HUMIDITY RESPONSES OF THE STABLE FLY, *STOMOXYS CALCITRANS* (L.) (DIPTERA: MUSCIDAE)

by

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INTRODUCTION

The stable fly, Stomoxys calcitrans (L.) is an obligatory haematophagous fly which feeds on the blood of many domestic animals and sometimes man. It is a vicious "biter" and after engorgement it leaves the animal and alights on a nearby fence or building where it digests its blood meal. This characteristic of remaining on the host for a short period of time makes the stable fly one of the most difficult livestock pests to control.

Losses in weight and milk production of cattle due to stable fly infestation are difficult to ascertain. Cutkomp and Harvey (1958) reported that treated beef cattle gained 1/4 pound per day more than cattle infested with horn flies and stable flies. Similarly, Cheng (1958) found that beef cattle infested with horn flies and stable flies gained 1/2 to 2/3 pounds per day less than the treated cattle. Freeborn et al. (1925) found that during one month's confinement in a heavy infestation of horn flies the loss in milk production was 1.4%; with house flies, 3.33% and with stable flies, 9.26%. Bruce and Decker (1958) were able to increase milk production 10-20% with the proper application of a good repellent spray formulation.

According to the 1965 Agriculture Handbook 291, the estimated annual loss caused by Stomoxys calcitrans in meat production is 74 million dollars and in milk production, 68 million dollars.

In addition to economic losses caused by exsanguination and annoyance, the stable fly is important as a disease vector and as an intermediate host of certain helminthic parasites (Habronema microstoma, Setaria cervi, and Hymenolepis carioca).

LITERATURE REVIEW

The life cycle and development of the stable fly has been studied by Newstead (1907), Mitzmain (1913), Bishopp (1913) and Parr (1962). According to Mitzmain (1913) and Newstead (1907), the eggs of the stable fly are laid in soggy, fermenting organic matter such as decaying hay, feed, or mixtures of hay and manure. Although this seems to be the most common type of media, in the coastal areas, stable flies breed freely in decomposing bay grasses (Simmons and Dove, 1941) and marine algae on the beaches (King and Lenert, 1936).

The eggs are pale white when first laid, but change to a deep cream color on exposure to air. One side of the egg is convex and the other side is concave with a groove down the side of the egg. The eggs are about 1 mm in length.

The reproductive potential of the stable fly appears to be high. Mitzmain (1913) found the maximum number of eggs laid by an individual to be 632 and that as many as twenty batches of eggs were laid by a single female fly with the number of eggs per batch ranging from 82 to 94. These results agree closely with those of Killough and McKinstry (1965). Contrary to this, Bishopp (1913) reported the greatest number of eggs deposited by a single female to be 278 with 3 being the greatest number of depositions. Parr (1962) reported that the average number of eggs per batch was 35.5 and that the female laid 10 or 11 batches. Herms (1964) noted that a group of stable flies which were fed only sugar water deposited no eggs so they apparently are not autogenous.

Parr (1962) found that the average hatch of stable fly eggs was 76.5 percent. Mitzmain (1913) found that the eggs hatched in 20-26 hours at a

temperature of 30° - 31° C and that eggs kept in a darkened closet hatched four to six hours sooner than eggs of the same batch kept at the same temperature in a lighted room.

Immediately after hatching the larvae begin to consume whatever food may be present. The color of the larvae at first is creamy white, but it quickly assumes the color of the ingested food (Mitzmain, 1913). Stable flies reared by Mitzmain (1913) remained in the larval stage an average of 12 days. This differs from Parr's (1962) findings of seven to eight days. The full grown stable fly maggott is about 10.00 mm long and 1.50 mm wide (Mitzmain, 1913). Immediately prior to pupation the larvae move to the drier part of the breeding material to pupate.

According to Parr (1962), the first indication of pupation is decreased mobility of the larva followed by a shortening and "fattening" of the body. Mitzmain (1913) found that a larva measuring 10 mm contracts to 5 mm and that the body is thickened from 1.5 mm to 2 mm. He also found that the female puparium is generally 0.5 mm longer than the male from the same batch and in forty instances, the males preceded the females in emergence from the puparia, usually by two days. Parr (1962) measured 100 puparia and reported the following mean values; length, 5.27 mm; width, 1.96 mm; weight, 11.23 mg.

Mitzmain (1913) reported that the imago usually emerges from the puparium in five days. He also found that the female is usually larger and lighter than the male in color and emerges with its long, tapering ovipositor everted which remains everted until her body dries in about one-half hour.

Herms (1964) has found that under laboratory conditions, with daily feedings on monkeys or rabbits, the average length of life of S. calcitrans is about 20 days. Mitzmain (1913) found the maximum life span for the female stable fly is 72 days and 94 days for the male.

The following description of the adult Stomoxys calcitrans was taken from La Pago (1962). The adult stable fly is about the size of the house fly and has a prominent proboscis which is held horizontally and is pointed forward. The M_{1+2} vein curves forward and the R_5 cell is open, ending at or behind the apex of the wing. The thorax is grey and has four longitudinal dark stripes. The lateral pair of stripes are narrow and do not reach the end of the scutum. The abdomen is shorter and broader than the housefly abdomen and is somewhat heart-shaped. The abdomen of the stable fly has three dark spots on each of the second and third segments, giving the abdomen a somewhat checkered appearance. Parr (1962) found the average weight of Stomoxys to be 8.6 mg.

Mitzmain (1913) reported that under laboratory conditions, stable flies will feed for the first time 6 to 8 hours after leaving the puparium; however, in nature he believed that blood is taken as early as one hour after emergence. The stable fly is essentially a blood feeder and it has never been observed in nature to feed on plant juices, although it will ingest sugar water under laboratory conditions.

Parr (1962) reported that a hungry Stomoxys calcitrans would ingest a blood meal about three times its body weight or 25.8 mg. This conflicts with data obtained by Lotmar (1948). She reported that Stomoxys ingests about 1 1/2 times its body weight, or 13 mg. The stable fly can engorge in three to four minutes when undisturbed (Mitzmain, 1913). Mitzmain (1913)

also observed that Stomoxys prefers domestic animals such as cattle and horses, but under laboratory conditions they will accept any host that would submit to its attacks.

Copulation of stable flies was reported by Parr (1962) to last as long as six minutes. Results obtained by Killough and McKinstry (1963) indicate that one-day-old males can successfully mate with five-day-old females and one-day-old females with five-day-old males. They noted, however, that more successful matings appeared to take place when both sexes were four or five days old. Harris et al. (1966) determined that one male stable fly may inseminate as many as nine females, with the average being 6.13. They observed that only 60 percent of the females had been inseminated in the first mating, although all had copulated. Their findings indicate that if sperm are transferred on the first mating the females will not mate again.

Nieschulz (1934) reported that the preferred temperature range of S. calcitrans was 27° - 30° C, with the largest percentage of flies aggregating at 29° C.

Although much research has been done on digestion of other blood sucking flies, very little work has been done on stable fly digestion. Champlain and Fisk (1956) reported that the enzymes trypsin, invertase, lipase, and amylase were found in the digestive tract of Stomoxys while pepsin and lactase were not found. They also found that the maximum proteolytic activity occurred about thirteen hours after a blood meal. According to Champlain and Fisk (1956) the ingested blood goes into the crop which functions simply as a storage organ and small quantities of blood are periodically released into the midgut. This finding conflicts

with that of Lotmar (1948) who reported that blood is never stored in the crop although sugar-water may be. This discrepancy may, in part, be explained by the work of Khan and Hopkins (unpublished, 1967). They found that in nature, the blood goes into the anterior part of the midgut and never into the crop. However, under laboratory conditions, the blood would sometimes go into the crop if the flies were starved for 24 to 36 hours before feeding.

Humidity responses of the stable fly have not been studied in great detail although other insects have been studied. Dakshinamurty (1948) reported that the house fly, Musca domestica, showed a preference for the drier humidities regardless of the alternative choices. Thomson (1938) reported that Culex fatigans showed a slight but regular avoidance of low humidities, provided a range not less than 40 percent relative humidity (R.H.) was present, with the exception of the avoidance of humidities above 95 percent R.H. MacGregor-Loaeza (1961) found that Anopheles albimanus, A. quadrimaculatus and to a certain extent Culex quinquefasciatus show a similar distribution; the unengorged females preferring the higher and engorged females the lower humidities. Both the unfed and engorged of Aedes aegypti preferred the lower degrees of R.H. Perttunen and Salmi (1956) demonstrated that the intensity of the humidity reaction of Drosophila melanogaster was correlated with the degree of the higher alternative humidity available rather than with the difference in humidity. When the higher alternative was any humidity between 100 percent and 87 percent R.H. the normal undesiccated specimens preferred the drier alternative. However, when the higher alternative humidity was between 77 and 20 percent R.H., the moister alternative was preferred.

A number of factors influence the response of insects to humidity. Desiccation of D. melanogaster reverses the original dry reaction to moist, and the original moist reaction was usually intensified (Perttunen and Salmi, 1956). Blatta orientalis, which normally prefers lower humidities, becomes hygropositive when desiccated (Gunn and Cosway, 1938). Tribolium confusum and T. castaneum gradually change with desiccation and starvation from an initial preference for lower humidities to a preference for higher humidities (Roth and Willis, 1951).

Wigglesworth (1941) reported that the R.H. at which Pediculus humanus corporus was conditioned prior to testing influenced the choice made when placed in the alternative chamber, and Dakshinamurty (1948) reported a similar difference in Musca domestica.

Perttunen and Ahonen (1946) reported that D. melanogaster of different ages reacted differently to humidity.

Another factor that influences the response to humidity is sex. Such differences were reported by Dakshinamurty (1948) and Roth and Willis (1951).

The identity of humidity receptors is not very well established. The responses of Tenebrio to humidity are abolished when the antennae are removed or completely covered. The most common sensillae are pits and pegs, the pegs being confined to the seven distal segments. The removal of the seven distal segments, greatly decreases the intensity of response, but complete abolition of a response requires the removal of the additional segments which bear only pits (Pielou, 1940). Roth and Willis (1951) report a similar correlation between the number of thin-walled sensillae and the intensity of response in Tribolium. Wigglesworth (1941) reported

that the humidity receptors of Pediculus humanus corporis were found on the fourth and fifth antennal segments. These tuft organs each consist of a minute cone bearing four delicate apical hairs. The humidity reactions of both sexes of Blatella germanica and the females of Aedes aegypti can be correlated with the distribution of thin-walled sensillae on the antennae (Roth and Willis, 1952). Perttunen and Syrjamake (1958) were able to abolish the humidity reaction of Drosophila melanogaster in undesiccated specimens by removal of the antennae. Bursell (1957) reported that the normal orthokenetic response to humidity was abolished when the thoracic spiracular filters were removed.

The objective of this study was to observe the reaction of Stomoxys calcitrans when subjected to a series of different humidities using the alternative chamber technique used by Gunn and Kennedy (1936).

METHODS AND MATERIALS

Rearing and Maintenance of the Stable Fly Colony. The stock culture originated from pupae obtained from the University of Nebraska (C. M. Jones, U.S.D.A., Lincoln, Nebraska) in the summer of 1965. Adults were kept in cages approximately 12 inches long, 8 inches wide, and 10 inches high. The bottom and back of the cages were 1 inch boards 12" by 8" and 10" by 8" respectively. They were attached at right angles with screws and finished with white enamel paint. The sides and top consisted of wire screen stretched over the two boards and stapled in place, leaving one end open. A nylon sleeve approximately 18 inches long was stapled onto the screen and wooden bottom of the open end, thus providing an easily accessible entrance. The sleeve was held closed with a rubber band when the cage was in use.

The rearing procedure of McGregor and Dreiss (1955) was utilized with a few modifications. The larval medium was prepared by mixing 1 part by volume of standard C.S.M.A. fly medium with 5 parts of wood shavings and moistening with water until one or two drops could be squeezed from a handful of the mixture. The moist medium was transferred to a one gallon crock filled to about 3/4 of its capacity. The eggs were stirred into the medium and the crock covered with a paper towel which was held in place with a rubber band. The crock was placed in the rearing room which was maintained at a constant temperature of 80° F, 50 percent relative humidity.

The eggs hatched in about 24 hours, and the larvae fed on the grain particles in the medium. Initially, when the larvae were small, the medium required stirring to prevent mold formation, however, when the larvae became large enough to keep the medium well mixed by normal activity, stirring was unnecessary. The top layer of dry shavings with pupae were removed from the crock and the pupae were recovered and placed in a paper cup inside a cage.

Fly emergence usually occurred over a 3 day period. The puparia were transferred to a new cage each day to insure uniform ages. All flies were fed outdated human blood obtained from a local hospital. The blood was placed in 1 1/4 oz. soufflé cups with a small styrofoam float to prevent entrapment of the flies. Fresh blood was added to the cages daily. A black cloth saturated with 10% ammonium carbonate was used as an oviposition site. The eggs were washed from the cloth into the culture medium.

Alternative Humidity Chambers. The alternative humidity chambers used were similar to those used by Gunn and Kennedy (1936). The chambers were made from round, clear, plastic boxes and were six inches in diameter and one and one-half inches deep. A piece of clear plastic, one-sixteenth inch thick, one inch wide, and six inches long was glued to the bottom and sides of the box making two water-tight chambers one inch deep. The glue used was made by dissolving clear plastic in chloroform. In addition to the piece of plastic dividing the box, two more pieces of plastic one-sixteenth inch thick, one inch wide and one-half inch long were glued to the sides and bottom of the box perpendicular to the dividing strip. The purpose of these two strips was to help support the false floor which fits into the upper part of the chamber. A five-sixteenth inch hole was drilled into the center of the lid to permit the entrance of the flies. The hole was ringed with vaseline and covered with a small piece of glass to make the chamber airtight.

The false floor was made by stretching white nylon mesh over the inner ring of a six-inch embroidery hoop and gluing it to the outer edge. The mesh could be held in place by the outer ring until the glue dried, at which time the outer ring was removed and discarded and the mesh was trimmed with a pair of scissors. The false floor was positioned on the supporting pieces of plastic giving the flies an area one-half inch high and six inches in diameter in which to move around. Ten flies were used in each test.

Prior to testing, the flies (except control groups) were conditioned for one hour at a known humidity (33% R.H. or 92% R.H.). These conditioning

chambers were identical to the alternative humidity chambers except they had no divider, but they did have a total of four false floor supporters.

All flies were sexed before testing. Sexing was done by holding the flies against a piece of screen wire with a vacuum and then transferring the desired sex to the plastic conditioning chambers with forceps. After conditioning for one hour, the flies were transferred to the alternative humidity chamber with another vacuum apparatus. The unconditioned flies were transferred from the screen directly to the alternative humidity chambers. The flies were given two minutes to acclimate after being placed into the alternative chamber before readings were taken. After this initial two-minute period, the number of flies on each side of the chamber was counted at two-minute intervals for a total of ten counts. Three replicates of each test were performed.

Salt Solutions. Saturated salt solutions producing specific relative humidities at 22° C (Peterson, 1959) are listed in Table 1. In addition, drierite and water were used to obtain zero and 100% R.H. respectively.

A saturated solution of the various salts was made with distilled water and then poured into the chambers to a level just below the false floors. The alternative humidity chambers were prepared by putting a solution of a different salt on the other side, thus giving the flies a "choice" between two different humidities. The chambers were allowed to set for an hour prior to each test so an equilibrium was established. There is, of course, a humidity gradient formed across the chamber.

Prior to testing, the flies were conditioned for 1 hour at either 33% R.H. or 92% R.H. There were unconditioned control groups tested. For the purpose of convenience, throughout this paper, the flies conditioned

at 33% R.H. will be called "dry" flies and those conditioned at 92% R.H. will be called "wet" flies, while those not conditioned will simply be called unconditioned flies.

The alternative humidity chambers containing the flies were placed on a stainless steel table situated 5' 8 3/8" below a fluorescent light when testing. The light intensity at the testing area was 35 foot candles. The temperature was held at 22 ± .5 degrees C for all conditioning and testing.

Experiment I. The stable flies were given a "choice" between two humidities which differed by approximately 20% R.H. The flies were subjected to the following R.H. combinations: 10:33%; 22:44%; 33:54%; 44:65%; 54:75%; 65:81%; 75:92%; and 81:100%.

Table 1. Humidities by saturated salt solutions.

Salt	Relative Humidity (%)
Zinc chloride	10
Potassium acetate	22
Calcium chloride	33
Potassium carbonate	44
Magnesium nitrate	54
Sodium nitrite	65
Sodium nitrate	75
Ammonium sulfate	81
Sodium carbonate	92

The following groups of flies were subjected to each of the above humidities:

(1) 1-day-old females conditioned at 92% R.H.

(2) 1-day-old females conditioned at 33% R.H.

(3) 1-day-old unconditioned females

(4) 1-day-old males conditioned at 92% R.H.

(5) 1-day-old males conditioned at 33% R.H.

(6) 1-day-old unconditioned males

(7) 7-day-old females conditioned at 92% R.H.

(8) 7-day-old females conditioned at 33% R.H.

(9) 7-day-old females unconditioned

(10) 7-day-old males conditioned at 92% R.H.

(11) 7-day-old males conditioned at 33% R.H.

(12) 7-day-old unconditioned males

The 7-day-old flies had access to food until the tests were performed, while the 1-day-old (24 hours) flies were not fed.

Experiment II. The stable flies were given a "choice" between two humidities which differed by approximately 50% R.H. The flies were subjected to the following R.H. combinations; 0:54%, 22:75%, and 54:100%.

The groups of flies tested were the same as those tested in Experiment I.

Experiment III. The flies were antennectomized immediately prior to testing, and then given a "choice" between two humidities which differed by approximately 50% R.H. The R.H. combinations used were the same as those used in Experiment II. In this experiment the flies were not preconditioned and only 7-day-old flies were tested.

Experiment IV. One-day-old female flies and seven-day-old male and female flies were not allowed to feed for a 24 hour period prior to testing. The one day males were not allowed to feed for 20 hours because they could not survive for a 24 hour period without food. The R.H. combinations used were the same as those in Experiment II. None of the flies were preconditioned.

RESULTS

Experiment I

Experiment I, as described in the Methods and Materials was conducted with 8 combinations of two relative humidities differing by 20 percent.

Test for Significance of Choice. The means of flies going to the lower humidity were analyzed by the "t" test to determine if the number was significantly different from the means of flies going to the other side of the gradient.

As can be seen in Table 2, between 50 and 70 percent of the one-day-old flies went to the lower humidity in all but one instance. Table 9 shows that in 25 instances, the means of the flies going to the lower humidity were significant at either the 1% or 5% level. The test in which the flies went to the higher humidity ("wet" females subjected to 10:33% R.H.) was not significant. All three groups of one-day-old flies ("wet", "dry", and unconditioned), exhibited the strongest "preference" for the lower R.H. when subjected to 81:100% R.H.

Table 3 shows that the seven-day-old flies showed a "preference" for the higher humidity in eight instances. However, Table 10 shows that

only two were significant ("dry" males subjected to 75:92% R.H. and "wet" males subjected to 22:44% R.H.). In 17 of the remaining 28 instances the means of the flies going to the lower humidity were significant at either the 1% or 5% level.

Test for Significance of Age. The means of one-day-old and seven-day-old flies going to the lower humidity were analyzed by the "t" test to determine if there was any difference due to age. The results of this analysis are in Table 11. No significant difference in response due to age was exhibited by either males or females.

Test for Significance of Sex. The means of male and female flies going to the lower humidity were analyzed by the "t" test to determine if there were any differences due to sex (Table 12). There was no significant difference in response found in either the one-day-old stable flies or the seven-day-old stable flies.

Test for Significance of Conditioning. By means of analysis of variance the effect of preconditioning was determined (Tables 2, 3 and 13). Those showing a significant difference were: one-day-old females subjected to 22:44% R.H., one-day-old females subjected to 75:92% R.H., and seven-day-old females subjected to 44:65% R.H. No males showed a significant difference because of preconditioning.

Experiment II

Test for Significance of Choice. The means of flies going to the lower humidity were analyzed by the "t" test to determine whether or not the "choice" was significant.

Table 2. Percentages of one-day-old stable flies preferring the low humidity in six relative humidity alternatives.[1]

Condition of flies	Sex	(%) Relative Humidity Alternatives					
		10:33	22:44	44:65	54:75	75:92	81:100
"Dry" Flies	♀	55	54	55	61	72	64
	♂	51	56	69	56	57	62
"Wet" Flies	♀	46	52	57	54	67	70
	♂	54	57	59	60	62	63
Unconditioned	♀	57	63	62	56	56	64
	♂	50	51	59	59	53	63

1 Determined from three replicates; 10 counts each.

Table 3. Percentages of seven-day-old stable flies preferring the low humidity in six relative humidity alternatives.[1]

Condition of Flies	Sex	(%) Relative Humidity Alternatives					
		10:33	22:44	44:65	54:75	75:92	81:100
"Dry" Flies	♀	54	52	59	54	60	60
	♂	51	49	59	57	41	61
"Wet" Flies	♀	58	48	49	56	49	56
	♂	62	43	63	52	44	58
Unconditioned	♀	51	57	56	52	58	53
	♂	53	50	60	55	47	69

1 Determined from three replicates; 10 counts each.

As seen in Tables 4 and 14, the number of one-day-old flies going to the lower humidity is greater than the number going to the higher humidity, and in each instance the "preference" is highly significant.

Tables 5 and 15 indicate that the number of seven-day-old flies going to the lower side of the humidity gradient was significantly different from the numbers of flies going to the higher side of the gradient in all but one test. Only the seven-day-old "dry" males subjected to 22:75% R.H. did not exhibit a significant "preference" for the lower humidity.

Test for Significance of Age. The means of one-day-old and seven-day-old flies going to the lower humidity were analyzed by the "t" test to determine if there was any difference due to age. The results of this analysis are in Table 16. As can be seen there is no significant difference in response due to age.

Test for Significance of Sex. The means of male and female flies going to the lower humidity were analyzed by the "t" test to determine if there were any differences in responses due to sex. The results of this analysis are in Table 17. As can be seen there is no significant difference in response due to sex.

Test for Significance of Preconditioning. The effect of preconditioning was determined by analysis of variance. The results may be seen in Tables 4, 5 and 18. Only the seven-day-old males subjected to 22:75% R.H. showed a significant difference.

Table 4. Percentages of one-day-old stable flies preferring the low humidity in three relative humidity alternatives.[1]

Condition of flies	Sex	(%) Relative Humidity Alternatives 0:54	22:75	54:100
"Dry" Flies	♀	67	64	61
	♂	68	69	77
"Wet" Flies	♀	63	71	65
	♂	61	65	72
Unconditioned	♀	68	65	61
	♂	64	64	59

1 Determined from three replicates; 10 counts each.

Table 5. Percentages of seven-day-old stable flies preferring the low humidity in three relative humidity alternatives.[1]

Condition of flies	Sex	(%) Relative Humidity Alternatives 0:54	22:75	54:100
"Dry" Flies	♀	64	63	61
	♂	65	50	63
"Wet" Flies	♀	67	61	64
	♂	69	61	60
Unconditioned	♀	60	65	66
	♂	71	64	64

1 Determined from three replicates; 10 counts each.

Experiment III

Test for Significance of Choice by Antennaeless Flies. The means of antennaeless flies going to the lower humidity were analyzed by the "t" test to determine if the number was significantly different from the number of flies going to the other side of the gradient. The results in Tables 6 and 19 showed a significant "preference" by both male and female flies for the lower humidity in all three gradients.

The "t" test was used to determine if the response of the antennaectomized flies differed significantly from the response of the flies with intact antennae. Table 20 indicates there is no significant difference in any of the groups.

Test for Significance of Sex. The "t" test was used to ascertain whether or not there was a significant difference between the responses of males and females. There was no significant difference between the responses of the sexes (Table 21).

Experiment IV

Test for Significance of Choice by Unfed Flies. The means of the starved flies going to the lower humidity were analyzed by the "t" test to determine whether they were significantly different from the means of the flies going to the higher side of the gradient. Tables 7 and 22 reveal that the one-day-old male flies subjected to 54:100% R.H. showed a significant difference among the one-day-old flies. This "preference" is not as great for the lower humidities as was found in the fed flies.

The seven-day-old unfed flies also show a reduction in the "preference" for the lower humidities (Tables 8 and 23) as compared with the fed seven-day-old flies.

Test for Significance of Sex and Age of Unfed Flies. The "t" test was used to determine if sex and age caused any significant difference in the response elicited. Tables 24 and 25 indicate that they did not.

DISCUSSION

As seen in Experiments I and II, Stomoxys calcitrans "chose" the lower humidity in most of the humidity gradients. These responses are quite similar to those observed by Dakshinamurty (1948) with the house fly and by Kennedy (1937) with the African migratory locust, which also "chose" the lower humidities. Dakshinamurty (1948) points out that the aggregation of these insects on the dry side of the humidity gradient may be due to two causes; either a "preference" for the environment containing dry air, or to the effects of dry air on the water balance of the insect. It is not known which of these is the case in Stomoxys, but the similar results obtained with the "wet", "dry" and unconditioned flies may indicate that at the time of testing the water balance of the flies in each of the three groups was approximately the same. In order for this to occur, during the conditioning period the flies must be closing their spiracles in the low humidity to conserve water, while opening them in the high humidity to avoid the accumulation of excess water in the body.

Correlation of this laboratory obtained data with the behavior of the flies in the field cannot be done at this time, nor can it be explained why a few groups of flies did not "prefer" the lower humidities.

The stable flies showed almost no significant difference in "choice" due to sex, as found in the house fly (Dakshinamurty, 1948), and Tribolium (Roth and Willis, 1951), or preconditioning, as found in the house fly

(Dakshinamurty, 1948), and Pediculus (Wigglesworth, 1941), or age as found in Drosophila melanogaster (Perttunen and Ahonen, 1946). There were a few groups of flies that showed a significant difference due to sex, age and/or preconditioning, but they can not be explained at this time.

The removal of the antennae of Drosophila melanogaster completely abolished their humidity reaction (Perttunen and Syrjamaki, 1958) indicating that the humidity receptors are located on the antennae. This is also the case in Tenebrio (Pielou, 1940), Aedes aegypti (Roth and Willis, 1952) and several other insects which have previously been mentioned.

However, the removal of the antennae from the stable flies did not cause them to react any differently to the humidity than did the stable flies with intact antennae. Both groups showed a significant "preference" for the lower humidities. This seems to indicate that the humidity receptors of Stomoxys are not located on the antennae and lends evidence to the fact that they may be located on the spiracular plates as is the case in Glossina (Bursell, 1957), a rather closely related genus.

The starvation of Tribolium castaneum resulted in a greatly reduced "preference" for the low humidity. After about five days of starvation the beetles reversed their "choice" and began to show a "preference" for the high humidity (Willis and Roth, 1950). The simultaneous starvation and desiccation of Tribolium confusum and Tribolium castaneum (Roth and Willis, 1951) resulted in a complete reversal from a "preference" for a low humidity to a "preference" for a higher humidity. Willis and Roth (1950) showed that the "preference" for a low or high humidity and the intensity of the reaction could be related to the degree of starvation and to the water balance of the insects.

Table 6. Percentages of seven-day-old antennaeless stable flies preferring the low humidity in three relative humidity alternatives.[1]

Condition of flies	Sex	(%) Relative Humidity Alternatives		
		0:54	22:75	54:100
Unconditioned	♀	51	55	55
	♂	57	54	65

[1] Determined from three replicates; 10 counts each.

Table 7. Percentages of starved one-day-old stable flies preferring the low humidity in three relative humidity alternatives.[1]

Condition of flies	Sex	(%) Relative Humidity Alternatives		
		0:54	22:75	54:100
Unconditioned	♀	52	47	60
	♂	50	54	66

[1] Determined from three replicates; 10 counts each.

Table 8. Percentages of starved seven-day-old stable flies preferring the low humidity in three relative humidity alternatives.[1]

Condition of flies	Sex	(%) Relative Humidity Alternatives		
		0:54	22:75	54:100
Unconditioned	♀	56	49	68
	♂	54	53	64

[1] Determined from three replicates; 10 counts each.

Although the starvation of Stomoxys did not cause a reversal in their humidity reactions it did greatly reduce the "preference" for the drier humidities, and it is possible that a reversal might have occurred if the stable flies had been simultaneously desiccated, as was the case in Tribolium.

SUMMARY AND CONCLUSIONS

Whether the humidity range was 20 percent R.H., as in Experiment I, or 50 percent, as in Experiment II, the flies generally "preferred" the lower humidity. There was practically no difference due to sex, age, or preconditioning. The flies seemed able to discriminate better between the highs and lows of the gradients when the range was 50 percent R.H. rather than 20 percent R.H.

The antennae seem to be of little importance in humidity detection and discrimination, and the location of the humidity receptors on the antennae is doubtful. It is very probable that the humidity receptors are located on the spiracular plates as in the Tsetse fly (Bursell, 1957).

Starvation for a 24 hour period will result in the near elimination of the "preference" for the lower humidities in one-day-old flies, and a decrease in the "preference" for the lower humidities in the seven-day-old flies.

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APPENDIX

Table 9. Test for significance of choice of one-day-old stable flies preferring the low humidity in six relative humidity alternatives (|t| values of data in Table 2).

Condition of flies	Sex	|t| values 10:33	22:44	44:65	54:75	75:92	81:100
"Dry" Flies	♀	1.863	0.949	2.513*	5.358**	8.164**	4.422**
	♂	0.435	1.792	7.119**	2.431*	2.628*	5.811**
"Wet" Flies	♀	1.748	0.958	2.346*	2.918**	8.614**	7.276**
	♂	1.158	3.928**	2.713*	1.676	3.307**	10.139**
Unconditioned	♀	2.898**	3.265**	2.662*	3.025**	2.705*	4.104**
	♂	0.899	0.231	5.292**	7.009**	0.699	4.104**

* $t_{.05,\ 29} = 2.045$, significance at the 5% level.

** $t_{.01,\ 29} = 2.756$, significance at the 1% level.

Table 10. Test for significance of choice of seven-day-old stable flies preferring the low humidity in six relative humidity alternatives (|t| values of data in Table 3).

Condition of flies	Sex	|t| values 10:33	22:44	44:65	54:75	75:92	81:100
"Dry" Flies	♀	2.336*	0.462	3.848**	1.425	3.428**	1.657
	♂	0.132	0.168	7.009**	4.156**	3.695**	2.063*
"Wet" Flies	♀	2.189*	0.560	0.433	2.565*	0.347	1.475
	♂	3.355**	2.235*	6.878**	0.651	0.991	2.628*
Unconditioned	♀	0.746	2.098*	3.033**	0.538	3.124**	0.649
	♂	0.847	0.0	2.738*	2.216*	0.878	8.219**

* $t_{.05,\ 29} = 2.045$, significance at the 5% level.

** $t_{.01,\ 29} = 2.756$, significance at the 1% level.

Table 11. Test for significance of age of one day versus seven-day-old stable flies preferring the low humidity in six relative humidity alternatives.

Condition of flies	Sex	|t| values					
		10:33	22:44	44:65	54:75	75:92	81:100
"Dry" Flies	♀	0.215	0.186	0.613	0.756	1.084	0.291
	♂	0.383	0.460	1.464	0.191	2.309	0.079
"Wet" Flies	♀	0.939	0.635	0.873	0.330	3.425	1.225
	♂	0.741	1.619	0.348	1.467	1.497	0.383
Unconditioned	♀	0.387	0.622	0.513	0.485	0.485	0.937
	♂	0.564	0.081	0.078	2.448	0.511	0.807

* $t_{.05,\ 2} = 4.303$, significance at the 5% level.

** $t_{.01,\ 2} = 9.925$, significance at the 1% level.

Table 12. Test for significance of sex of male versus female stable flies preferring the low humidity in six relative humidity alternatives.

Condition of flies	Age	|t| values					
		10:33	22:44	44:65	54:75	75:92	81:100
"Dry" Flies	1 Day	0.518	0.324	1.672	0.783	2.101	0.471
	7 Day	0.212	0.171	0.0	0.358	1.692	0.069
"Wet" Flies	1 Day	0.920	1.083	0.275	0.837	0.683	0.672
	7 Day	0.846	0.623	1.569	0.896	0.486	0.217
Unconditioned	1 Day	1.265	1.202	0.246	3.674	0.288	0.061
	7 Day	0.303	0.525	0.468	0.347	1.364	1.322

* $t_{.05,\ 2} = 4.303$, significance at the 5% level.

** $t_{.01,\ 2} = 9.925$, significance at the 1% level.

Table 13. Test for significance of conditioning by analysis of variance for data in Tables 2 and 3.

Age of flies	Sex	"F" values					
		10:33	22:44	44:65	54:75	75:92	81:100
One Day	♀	4.153	20.581**	0.804	2.902	8.107*	0.942
	♂	0.500	1.348	1.304	0.733	1.034	0.130
Seven Day	♀	0.651	0.987	5.714*	0.283	3.391	0.265
	♂	0.854	0.363	0.749	2.038	0.419	0.915

* $F_{2,6} = 5.14$, significance at the 5% level.

** $F_{2,6} = 10.92$, significance at the 1% level.

Table 14. Test for significance of choice of one-day-old stable flies preferring the low humidity in three relative humidity alternatives (|t| values of data in Table 4).

Condition of flies	Sex	|t| values		
		0:54	22:75	54:100
"Dry" Flies	♀	8.1086**	4.3419**	4.4746**
	♂	3.8226**	7.5037**	9.0711**
"Wet" Flies	♀	6.303**	4.876**	6.559**
	♂	5.373**	6.494**	6.618**
Unconditioned	♀	7.480**	4.542**	2.384*
	♂	4.609**	5.015**	2.779**

* $t_{.05,\ 29} = 2.045$, significance at the 5% level.

** $t_{.01,\ 29} = 2.756$, significance at the 1% level.

Table 15. Test for significance of choice of seven-day-old stable flies preferring the low humidity in three relative humidity alternatives (|t| values of data in Table 5).

Condition of flies	Sex	|t| values 0:54	22:75	54:100
"Dry" Flies	♀	2.957**	3.314**	2.471*
	♂	4.919**	0.186	2.877**
"Wet" Flies	♀	4.308**	3.463**	6.999**
	♂	5.323**	17.107**	3.743**
Unconditioned	♀	2.692*	3.168**	3.672**
	♂	5.478**	7.005**	4.616**

* $t_{.05,\ 29} = 2.045$, significance at the 5% level.

** $t_{.01,\ 29} = 2.756$, significance at the 1% level.

Table 16. Test for significance of age of one-day versus seven-day-old stable flies preferring the low humidity in three relative humidity alternatives.

Condition of flies	Sex	|t| values 0:54	22:75	54:100
"Dry" Flies	♀	0.203	0.104	0.026
	♂	0.239	3.089	1.455
"Wet" Flies	♀	0.377	1.218	0.123
	♂	0.884	0.696	1.515
Unconditioned	♀	1.471	0.053	0.483
	♂	0.719	0.092	0.372

** $t_{.01,\ 2} = 9.925$, significance at the 1% level.

Table 17. Test for significance of sex of male versus female stable flies preferring the low humidity in three relative humidity alternatives.

Condition of flies	Age	|t| values 0:54	22:75	54:100
"Dry" Flies	One Day	0.1182	0.8070	1.4837
	Seven Day	0.1078	1.2365	0.1367
"Wet" Flies	One Day	0.5762	1.4705	1.0857
	Seven Day	0.1176	0.0	0.4273
Unconditioned	One Day	1.0880	0.1022	0.1897
	Seven Day	2.9810	0.0947	0.2633

* $t_{.05,\ 2} = 4.303$, significance at the 5% level.

** $t_{.01,\ 2} = 9.925$, significance at the 1% level.

Table 18. Test for significance of conditioning by analysis of variance from data in Tables 4 and 5.

Age	Sex	"F" values 0:54	22:75	54:100
One Day	♀	2.53	1.889	0.298
	♂	0.538	0.728	0.375
Seven Day	♀	0.446	0.155	0.301
	♂	0.717	6.996*	0.298

* $F_{2,6} = 5.14$, significance at the 5% level.

Table 19. Test for significance of choice of seven-day-old antennaeless stable flies preferring the low humidity in three relative humidity alternatives (|t| values of data in Table 8).

Sex	|t| values 0:54	22:75	54:100
♀	3.49**	4.33**	2.66**
♂	2.19*	2.32*	4.92**

* $t_{.05,29} = 2.045$, significance at the 5% level.

** $t_{.01,29} = 2.756$, significance at the 1% level.

Table 20. Test for significance of antennectomy of flies with antennae versus antennaeless flies preferring the low humidity in three relative humidity alternatives.

Sex	|t| values 0:54	22:75	54:100
♀	1.682	0.987	1.053
♂	1.844	0.122	1.021

* $t_{.05,2} = 4.303$, significance at the 5% level.

** $t_{.01,2} = 9.925$, significance at the 1% level.

Table 21. Test for significance of sex of antennaeless male versus female flies preferring the low humidity in three relative humidity alternatives.

	Relative Humidity Alternatives 0:54	22:75	54:100
|t| values	0.7038	0.1253	0.9706

* $t_{.05,2} = 4.303$, significance at the 5% level.

** $t_{.01,2} = 9.925$, significance at the 1% level.

Table 22. Test for significance of choice of starved one-day-old stable flies preferring the low humidity in three relative humidity alternatives (|t| values of data in Table 9).

Sex	|t| values		
	0:54	22:75	54:100
♀	0.637	0.827	1.739
♂	0.833	1.581	3.993**

* $t_{.05,\ 29} = 2.045$, significance at the 5% level.

** $t_{.01,\ 29} = 2.756$, significance at the 1% level.

Table 23. Test for significance of choice of starved seven-day-old stable flies preferring the low humidity in three relative humidity alternatives (|t| values of data in Table 10).

Sex	|t| values		
	0:54	22:75	54:100
♀	1.898	0.550	5.627**
♂	2.513*	0.609	8.051**

* $t_{.05,\ 29} = 2.045$, significance at the 5% level.

** $t_{.01,\ 29} = 2.756$, significance at the 1% level.

Table 24. Test for significance of age of starved one-day-old versus seven-day-old stable flies preferring the low humidity in three relative humidity alternatives.

Sex	|t| values 0:54	22:75	54:100
♀	0.4323	0.1852	1.237
♂	0.6432	0.2193	0.4040

* $t_{.05,\ 2} = 4.303$, significance at the 5% level.

** $t_{.01,\ 2} = 9.925$, significance at the 1% level.

Table 25. Test for significance of sex of starved male versus starved female stable flies preferring the low humidity in three relative humidity alternatives.

Age	|t| values 0:54	22:75	54:100
One Day	0.2138	1.076	1.2896
Seven Day	0.225	0.0534	0.0682

* $t_{.05,\ 2} = 4.303$, significance at the 5% level.

** $t_{.01,\ 2} = 9.925$, significance at the 1% level.

Experiment I

Table 26. Summary of the total fly count of one-day-old stable flies for each replicate for six relative humidity alternatives.[1]

Relative Humidity Alternatives	Conditioned at 33% RH		Conditioned at 92% RH		Unconditioned	
	Female	Male	Female	Male	Female	Male
A - 10:33%	60:40	51:49	49:51	62:38	59:41	47:53
	52:48	58:42	50:50	50:50	52:48	48:52
	54:46	45:55	38:62	51:49	59:41	54:46
B - 22:44%	53:47	53:47	53:47	53:47	59:41	44:56
	60:40	58:42	48:52	60:40	59:41	58:42
	49:51	57:43	54:46	58:42	72:28	51:49
C - 44:65%	54:46	74:26	56:44	47:53	62:38	57:43
	60:40	71:29	55:45	71:29	51:49	57:43
	52:48	61:39	59:41	59:41	74:26	64:36
D - 54:75%	65:35	50:50	48:52	63:37	56:44	59:41
	60:40	56:44	56:44	58:42	55:45	59:41
	59:41	62:38	59:41	58:42	56:44	58:42
E - 75:92%	67:33	53:47	73:27	54:46	60:40	59:41
	70:30	59:41	65:35	66:34	53:47	42:58
	79:21	59:41	64:36	65:35	56:44	59:41
F - 81:100%	64:36	62:38	77:23	61:39	61:39	66:34
	67:33	59:41	74:26	62:38	63:37	58:42
	61:39	65:35	58:42	65:35	67:33	66:34

1 Fly count = number per 10 flies on each side of the chamber for 10 consecutive counts.

Experiment I

Table 27. Summary of the total fly count of seven-day-old stable flies for each replicate for six relative humidity alternatives.[1]

Relative Humidity Alternatives	Conditioned at 33% RH		Conditioned at 92% RH		Unconditioned	
	Female	Male	Female	Male	Female	Male
A - 10:33%	58:42	65:35	63:37	14:86	52:48	58:42
	49:51	35:65	67:33	47:53	51:49	51:49
	45:55	52:48	45:55	53:47	52:48	50:50
B - 22:44%	53:47	49:51	45:55	34:66	65:35	61:39
	58:42	34:66	47:53	50:50	55:45	48:52
	62:38	64:36	53:47	44:56	50:50	41:59
C - 44:65%	55:45	58:42	58:42	61:39	58:42	52:48
	59:41	59:41	41:59	67:33	57:43	67:33
	62:38	59:41	48:52	62:38	54:46	61:39
D - 54:75%	52:48	54:46	54:46	47:53	60:40	56:44
	45:55	61:39	58:42	54:46	45:55	53:47
	64:36	57:43	57:43	55:45	51:49	55:45
E - 75:92%	60:40	47:53	46:54	54:46	58:42	40:60
	69:31	41:59	50:50	34:66	59:41	55:45
	50:50	35:65	50:50	43:57	58:42	46:54
F - 81:100%	46:54	47:53	50:50	71:29	63:37	71:29
	58:42	66:34	57:43	50:50	42:58	73:27
	75:25	70:30	60:40	54:46	55:45	63:37

[1] Fly count = number per 10 flies on each side of the chamber for 10 consecutive counts.

Experiment II

Table 28. Summary of the total fly count of one-day-old stable flies for each replicate for three relative humidity alternatives.[1]

Relative Humidity Alternatives	Conditioned at 33% RH		Conditioned at 92% RH		Unconditioned	
	Female	Male	Female	Male	Female	Male
A - 0:54%	71:29	58:42	59:41	64:36	73:27	64:36
	68:32	78:22	63:37	63:37	65:35	56:44
	61:39	68:32	66:34	57:43	65:35	73:27
B - 22:75%	65:35	64:36	74:26	69:31	73:27	67:33
	64:36	75:25	69:31	63:37	67:33	67:33
	63:37	67:33	71:29	64:36	56:44	59:41
C - 54:100%	59:41	67:33	71:29	69:31	71:29	67:33
	56:44	79:21	64:36	72:28	55:45	46:54
	67:33	84:16	59:41	75:25	58:42	63:37

Table 29. Summary of the total fly count of seven-day-old stable flies for each replicate for three relative humidity alternatives.[1]

Relative Humidity Alternatives	Conditioned at 33% RH		Conditioned at 92% RH		Unconditioned	
	Female	Male	Female	Male	Female	Male
A - 0:54%	62:38	60:40	61:39	64:36	58:42	72:28
	53:47	70:30	60:40	78:22	58:42	68:32
	77:23	66:34	81:19	65:35	63:37	72:28
B - 22:75%	64:36	51:49	66:34	63:37	56:44	60:40
	53:47	47:53	51:49	54:46	64:36	61:39
	72:28	51:49	65:35	65:35	74:26	70:30
C - 54:100%	72:28	58:42	64:36	63:37	71:29	69:31
	62:38	65:35	69:31	65:35	62:38	55:45
	49:51	65:35	58:42	51:49	65:35	67:33

[1] Fly count = number per 10 flies on each side of the chamber for 10 consecutive counts.

Experiment III

Table 30. Summary of the total fly count of antennectomized stable flies for each replicate for three relative humidity alternatives.[1]

Relative Humidity Alternatives	Females	Males
A - 0:54%	46:54	52:48
	51:49	53:47
	55:45	65:35
B - 22:75%	58:42	60:40
	52:48	46:54
	56:44	57:43
C - 54:100%	59:41	66:34
	62:38	67:33
	45:55	61:39

Experiment IV

Table 31. Summary of the total fly count of starved stable flies for each replicate for three relative humidity alternatives.[1]

Relative Humidity Alternatives	Females		Males	
	1 day	7 day	1 day	7 day
A - 0:54%	58:42	50:50	52:48	52:48
	50:50	53:47	44:56	54:46
	48:52	66:34	55:45	57:43
B - 22:75%	49:51	54:46	55:45	56:44
	41:59	41:59	52:48	51:49
	52:48	52:48	54:46	52:48
C - 54:100%	57:43	62:38	66:34	63:37
	64:36	73:27	70:30	61:39
	58:42	69:31	63:37	67:33

[1] Fly count = number per 10 flies on each side of the chamber for 10 consecutive counts.

HUMIDITY RESPONSES OF THE STABLE FLY, *STOMOXYS CALCITRANS* (L.) (DIPTERA: MUSCIDAE)

by

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B. S., Stephen F. Austin State College, 1965

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The responses of Stomoxys calcitrans were determined when subjected to a series of relative humidity combinations using the alternative chamber technique described by Gunn and Kennedy (1936).

Experiment I consisted of allowing the flies a "choice" between two humidities differing by approximately 20 percent R.H. The R.H. gradients used were 10:33%; 22:44%; 33:54%; 44:65%; 54:75%; 65:81%; 75:92% and 81:100% R.H.

Statistical analyses revealed that one and seven-day-old males and females usually "preferred" the lower humidity. Sex, age, or preconditioning had little, or no, effect.

Experiment II consisted of subjecting the flies to a humidity gradient in which the high and low humidities differed by approximately 50% R.H. The R.H. gradients used were 0:54% R.H.; 22:75% R.H.; and 54:100% R.H.

It was determined by statistical analyses that, with but one exception, the one and seven-day-old male and female flies "preferred" the lower humidities. Differences in responses due to sex, age, and preconditioning were almost nonexistent.

In Experiment III, flies were antennectomized and subjected to the following humidity gradients: 0:54%; 22:75%; and 54:100% R.H. Statistical analyses showed that the antennectomized flies responded the same as the flies with intact antennae, thus eliminating the antennae as the location of the humidity receptors.

In Experiment IV, flies were deprived of food for 24 hours (except one-day-old males which were deprived of food for 20 hours) and subjected to the following humidity gradients: 0:54%; 22:75%; and 54:100% R.H. It was observed that the flies deprived of food did not exhibit as great a

"preference" for the lower humidities as the flies not deprived of food. There were no differences in responses due to sex or age in these flies.

Whether the humidity range was 20 percent R.H., as in Experiment I, or 50 percent, as in Experiment II, the flies almost always "preferred" the lower humidity. There was practically no difference in responses due to sex, age, or preconditioning. The flies appeared more dicriminatory between the highs and lows of the gradients when the range was 50 percent R.H. rather than 20 percent R.H.

The antennae seem to be of no importance in humidity detection and it is very probable that the humidity receptors are located on the spiracular plates as in the Tsetse fly (Bursell, 1957).

Deprivation of food for a 24 hour period will result in the near elimination of the "preference" for the lower humidities in one-day-old flies, and a decrease in the "preference" for the lower humidities in the seven-day-old flies.