THE HUMIDITY RESPONSES OF *TRIBOLIUM CONFUSUM* JACQUELIN DUVAL IN WHEAT FLOUR, SAND, AND AIR[1]

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ABSTRACT

Larval and adult *Tribolium confusum* were tested in flour and sand substrates at various relative humidities, ranging from 10% to 90%. Both life stages preferred a flour environment of from 10% to 50% relative humidity. In sand, however, the insects selected more humid environments.

Investigations of survival in sand, flour, and air environments indicated that two factors may be operating in the preference for a wet-sand environment: (1) desiccation, and (2) loss of nutrition and source of metabolic water. Insects presented with two air streams, each of 35% relative humidity or greater, selected the drier air stream. In a humidity gradient in air, both life stages indicated a preference for relative humidities of 10% to 25%. Data from an exploratory experiment indicated that adult beetles were not responding to humidity through a kinetic mechanism, though larvae may have been responding kinetically.

The three major conclusions were that: (1) both larval and adult *Tribolium confusum* preferred a dry-flour environment, (2) this preference was reversed if the nature and nutritional content of the medium were altered, and (3) the insects selected an air environment with relative humidities between 10% to 25%.

INTRODUCTION

Tribolium confusum inhabits flour and other grain products. The humidity

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reactions of both *T. confusum* and *T. castaneum* have been examined in conditioned air streams (Willis and Roth, 1950; Roth and Willis, 1951a), but no extensive examination has been made of the reactions of the beetles to media acclimated at different humidities.

Some work has been done in this area. Willis and Roth (1950) found that *T. confusum*, tested with two air streams of different humidities, moved to the air stream of lower humidity, if at least one of the air currents was higher than 30% relative humidity (R.H.). At relative humidities of less than 30% there was a reversal, with the beetles preferring the air stream of higher relative humidity. Only adult beetles were tested in this study. The discrimination pattern broke down when the two humidities differed by less than 5%. Further investigation by the same team (Roth and Willis, 1951a) confirmed the preference of *T. castaneum* for lower relative humidities and stated similar findings for *T. confusum*. Experiments led to the conclusion that starvation and/or dessication could reverse this preference, producing a preference for higher relative humidities. Roth and Willis (1951a) concluded that the beetles' humidity reactions were determined by the water balance of the organism. Their studies also revealed an attraction of *T. castaneum* to whole-wheat flours with relative humidities ranging from only 7% to 15%, an attraction that was reversed by starvation (Roth and Willis, 1951b). *Tribolium* has hygroreceptors located on the antennae (Roth and Willis, 1951b). Observations of the movements of the head region and antennae of these insects could provide some insight as to the mechanism used in selecting a given humidity.

The present study had two purposes: (1) to determine the humidity preferences of larval and adult *Tribolium confusum* in several different media, and (2) to test several possible mechanisms which could mediate the observed humidity reactions. To accomplish these purposes, five experiments were conducted. The humidity preferences of *Tribolium* were tested in sand and in flour (Experiment I) and in air (Experiments IV and V). One experiment (IV) tested the immediate preference of the insects for one of two air streams, as in the experiments of Willis and Roth (1950) and Roth and Willis (1951a), while the other tested humidity preferences in an air gradient (V). Survivorship of larval and adult *T. confusum* was investigated in sand, flour, and air (Experiment III) and at different humidities to determine the effects of both the medium and of relative humidity on survival. The possible involvement of a kinetic mechanism in the humidity reactions of the insects was treated by recording the percentage of insects moving at different relative humidities (Experiment II).

GENERAL METHODS

Stock colonies of *Tribolium confusum* (Carolina Biological Supply Company) were maintained in a medium of 97% whole-wheat flour and 3% non-active yeast. All colonies were housed in an incubator maintained at $27 \pm 2°C$ and $60 \pm 10\%$ R.H. The techniques used for culturing *T. confusum* were those of Good (1936) and of Park (1932). All experimental animals were either adults between one and two months of age, or middle instar larvae (under the culture conditions used, there were six to eight instars for *T. confusum*. Pilot experiments indicated no sex differences in humidity responses, a result in agreement with earlier findings for both *T. confusum* and *T. castaneum* (Willis and Roth, 1950, Roth and Willis, 1951a). Accordingly, the sexes were not separated during these investigations. All experiments were conducted on a level surface at $27 \pm 1°C$, and under constant overhead (fluorescent) lighting. Each insect was used only once in an experiment, and was then discarded.

EXPERIMENT I

The purposes of this experiment were: (1) to determine the humidity preferences of *Tribolium confusum* in whole-wheat flour, and (2) to make similar determinations when the flour medium had been replaced by non-nutrient, fine-grained sand. The sand was powdered to the same grain size as the flour and in itself had no observable effects on the beetles, other than providing a non-nutritional medium.

Sand and flour were acclimatized for one week at 10%, 25%, 50%, 75%, and 90% R.H. in desiccators containing aqueous solutions of H_2SO_4 (after Wilson, 1911). The water contents of the sand and flour media were checked by weighing portions of equal volume. Each sample of sand and flour was found to contain moisture in direct proportion to that in the desiccator in which it had been acclimatised, and samples of each of thetwo media at the same relative humidity were shown to contain equal quantities of moisture.

Representatives of each of the two life stages (larvae and adults) were tested using portions of each of the two media (flour and sand) at each of the different relative humidities. Thus, ten pairs of media were tested for each type of medium, corresponding to the 10 possible combinations of the five relative humidities in the desiccator environments. These ten pairs were:

10% R.H. versus 25% R.H.;
10% R.H. versus 50% R.H.;
10% R.H. versus 75% R.H.;
10% R.H. versus 90% R.H.;
25% R.H. versus 50% R.H.;
25% R.H. versus 75% R.H.;
25% R.H. versus 90% R.H.;
50% R.H. versus 75% R.H.;
50% R.H. versus 90% R.H.; and
75% R.H. versus 90% R.H.

For each pair of humidities, six replicates were conducted, with 50 insects per replicate.

The insects were tested using the following procedure. Two portions (20 cc) of media from the two humidities to be tested were placed on opposite sides of the bottom of a rectangular glass box 4″ by 3″ by 2½″ deep, with fitted glass lid. The half of the box to which each media portion was assigned was determined by a sequence of random numbers. The portions of each of the two media to be tested were laid in opposite ends of the box and smoothed, leaving a one-inch-wide clear strip across the middle of the bottom of the box. The experimental insects were then placed in this center strip and the lid was replaced. After two hours the two media portions were removed, one at a time, from the box, and the number of insects present in each half was recorded.

Control trials were run using the same medium at the same relative humidity on both sides of the box. No significant differences were found between the distributions of the insects (larvae or adults) on the two sides on any control trial.

The results of Experiment I are shown in Table 1 as the percentage of insects found on the side covered with the medium of lower relative humidity. A value greater than 50 percent means that more than half of the organisms were found on the side with lower relative humidity, which is interpreted to indicate a preference for this moisture condition by the insects. Chi-square tests were run for each pair of media tested, with an expected frequency of 25 insects locating in each half of the box (Siegel, 1965). Those cells where the X^2 value was significant ($p<.01$) are indicated by an asterisk in Table 1. A X^2 value was also calculated for each life stage and for each medium (bottom row of Table 1). Both life stages preferred a lower relative humidity in the flour medium, and a higher relative humidity in the sand. In flour, the preference for the drier of two humidities was reversed when relative humidities of 10% and 25% were paired. For this pairing, both larvae and adults selected the medium of higher relative humidity. The X^2 values in the bottom row of Table 1 indicate that the preference for sand of higher relative humidity was more pronounced ($X^2=85.1$ for adults and $X^2=$

75.8 for larvae) than was the preference for a dryer flour medium ($X^2=31.2$ for adults and $X^2=51.5$ for larvae).

This first experiment demonstrated: (1) that both larval and adult forms of *Tribolium confusum* exhibited similar humidity preferences in both sand and flour media, (2) that the insects preferred a flour medium with a relative humidity of from 10% to 50%, and (3) that the insects consistently selected a higher relative humidity in the non-nutrient sand environment than they did in the flour.

TABLE 1

Humidity preferences of larval and adult Tribolium confusum *in sand and in flour. Data are presented as the percentage of the insects found in the portion of the medium with the lower relative humidity.*

% R.H. of two media portions	Flour		Sand	
	adults	larvae	adults	larvae
10% vs. 25%	41.2*	38.0*	51.3	51.4
10% vs. 50%	45.0	53.7	38.1*	41.5*
10% vs. 75%	61.5*	71.2*	29.4*	23.6*
10% vs. 90%	59.7*	64.8*	17.9*	30.6*
25% vs. 50%	44.7	49.3	39.5*	37.5*
25% vs. 75%	60.9*	65.1*	32.8*	41.7*
25% vs. 90%	73.0*	70.2*	29.4*	25.9*
50% vs. 75%	67.5*	59.8*	39.1*	19.4*
50% vs. 90%	60.6*	81.5*	16.5*	20.6*
75% vs. 90%	59.5*	63.9*	22.5*	41.8*
X^2	31.2	51.5	85.1	75.8
P	<.01	<.01	<.01	<.01

*Significant at the .01 level.

EXPERIMENT II

This experiment was designed to test the possible involvement of a kinetic mechanism in the humidity reactions of larval and adult *Tribolium confusum*. Kinesis is here defined as a random change in rate of movement in response to stimulus intensity. In contrast, taxes are defined as orientations or responses which are directed with respect to an external stimulus (see Fraenkel and Gunn, 1961).

The test apparatus consisted of an inverted petri dish, 150 mm by 15 mm in size, with no. 6-mesh silk bolting-cloth stretched over the larger half, the smaller half inverted on top as a lid. The lower half of the dish was filled with sulfuric acid solutions of different concentrations (aqueous) to produce relative humidities in the test apparatus of 1%, 10%, 25%, 35%, 50%, 65%, 75%, 90%, and 99% R.H. (see Wilson, 1911). The first solution in the series (1% R.H.) was pure sulfuric acid and the last (99% R.H.) was distilled water.

Larvae and adults were tested separately, with three replicates for each humidity and 10 insects per replicate. Prior to each trial, the insects were placed on the bolting cloth, the lid was replaced, and a one-hour equilibration period followed. During the subsequent one-hour experimental period, five-second observations were made every five minutes. During each observation the number of insects (of the 10) moving was recorded.

The results were analyzed using a two-way analysis of variance (Li, 1964), with three replicates per cell. The main treatments were the nine humidities and the two life stages. The value recorded was the mean number of animals moving per five-second sample, calculated from the 12 samples obtained during the one hour of observation.

TABLE 2

Mean number of insects moving, out of the total of 10 insects, during a five-second sample (±1 S.E.).

		Relative Humidity (%)								
		1	10	25	35	50	65	75	90	99
Adult	$\bar{x}$	5.9	2.4	3.0	2.9	3.1	3.0	2.3	1.2	1.1
	S.E.	0.3	0.3	0.4	0.2	0.5	0.3	0.3	0.2	0.1
Larvae	$\bar{x}$	2.5	3.1	3.5	3.1	2.9	3.7	4.2	3.6	5.2
	S.E.	0.2	0.2	0.5	0.6	0.3	0.3	0.5	0.4	0.9

The results and analyses of the experiment are shown in Tables 2, 3, and 4. Application of Duncan's New Multiple Range Test (Li, 1964) showed three separate reaction groups for adult beetles: high activity at 1% R.H., decreased activity between 10% and 75% R.H., and relatively little activity at 90% and 99% R.H. (Table 4). If there were a kinetic mechanism involved, I would expect the adult beetles to have moved less at lower relative humidities and more at high humidities; just the opposite was observed (Table 4). Thus the data provide little support for the hypothesis that the humidity reactions of the adult beetles involve a kinetic mechanism.

TABLE 3

Analysis of variance of the number of insects moving per five-second sample.

Source	DF	MS	F	Prob.
Life Stage	1	6.40	29.09	$<.01$
% R.H.	8	2.05	9.32	$<.01$
Life Stage x % R.H.	8	5.90	26.82	$<.01$
Error	36	0.22		
Total	53	(SS=77.90)		

In the analysis of variance (Table 3), terms both for life stages and for humidities, and also the interaction term (life stage by percent R.H.) were significant ($p<.05$). This indicated that larvae were reacting differently to the different relative humidities than were adult beetles. The Duncan's breakdown of the means for the larvae (Table 4) is difficult to interpret. However, it is clear that the activity was highest under the four highest relative humidities. This suggests

TABLE 4

Duncan's New Multiple Range Test on the mean number of insects moving per five-second sample. Those means not subtended by the same line are significantly different at the .01 level.

(1) Adults									
% R.H.	99	90	75	10	35	25	50	65	1
Mean	1.1	1.2	2.3	2.4	2.9	3.0	3.1	3.4	5.9
(2) Larvae									
% R.H.	1	50	10	35	25	90	65	75	99
Mean	2.5	2.9	3.1	3.1	3.5	3.6	3.7	4.2	5.2

that larval *Tribolium confusum* may be using a kinetic mechanism to determine humidity preference.

EXPERIMENT III

The purpose of this experiment was to study the combined effects of substrate and substrate moisture level on survival. Adult and larval *Tribolium confusum* were tested at five humidities (10%, 25%, 50%, 75%, and 90% R.H.). Individual insects were isolated in small glass vials (3″ high by 1″ in diameter) containing either flour or sand acclimatized at the different humidities, or no medium (air). The vials were placed in desiccators maintained at the five different humidity levels by aqueous sulfuric acid solutions. Each insect was weighed prior to the start of the experiment and at death. The weight loss and number of days of survival were recorded for each organism. Ten adult beetles and ten larvae were tested in each humidity-medium combination.

TABLE 5

Mean number of days of survival of larval and adult Tribolium confusum *in sand, flour, and air environments (±1 S.E.).*

Group	Relative Humidity (%)				
	10	25	50	75	90
Adults					
sand	5.3(0.3)	5.6(0.3)	7.9(0.1)	6.8(0.1)	9.5(0.4)
flour	indefinite survival and reproduction at all humidities				
air	12.2(2.3)	12.8(2.7)	16.1(0.2)	15.8(0.1)	17.3(0.3)
Larvae					
sand	2.0(0.2)	1.8(0.3)	2.9(0.3)	4.3(0.8)	3.9(0.7)
flour	transformed into pupae and adults at all humidities				
air	7.0(1.4)	14.0(5.6)	to pupae, then died	to pupae, then died	to pupae, then died

The results are shown in Table 5 as the mean number of days of survival for the insects in each group. In flour, adult beetles survived and reproduced at all humidity levels for more than six weeks. Larvae molted, pupated, metamorphosed, and followed adult patterns at all humidities in flour. All insects in sand or in air (no medium) died after some interval of time. Adult survival in sand increased from a mean of 5.3 days at 10% R.H. to 9.5 days at 90% R.H. Similarly, larvae lived an average of 2.0 days in sand at 10% R.H., but survived 3.9 days at 90% R.H. Survival of both larvae and adults in air was much longer than in sand; adults lived 12.2–17.3 days on the average, and larvae 7.0–14.0 days. Larvae pupated at higher relative humidities in air, but the pupae did not metamorphose and died within three days.

Weights taken of all larvae at death showed a dramatic (40% average) decline in body weight for those living in sand; larvae living in the air environment lost only 18% of their body weight. Adult beetles lost 20% of their body weight in sand and 11% in air. All these results suggest two factors which may be operative in the survival of *Tribolium:* (1) desiccation which was very pronounced in sand and also occurred in air at low relative humidities, and (2) a loss of nutrition, and thus of metabolic water, in both sand and air environments.

EXPERIMENT IV

The immediate humidity preferences of larval and adult *Tribolium confusum* were tested using a horizontal T-maze. The T used had a 2½-inch stem and 2-inch arms. A series of flasks was established with the same nine different aqueous sulfuric acid solutions used in Experiment II. Two flasks were used in series for each of the nine humidity levels. Air flowed from a tap, through the two flasks, and then to one arm of the T. Each humidity could thus be paired with each other humidity, with air flows passing into opposite arms of the T. Air flow was maintained constant on each side of the maze by counting air bubbles per unit time in the acid-solution flasks.

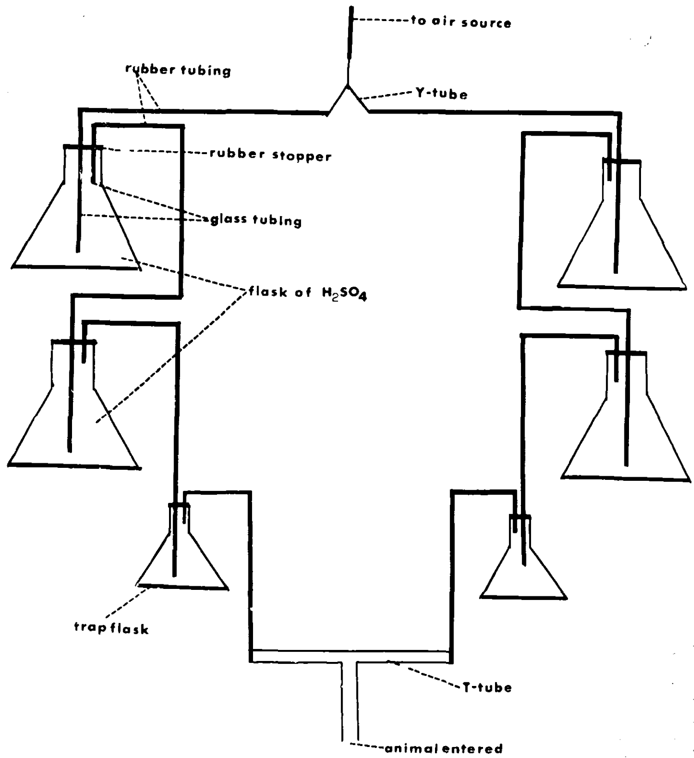

Figure 1 T-Maze Apparatus

Individual insects were introduced at the base of the T from where they moved to the junction; here the insect had to make a choice. A test was terminated when the insect had passed a mark one and one-half inches down either side arm of the T. Each trial consisted of only one such choice, and each insect was tested only once. A clean T-tube was used for each trial. The positions of the air hoses were changed after each trial, according to a sequence of random numbers.

The 36 possible combinations of the nine humidities used were each tested with 20 larvae and 20 adult beetles. The number of insects selecting the air stream of lower relative humidity in each pair presented was recorded. Significance was determined using sign tests and the binomial distribution (Siegel, 1956).

Both larval and adult *Tribolium confusum* chose the drier air stream ($p < .01$) for each pair of humidities when at least one air stream was above 25% R.H. For pairs where both air currents were at or below 25% R.H., the insects did not show a significant preference. These results are in agreement with those of Willis and Roth (1950) and of Roth and Willis (1951a) for both *T. confusum* and *T. castaneum*, confirming their earlier work for *T. confusum* and also demonstrating that larval *T. confusum* behave like adult insects of both species when presented with two air currents of different humidities (tables 6 and 7).

TABLE 6

Number of adult Tribolium confusum *out of 20 selecting the drier air stream of two presented.*

% R.H. Air Stream No. 2	% R.H. Air Stream No. 1: 1	10	25	35	50	65	75	90	99
1	X								
10	10	X							
25	9	11	X						
35	15*	19*	14	X					
50	15*	16*	16*	17*	X				
65	16*	16*	15*	19*	17*	X			
75	16*	15*	16*	15*	15*	15*	X		
90	15*	15*	16*	18*	15*	16*	15*	X	
99	17*	18*	17*	15*	17*	15*	16*	16*	X

*Significant at the .05 level.

TABLE 7

Number of larval Tribolium confusum *out of 20 selecting the drier air stream of two presented.*

% R.H. Air Stream No. 2	% R.H. Air Stream No. 1: 1	10	25	35	50	65	75	90	99
1	X								
10	8	X							
25	11	13	X						
35	17*	15*	15*	X					
50	16*	16*	17*	18*	X				
65	16*	14	15*	15*	16*	X			
75	15*	15*	15*	16*	15*	15*	X		
90	20*	20*	18*	20*	15*	16*	18*	X	
99	18*	18*	17*	16*	15*	20*	18*	15*	X

*Significant at the .05 level.

EXPERIMENT V

In the final experiment, the humidity preferences of larval and adult beetles were tested in a humidity gradient in air. The gradient apparatus consisted of a wooden box 9 inches by 4 inches by ¾ inches deep. Nine rows of four vials (1 inch in diameter and ¾ inches deep) were placed in the box, each row containing a different H_2SO_4 solution. The same nine humidities used in Experiment II were used here, arranged in order to create a gradient from 1% to 99% R.H. Silk bolting-cloth was stretched over the top of the box and a glass lid with a rim of ½-inch-thick foam rubber was placed on top, leaving an air space of ⅜ inch. To insure that the chamber was sealed, vaseline was applied to the junction between the lid and the box.

In each trial, 10 insects were placed on the cloth, and the lid was placed over the apparatus and sealed; a one-hour equilibration period followed. During the subsequent two-hour experimental period, the location of each insect was noted at ten-minute intervals. Three replicates were run for each life stage, with 10 insects per replicate.

Examination of the data indicated that insects established their humidity preferences during the first two 10-minute intervals and that these preferences remained stable thereafter. From these data, a mean percentage of the insects locating in each region of the humidity gradient was calculated, based on the 12 observations made in each trial and across the three replicates conducted for each life stage.

These data are presented in Table 8. More than 50% of both larvae and adults remained located in the 10%-R.H. band of the gradient. For adult beetles, an additional 28.6% of the insects located in the 25%-R.H. band. Hence, 86.3% of all adult beetles selected the 10–25%-R.H. region of the gradient. Of the larvae, on the other hand, 28% selected the 50–99% R.H. bands of the gradient.

TABLE 8

Humidity preferences of larval and adult Tribolium confusum *in a gradient in relative humidity from 1% to 99%. Data are given as the mean percentage of insects locating in the area of each relative humidity (±1 S.E.).*

		% Relative Humidity								
		1	10	25	35	50	65	75	90	99
Larvae										
	$\bar{x}$	2.8	59.9	4.7	3.0	6.7	8.6	1.9	3.6	8.3
	S.E.	0.3	7.9	2.2	1.7	2.2	3.8	1.2	0.7	3.4
Adults										
	$\bar{x}$	2.2	57.7	28.6	2.9	2.5	0.3	1.1	2.9	3.6
	S.E.	1.2	7.7	9.4	1.8	0.9	0.1	0.1	0.8	3.2

DISCUSSION

The following points arise from these experiments.

(1) Larval and adult individuals of *Tribolium confusum* preferred a wheat-flour environment of from 10% to 50% R.H., but consistently selected an environment of higher relative humidity in non-nutrient sand. The preference for a drier (10–50% R.H.) flour environment may be attributed to the ease with which the insects of both life stages could burrow and tunnel in a drier medium; at higher humidities, flour becomes compacted into lumps and is more difficult to penetrate. In flour at 75–90% R.H., larvae and adults remained on top of the medium, or tunneled along the sides of the container.

A portion of the explanation for the selection of higher humidities in sand environments was obtained in Experiment III. Here desiccation and loss of a source of nutrition and metabolic water were critical to the selection of a humid environment. This point is supported by the fact that insects survived nearly twice as long in sand with 90% R.H. than in sand with 10% R.H. Further investigations of the desiccation occuring in sand could be conducted by obtaining water-loss data for *Tribolium* maintained for varying periods of time in sand at different relative humidities. These values could be compared with similar data for beetles maintained only in air, or only in flour, to provide an estimate for the overall desiccatory effect of the sand. Because of the presence of a complete exoskeleton in the adults as compared with the larvae, different rates of water loss would be expected for the two life stages. This was in fact observed in Experiment III, where adults survived longer than larvae in sand and air.

(2) The adult beetles do not orient kinetically. For adult *Tribolium*, the rate of movement was not positively correlated with stimulus intensity. Observations were made of adult beetles' behavior during Experiments II, IV, and V. These insects used side-to-side movements of head and antennae as they moved about the apparatus. This suggests that they were orienting tropo-tactically. Fraenkel and Gunn (1961) define tropo-taxis as an orientation in which the path of the animal is straight and directly towards or away from the source of stimulation.

Klino-kinesis, which results in a wavy pathway, can be eliminated, because the insects appeared to traverse a direct line path. Further work will be required to define the adult-orientation mechanism.

Larval *Tribolium confusum* appeared to orient using a kinetic mechanism. The data from Experiment II do not permit confirmation or rejection of this hypothesis. Larvae did not exhibit side-to-side head movements comparable to those seen in adult beetles. Further investigations of orientation in larval *T. confusum* might utilize a humidity gradient to study the amount of time spent in the various gradient regions, and the rates of movement into and out of the various bands of the gradient.

(3) In an immediate two-choice test situation, insects of both life stages selected the drier air stream for pairs of humidities above 25% R.H. Below 25% R.H., the selections were variable. In a longer two-hour test in such a gradient, insects of both life stages located in the 10–25%-R.H. region. Roth and Willis (1951b) described in detail the hygroreceptors of these insects. If some (or all) of these receptors operate through a desiccation principle, this might explain the selection of the less extreme 10–25%-R.H. range.

(4) The difference in survival of larvae and adults in Experiment III and the difference in larval preference in the gradient-test apparatus require explanations. The presence of an exoskeleton in the adult *Tribolium confusum* could account for these differences in behavior; water loss should be reduced with an exoskeleton, which is presumably the reason why adult beetles lived over twice as long in sand at each humidity level as did larvae. In the gradient experiment, the lack of an exoskeleton could account for the selection of an environment of 50% or greater R.H. by nearly 30% of the larvae, as compared with only 10% of the adults.

These experiments show that both larval and adult *Tribolium confusum* exhibit humidity preferences similar to those found for *T. castaneum* (Willis and Roth, 1950) and indicated for *T. confusum* (Roth and Willis, 1951a). The adult and larval stages also show similar humidity preferences in both flour and sand media. Finally, I would conclude from this work that the nature of, the water content of, and the nutritional value of the medium are all important variables in determining the humidity preferences in larval and adult *T. confusum*.

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REFERENCES

Fraenkel, G. S. and **D. S. Gunn.** 1961. *The orientation of animals.* Dover Publications Inc., New York.

Good, N. E. 1936. The flour beetles of the genus Tribolium. U.S. Dept. Agr. Tech. Bull. No. 498.

Li, J. C. R. Statistical Inference I. Edwards Brothers Inc., Ann Arbor, Michigan.

Park, Thomas. 1932. Studies in population physiology: The relations of numbers to initial population growth in *Tribolium confusum* (Duval). Ecology 13(2): 172–181.

Roth, L. M. and **E. R. Willis.** 1951a. The effects of desiccation and starvation on the humidity behavior and water balance of *Tribolium confusum* and *Tribolium castaneum.* Jour. Expt. Zool. 118: 337–361.

Roth, L. M. and **E. R. Willis.** 1951b. Hygroreceptors in adults of Tribolium (Coleoptera, Tenebrionidae). Jour. Expt. Zool. 116: 527–570.

Siegel, S. 1965. Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Co. Inc., New York.

Willis, E. R. and **L. M. Roth.** 1950. Humidity reactions of *Tribolium castaneum* (Herbst) Jour. Expt. Zool. 115(3): 561–587.

Willis, E. R. and **L. M. Roth.** 1951. Attraction of *Tribolium castaneum* to flour. Jour. Econ. Entomol. 43: 927–932.

Wilson, R. E. 1911. Humidity control by means of sulfuric acid solutions with critical compilation of vapor pressure data. Jour. Ind. Eng. Chem. 13: 326–331.