, '48).

The intensity of the dry reaction of non-desiccated adults of *T. castaneum* was a function of the higher alternative humidity and to a limited extent of the humidity range. The former relationship extended from about 30 to 100% R.H. A similar relationship of the dry reaction to the higher humidity has been found at the moist end of the relative humidity range with other non-desiccated arthropods, e.g. *Culex* (Thomson, '38); *Tenebrio* (Pielou and Gunn, '40); *Pediculus* (Wigglesworth, '41); and *Ixodes* (Lees, '48). An analogous situation in which the intensity of the *wet* reaction is related to the higher humidity, has been found with larvae of the wireworms *Agriotes obscurus* and *A. lineatus* (Lees, '43), appears in Begg and Hogben's ('46) data on the wet reaction of non-desiccated males of *Drosophila* (when 0% R.H. was paired with increasing alternative humidities), and is one component of the wet reaction of the onychophoran *Peripatopsis moseleyi* (Bursell and Ewer, '50).

In the drier portion of the humidity range, from 0 to about 30% R.H., there was an inverse relationship between the intensity of the dry reaction of *T. castaneum* and the higher

humidity when the alternative humidities differed by 15% R.H. This reaction is reminiscent of that reported for *Ptinus* (Bentley, '44) except that the reaction of *Tribolium* was less intense. Both *Tribolium* and *Ptinus* in alternative humidities differing by 15% R.H. gave intense reactions at both the dry and the wet ends of the humidity range; however, the least intense reaction of *Tribolium* occurred between 30 and 45% R.H. while that of *Ptinus* occurred in the gradient between 75 and 90% R.H.

The dry reaction of *T. castaneum* was not related to the humidity range when 100% R.H. was paired with either 0% R.H. or 90% R.H. However, the intensity of the dry reaction appeared to be related to the difference between alternative humidities when 75% R.H. was paired with decreasing humidities in the range from 75 to 45% R.H. A similar relationship of the dry reaction to the range of the humidity gradient has been found with the African migratory locust *Locusta migratoria migratorioides* (Kennedy, '37). An analogous relationship between the intensity of the *wet* reaction and the range of the humidity gradient has been found with *Agriotes* (Lees, '43), appears in data on non-desiccated males of *Drosophila* (when 100% R.H. was paired with decreasing alternative humidities) (Begg and Hogben, '46), and is a second component of the wet reaction of *Peripatopsis* (Bursell and Ewer, '50).

Starvation with or without accompanying desiccation reduced the intensity of the dry reaction of *T. castaneum*. After 2-3 days of starvation under conditions of low humidity, the dry reaction was reversed, and the ensuing wet reaction increased in intensity with the period of starvation. Since desiccated adults of *Tribolium* lost water at a relatively constant rate until death, the correlation between the water loss of desiccated beetles and the index of reaction seems fairly obvious. However, non-desiccated beetles starved in a moist atmosphere lost relatively little water; the average water content (based on initial weight) of non-desiccated beetles at death after 1-3 weeks of starvation was about equal to that

of desiccated beetles starved one day. Beetles starved dry for only one day gave a very intense dry reaction, while non-desiccated beetles starved one week exhibited a much less intense dry reaction. This dry reaction of non-desiccated beetles decreased slightly in intensity with increasing starvation, even though the water content of these beetles remained nearly constant and at nearly the same level as that found in beetles starved dry one day. Thus the intensity of the dry reaction of non-desiccated beetles was a function of the period of starvation. A similar starvation effect might be reflected in the changing index of reaction of desiccated beetles, being superimposed on the part of the intensity of reaction that was a function of the degree of desiccation.

The direction (toward wet or dry) of the humidity reactions of *T. castaneum* may possibly be correlated with the proportion of body water in the tissues. The dry reactions of non-desiccated beetles starved two to three weeks and of desiccated beetles starved less than three days were paralleled by an increase in body water relative to solids. Reversal of the reaction from dry to wet among desiccated beetles starved three or more days was paralleled by a decreasing proportion of body water. Thus the respective humidity reactions tended to favor restoration of a state of normal water balance. Lees ('48) has shown that the humidity behavior of the tick *Ixodes* is influenced by the physiological state of the animal. Unfed ticks with a normal water balance avoid higher humidities; after desiccation the tick is intensely active in dry air but comes to rest in moist air where the water balance is restored by water uptake through the cuticle.

In most instances in which dry reactions have been observed, the insects were presumably either in a state of normal water balance or had been watered prior to testing their humidity reactions. Only a few insects have been reported to give a dry reaction after having been conditioned in a "dry" atmosphere or starved prior to testing. Hungry females of *Culex*, which had been deprived of moist food one day, weakly avoided high humidities unless the difference was very large, e.g. between

10 and 60% R.H. (Thomson, '38). *Pediculus* avoided the higher humidity of alternative pairs at both ends of the humidity range after having been exposed 1-3 hours to the lower humidity, although this reaction occasionally was reversed during the test (Wigglesworth, '41). Both males and females of *Musca* gave dry reactions after conditioning at 30% R.H. for 30 minutes at 25°C. (Dakshinamurty, '48). It seems little likely that the brief periods of "dry" conditioning, to which these insects were subjected, would have altered their water balance appreciably. However, Kennedy ('37) concluded that the preference of *Locusta* for dry air was not destroyed by starving for three days in dry cages (either at 15-18% or 24-27% R.H.), although the preference may have been reduced in intensity. What may have been operating in these cases is the usual preference for the lower of alternative humidities that is shown by non-desiccated insects.

SUMMARY

1. The humidity reactions of the red flour beetle *Tribolium castaneum* have been studied with the aid of an improved olfactometer in which the insects were given a choice between two humidities that were presented in various combinations covering the relative humidity range.

2. Unstarved, non-desiccated beetles could discriminate between humidities differing by 15% R.H. or more over the entire relative humidity range. However, with a choice between humidities that differed by only 5% R.H., these beetles discriminated weakly between 0 and 5% R.H., not at all between 40 and 45%, 45 and 50%, or 50 and 55% R.H., but intensely between 95 and 100% R.H.

3. The intensity of the dry reaction (preference for the lower humidity) of unstarved, non-desiccated beetles, given alternative humidities between which they could discriminate, was a function of the higher humidity from about 30 to 100% R.H.

4. The preference for the lower or higher humidity and the intensity of the humidity reaction of beetles given a choice

between 15 and 75% R.H. were related to the degree of starvation and to the water balance of the insects. Among both desiccated and non-desiccated beetles the dry reaction decreased in intensity with the period of starvation. The dry reaction of desiccated beetles ceased after three days of starvation, becoming an intense wet reaction (preference for the higher humidity) after 5 days. However, non-desiccated beetles continued to show a dry reaction even after two weeks of starvation. The wet reaction of beetles desiccated three or more days was reversed from wet to dry by providing the insects with water for about an hour before testing.

5. The water content, based on the initial live weight, of adult beetles starved in dry air was a function of the period of starvation; however, this relationship was not found among beetles starved in very moist air. During starvation there was an increase in the water content of the tissues, based on the final live weight, relative to solids both in desiccated beetles (first 3 days only) and in those held in a moist atmosphere.

LITERATURE CITED

- BAILEY, C. H. 1925 The chemistry of wheat flour. The Chemical Catalog Co., New York.
- BEGG, M., AND L. HOGBEN 1946 Chemoreceptivity of *Drosophila melanogaster*. Proc. Roy. Soc. London, Series B, 133: 1-19.
- BENTLEY, E. W. 1944 The biology and behaviour of *Ptinus tectus* Boie (Coleoptera, Ptinidae), a pest of stored products. V. Humidity reactions. J. Exp. Biol., 20: 152-158.
- BURSELL, E., AND D. W. EWER 1950 On the reactions to humidity of *Peripatopsis moseleyi* (Wood-Mason). Ibid., 26: 335-353.
- DAKSHINAMURTY, S. 1948 The common house-fly, *Musca domestica*, L., and its behaviour to temperature and humidity. Bull. Ent. Res., 39: 339-357.
- DE MEILLON, B. 1937 Studies on insects of medical importance from southern Africa and adjacent territories (part IV). 3. Some reactions of *Anopheles gambiae* and *Anopheles funestus* to environmental factors. Publ. S. African Inst. Med. Res., 7: 313-327.
- DETHIER, V. G. 1947 Chemical insect attractants and repellents. The Blakiston Co., Philadelphia.
- DUNMORE, F. W. 1939 An improved electric hygrometer. J. Res. U. S. Nat. Bur. Stand., 23: 701-714.
- FRAENKEL, G., AND M. BLEWETT 1944 The utilization of metabolic water in insects. Bull. Ent. Res., 35: 127-139.

- FRAENKEL, G., AND D. L. GUNN 1940 The orientation of animals. Oxford University Press, Oxford.
- GUNN, D. L., AND C. A. COSWAY 1938 The temperature and humidity relations of the cockroach. V. Humidity preference. *J. Exp. Biol.*, *15*: 555-563.
- GUNN, D. L., AND J. S. KENNEDY 1936 Apparatus for investigating the reactions of land arthropods to humidity. *Ibid.*, *13*: 450-459.
- GUNN, D. L., AND D. P. PIELOU 1940 The humidity behaviour of the mealworm beetle, *Tenebrio molitor* L. III. The mechanism of the reaction. *Ibid.*, *17*: 307-316.
- HOSKINS, W. M., AND R. CRAIG 1934 The olfactory responses of flies in a new type of insect olfactometer. I. Theory and design of the olfactometer. *J. Econ. Ent.*, *27*: 1029-1036.
- KENNEDY, J. S. 1937 The humidity reactions of the African migratory locust, *Locusta migratoria migratorioides* R. and F., gregarious phase. *J. Exp. Biol.*, *14*: 187-197.
- LEES, A. D. 1943 On the behaviour of wireworms of the genus *Agriotes* Esch. (Coleoptera, Elateridae). I. Reactions to humidity. *Ibid.*, *20*: 43-53.
- LEES, A. D. 1948 The sensory physiology of the sheep tick, *Ixodes ricinus* L. *Ibid.*, *25*: 145-207.
- PETERSON, A. 1947 A manual of entomological equipment and methods, Parts I and II. Edwards Brothers, Inc., Ann Arbor, Michigan.
- PIELOU, D. P., AND D. L. GUNN 1940 The humidity behaviour of the mealworm beetle, *Tenebrio molitor* L. I. The reaction to differences of humidity. *J. Exp. Biol.*, *17*: 286-294.
- ROTH, L. M., AND E. R. WILLIS Hygroreceptors in adults of *Tribolium* (Coleoptera, Tenebrionidae). To be published.
- THOMSON, R. C. M. 1938 The reactions of mosquitoes to temperature and humidity. *Bull. Ent. Res.*, *29*: 125-140.
- WIETING, J. O. G., AND W. M. HOSKINS 1939 The olfactory responses of flies in a new type of insect olfactometer. II. Responses of the housefly to ammonia, carbon dioxide and ethyl alcohol. *J. Econ. Ent.*, *32*: 24-29.
- WIGGLESWORTH, V. B. 1941 The sensory physiology of the human louse *Pediculus humanus corporis* DeGeer (Anoplura). *Parasitology*, *33*: 67-109.
- WILLIS, E. R. 1947 The olfactory responses of female mosquitoes. *J. Econ. Ent.*, *40*: 769-778.
- WILLIS, E. R., AND L. M. ROTH 1951 The attraction of *Tribolium castaneum* (Herbst) to flour. *J. Econ. Ent.* (in press).
- WILSON, R. E. 1921 Humidity control by means of sulfuric acid solutions, with critical compilation of vapor pressure data. *J. Ind. Eng. Chem.*, *13*: 326-331.