Temperature Gradient on *Tribolium castaneum* (Coleoptera: Tenebrionidae) Adult Dispersal in Stored Wheat

DAVID W. HAGSTRUM, PAUL W. FLINN, AND JERRY J. GAFFNEY[1]

Grain Marketing and Production Research Center, USDA-ARS, 1515 College Avenue, Manhattan, KS 66502

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ABSTRACT The dispersal behavior of *Tribolium castaneum* (Herbst) adults along a 22–36°C temperature gradient in stored wheat was monitored over a 20-h period with 8 microphones. Males tended to disperse more readily than females but both preferred temperatures of >30°C. Single adults generally did not stay at one location very long, and the average time of 9.7 min spent at the warm end with a temperature gradient was longer than that of 4.4 min spent at that location without a temperature gradient. An adult moved to a location 1, 2, and 3 microphones (7.8, 15.6, and 23.4 cm) away from where they were previously detected in 88.2, 10.6, and 1.2% of the cases. The temperature preference was much more evident with groups of 6 adults than with single adults because the presence of other adults apparently increased the time that adults spent at preferred temperatures. Observations on the times spent at different temperatures, the distances moved, and the influence of the sex of an adult and the presence of other adults provide the information needed to develop a predictive model for adult dispersal in response to the temperature gradients that occur in stored grain during the autumn and spring.

KEY WORDS *Tribolium castaneum*, temperature preference, dispersal behavior, stored products

TEMPERATURE PREFERENCES OF insects are important because insects have a limited ability to regulate their body temperature and temperature determines developmental time, fecundity, and population growth rate. Adult *Tribolium castaneum* (Herbst) favor temperatures around 30°C (Graham 1958, Amos et al. 1968, Amos and Waterhouse 1969, Yinon and Shulov 1970, King and Dawson 1973, Langer and Young 1976) at which the developmental times of their offspring are short (Howe 1956) and their fecundities and population growth rates are high (Howe 1962, Lhaloui et al. 1988). In most of these temperature preference studies, 30 to several hundred adults were used and their distributions along the temperature gradient were recorded after 20 min to 48 h. The study by Amos et al. (1968) recorded the locations of single adults at 5-min intervals. Only the study by Amos and Waterhouse (1969) examined the effects of the sex of adults on temperature preference. Studies were generally done without food or with only a thin layer of flour except for the study by King and Dawson (1973), which used a 2.5-cm layer of flour.

The dispersal of adult *T. castaneum* in bulk stored wheat has been studied at constant temperatures (Surtees 1964, Hagstrum 1995) but has not been studied with temperature gradients. In the autumn and spring, temperature gradients occur in stored wheat as grain cools or warms, respectively, from the outside to the center. The gradient increases gradually reaching as much as 7–10°C/m (Hagstrum 1987). Flinn et al. (1992) developed and validated a spatial model for predicting population growth of *Cryptolestes ferrugineus* (Stephens) in stored wheat at different locations along the temperature gradient. To accurately predict the population growth of this and other species in the autumn and spring, more information is needed on the influence of a temperature gradient on adult dispersal between locations in the grain bin.

Microphones have been used to monitor insect populations in bulk stored wheat (Hagstrum et al. 1996) and provide a method for monitoring the dispersal of a single adult along a temperature gradient within a grain mass. For *T. castaneum*, the detection of insect sounds has been shown to decrease as the distance between an insect and a microphone increases, and to be directly proportional to the number of insects (Hagstrum et al. 1991).

In this article, we report on the dispersal behavior of a single adult *T. castaneum* and groups of adults in response to a 14-degree temperature gradient in 3-kg lots of wheat using microphones to monitor the distances moved and the times spent at different temperatures during a 20-h period. The effects of

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[1] Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, P. O. Box 14565, Gainesville, FL 32604. Current address: Gaffney Engineering Company, 5530 N.W. 97th Street, Gainesville, FL 32653.

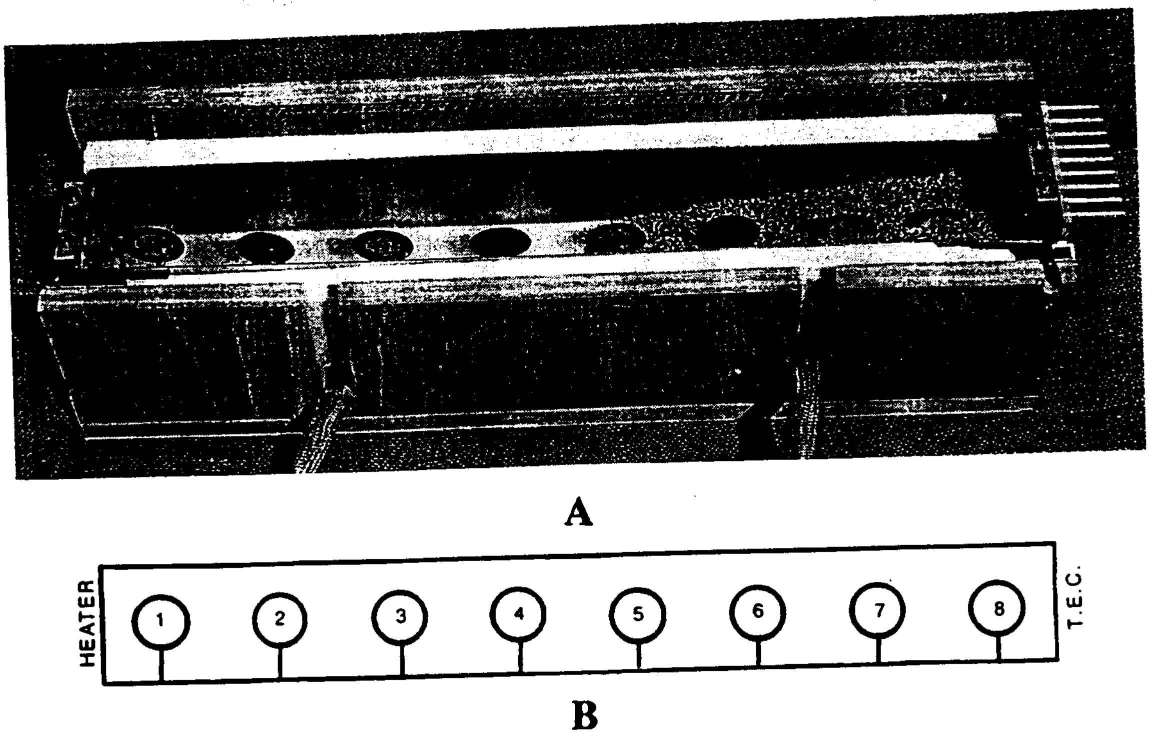

Fig. 1. Representations of the temperature gradient trough (7.6 by 7.6 by 61 cm) showing the locations (A) and numbering (B) of the microphones relative to the locations of the heater and the thermoelectric cooler (T. E. C.). The gradient trough was placed in a foam-lined plywood box for insulation and a portion of the 3 kg of wheat that it will hold was added at one end.

the sex of an adult and the presence of other adults on dispersal behavior were examined.

Materials and Methods

The temperature preferences of adult *T. castaneum* were determined by monitoring their dispersal behavior in 3 kg of 12.9% moisture content hard red winter wheat, *Triticum aestivum* L., in a stainless steel trough (7.6 by 7.6 by 61 cm) that was closed at both ends (Fig. 1). Eight microphones were mounted 7.6 cm apart to the bottom of the trough. The trough had a stainless steel cover that was fitted tightly against the grain. The cover had holes through which thermistors could be inserted to check the grain temperature at microphone locations. Grain moisture contents of samples from the center, and the warm and cold ends of the gradient trough were checked at the end of tests using a moisture meter (Model GAC II, Dickey-John, Auburn, IL).

The gradient trough was heated at one end with a power resistor (ME284-HS50-4, Mouser Electronics, Mansfield, TX) and cooled at the other end with a thermoelectric cooler (CP1.4-127-06L, Melcor, Trenton, NJ). The heater was operated using a 12 VDC, 12.5-ampere power supply (TS9980-ND, Digi-Key, Thief River Falls, MN) and the thermoelectric cooler was operated using a 12 VDC, 5.1-ampere power supply (PW9982-ND, Digi-Key). Temperature gradients were maintained with several devices but the only commercially available one is the dual input temperature controller (Model CN3240, Omega Engineering, Stamford, CT). Temperatures at each microphone location were measured at the beginning and the end of each test with thermistor probes (Model 8523, Cole-Parmer, Chicago, IL). Average temperatures were 36, 31, 27.5, 26, 25, 24, 23, and 22°C at microphones 1–8, respectively, with a temperature gradient, and 23.5°C without a temperature gradient. Temperatures were maintained within ±1°C.

Insects reared on a wheat flour and yeast diet at 27°C and 70% RH were segregated by sex as pupae. Adults were held on unused rearing diet for 20 d and a single male or female, groups of 6 males or 6 females, or 3 male–female pairs were then introduced in the center of the gradient trough at a depth of 3.8 cm in the wheat. Additional studies were done with 6 males or 6 females introduced between microphones 7 and 8. Each of these treatments was replicated 5–7 times with and without the temperature gradient. The locations of adults were determined during a 20-h period by checking each of the 8 piezoelectric microphones (9D0576, BNF Enterprises, Peabody, MA) for 10 s every 80 s using the software and equipment described by Hagstrum et al. (1991). The signal from each microphone was

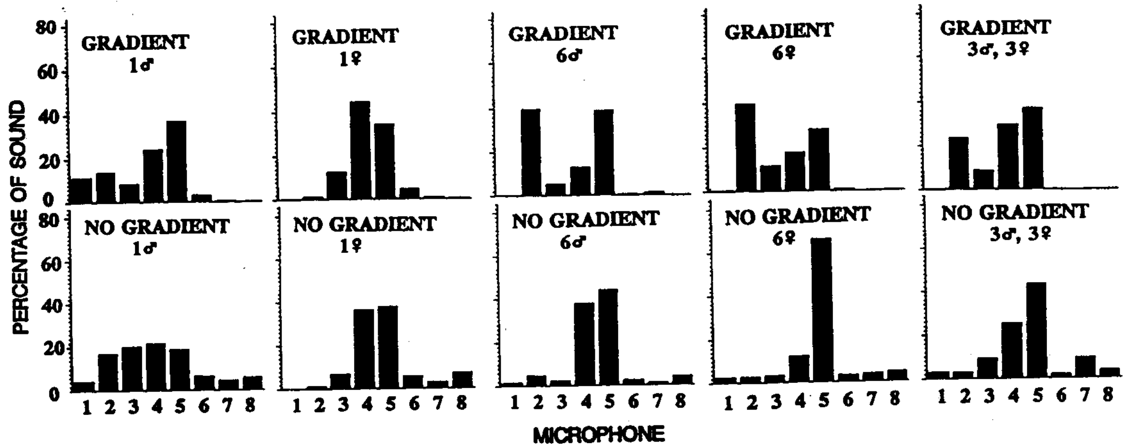

Fig. 2. Frequency distributions for percentages of total sounds from a single adult *T. castaneum* male or female, groups of 6 males or 6 females, and 3 male–female pairs detected at different locations along the temperature gradient or along the trough with no temperature gradient during the last 16 h of the test.

amplified (Bruel & Kjaer Model 2610, Marlborough, MA), the number of sounds was counted (Hewlett-Packard universal counter model 5316A, Wichita, KS), an IBM-compatible computer stored the data, and an 8-channel multiplexer (Bruel & Kjaer Model 2811) switched to the next microphone.

Data were analyzed using commercial statistical analysis software (SAS Institute 1996). The effects of sex (SEX), density (DEN), and temperature gradient (GRAD) on the percentages of adult sounds detected near each microphone location (MIC) were analyzed using PROC MIXED. Each replicate of the experiment was considered a TEST. The design of this experiment was a split-plot with TEST as the whole-plot experimental unit for combinations of SEX, DEN, and GRAD, and TEST(SEX DEN GRAD) as the appropriate error term. The microphone location within a TEST was the experimental unit for MIC and the interactions of MIC with SEX, DEN and GRAD, and TEST*MIC(SEX DEN GRAD) was the appropriate error term. For this analysis, the data for the first 4 h of the test period were excluded, and the percentages of total adult sounds detected at each microphone location during each of the remaining four 4-h periods were used. The statement LSMEANS MIC SEX DEN GRAD/DIFF was used to obtain t-tests for 4,560 possible pairwise comparisons. Squared t values are equal to F values with 1, 266 df and only the error degrees of freedom is generally reported for the t-test. Only 384 of the t-tests were of interest and these were for the comparisons of all combinations of the 8 microphones for 1 male with a gradient, 1 male without a gradient, 1 female with a gradient, 1 female without a gradient, 6 males with a gradient, 6 males without a gradient, 6 females with a gradient, 6 females without a gradient, 3 pair with a gradient and 3 pair without a gradient; male versus female for 1 adult with a gradient and 1 adult without a gradient; male versus female versus 3 pair for 6 adults with a gradient and 6 adults without a gradient; and a gradient versus no gradient for 1 male, 1 female, 6 males, 6 females, and 3 pairs. Chi-square tests were used to compare the frequency distributions of the times spent at different microphone locations.

Results

The percentages of adult sounds detected at each of the 8 microphone locations for a single adult male *T. castaneum* or groups of males in a temperature gradient or without a temperature gradient (Fig. 2) were generally not significantly different from those for females, and similar distributions were observed with 3 male–female pairs (t = 0.01–2.06, df = 266, P = 0.04–0.99). However, in studies with a temperature gradient, single females tended to disperse less and were detected significantly more often near microphone 4 (26°C) where they were introduced than single males (t = 2.85, df = 266, P = 0.005). Pairwise comparisons were used because MIC*GRAD (F = 1.69; df = 7, 266; P = 0.11), MIC*DEN (F = 1.94; df = 7, 266; P = 0.06) and MIC*GRAD*DEN (F = 1.74; df = 7, 266; P = 0.10) interactions approached significant levels. MIC was significant (F = 28.15; df = 7, 266; P = 0.0001), but the other main effects and interactions were not ($F < 1.13$; df = 1–14, 266; $P > 0.25$). Data for the first 4 h of the tests were not used for this analysis because adult activity was apparently increased by the disturbance of introducing them into the grain and significantly more adult sounds tended to be detected during this period than later (Table 1).

For single adults of either sex, the percentages of sounds detected at each of the 8 microphone locations with a temperature gradient were not significantly different from those without a temperature gradient (t = 0.01–1.67, df = 266, P = 0.09–0.99) (Fig. 2). With groups of 6 males (t = 3.25, df = 266,

Table 1. Ratio of total adult sounds detected during the first 4-h period compared with average of those detected during subsequent 4-h periods

Density	Sex	Ratio	F	P
		Gradient		
1	Female	1.9	2.94	0.0234
1	Male	7.5	16.12	0.0001
6	3 pairs	1.2	2.31	0.0748
6	Female	1.2	0.14	0.9657
6	Male	3.7	4.39	0.0022
		No gradient		
1	Female	3.7	8.87	0.0001
1	Male	8.6	4.79	0.0016
6	3 pairs	1.4	0.72	0.5785
6	Female	2.7	2.01	0.1120
6	Male	2.0	1.87	0.1162

$P = 0.001$) or 6 females ($t = 2.23$, df = 266, $P = 0.03$), adults were detected significantly more at the warm end near microphone 2 (31°C) with a temperature gradient than near microphone 2 without a temperature gradient. With 3 male–female pairs, adults were detected more near microphone 2 with than without a gradient but differences were not significant ($t = 0.11$–1.68, df = 266, $P = 0.09$–0.91) for any of the microphone locations. Although not statistically significant, as seen in Fig. 2, adults were consistently detected less at the cold end near microphones 6, 7, and 8 with a temperature gradient than without a temperature gradient ($t = 0.08$–0.97, df = 266, $P = 0.33$–0.93). With a temperature gradient, introducing 6 males or 6 females between microphones 7 and 8 (22–23°C) resulted in 52% of their sounds being detected at these locations and 40% at the warm end near microphones 1 and 2 (31–36°C).

The location of a single adult was monitored with and without a temperature gradient during a 20-h period (Fig. 3). Several males moved to the warm end of the temperature gradient within 1, 3, or 19 h and stayed there during the remainder of the test period as shown for one male in Fig. 3A. Another male moved to the warm end of the gradient and then returned to the center within the 1st h. In more than half of all tests, females stayed in the center where they were introduced during most of the test period as shown in Fig. 3B. More dispersal was observed without a temperature gradient (Fig. 3 C and D). During an 80-s period, adults moved to locations 1, 2, and 3 microphones (7.8, 15.6, and 23.4 cm) away from where they were previously detected in 88.2, 10.6, and 1.2% of the observed moves, respectively.

From the data in Fig. 3, the times spent near each microphone can be calculated (Fig. 4). With a temperature gradient, adults spent significantly more time (mean = 9.7 min, standard deviation = 18.2, maximum = 125.3 min) during each visit to the warm end near microphones 1 and 2 than was spent at these locations (mean = 4.4 min, standard deviation = 5.4, maximum = 46.7 min) without a temperature gradient ($\chi^2 = 15.03$, df = 6, $P = 0.02$). Differences between tests with a temperature gradient and those without a temperature gradient were less significant ($\chi^2 = 12.58$, df = 6, $P = 0.05$) for microphones 3–6 (mean = 4.9 versus 4.6 min) and not significant ($\chi^2 = 2.49$, df = 5, $P = 0.78$) for microphones 7 and 8 (mean = 4.1 versus 4.3 min). With a temperature gradient, the times spent near microphones 1 and 2 were significantly longer than those spent near microphones 3–6 ($\chi^2 = 68.65$, df = 6, $P = 0.0001$) or microphones 7 and 8 ($\chi^2 = 13.79$, df = 6, $P = 0.03$). Without a temperature gradient, the times spent near each group of microphones were similar ($\chi^2 = 2.40$–4.03, df = 6, $P = 0.67$–0.79).

During the 20-h test period, the average grain moisture contents at the warm end of the temperature gradient decreased only slightly from 12.9 to 12.7%. Moisture contents were unchanged in the center and at the cold end of the gradient.

Discussion

As in previous studies with a thin layer of diet, adult male and female *T. castaneum* in a mass of stored wheat both preferred temperatures around 30°C. Six males, 6 females, or 3 male–female pairs spent more time near microphone 2 (31°C), and single males or single females spent more time near microphones 1 and 2 (31–36°C) with than without a temperature gradient. There was also a tendency for adult sounds to be detected infrequently near the cold end of the temperature gradient. Temperature preference was much more evident with groups of 6 adults than with single adults. The presence of other adults apparently increased the time that adults spent at a preferred temperature. Inclusion of the influence of other adults on temperature preference will be important for accurately predicting adult distribution in grain during the autumn and spring.

Adult *T. castaneum* may have spent more time at both the preferred temperatures and the locations where adults were introduced because of an aggregation pheromone produced by both sexes in their frass which is attractive to both sexes (Suzuki 1985). This pheromone has not been identified and a synthetic version is not available for further investigation of its influence on temperature preference. Aggregation that occurred when only females were present, could not have been due to the identified aggregation pheromone that is attractive to both sexes because it is produced only by males (Suzuki and Sugawara 1979, Rangaswamy and Sasikala 1991). The tendency for *T. castaneum* in the current study and an earlier study by Yinon and Shulov (1970) to spend more time at the cold end of the temperature gradient only when introduced there may also be explained by reduced activity in response to the presence of aggregation pheromone. Yinon and Shulov (1970) explained the higher numbers at the warm and the cold ends as a thigmotactic response to the corners of the temperature gradient trough and by some observed mortality at these

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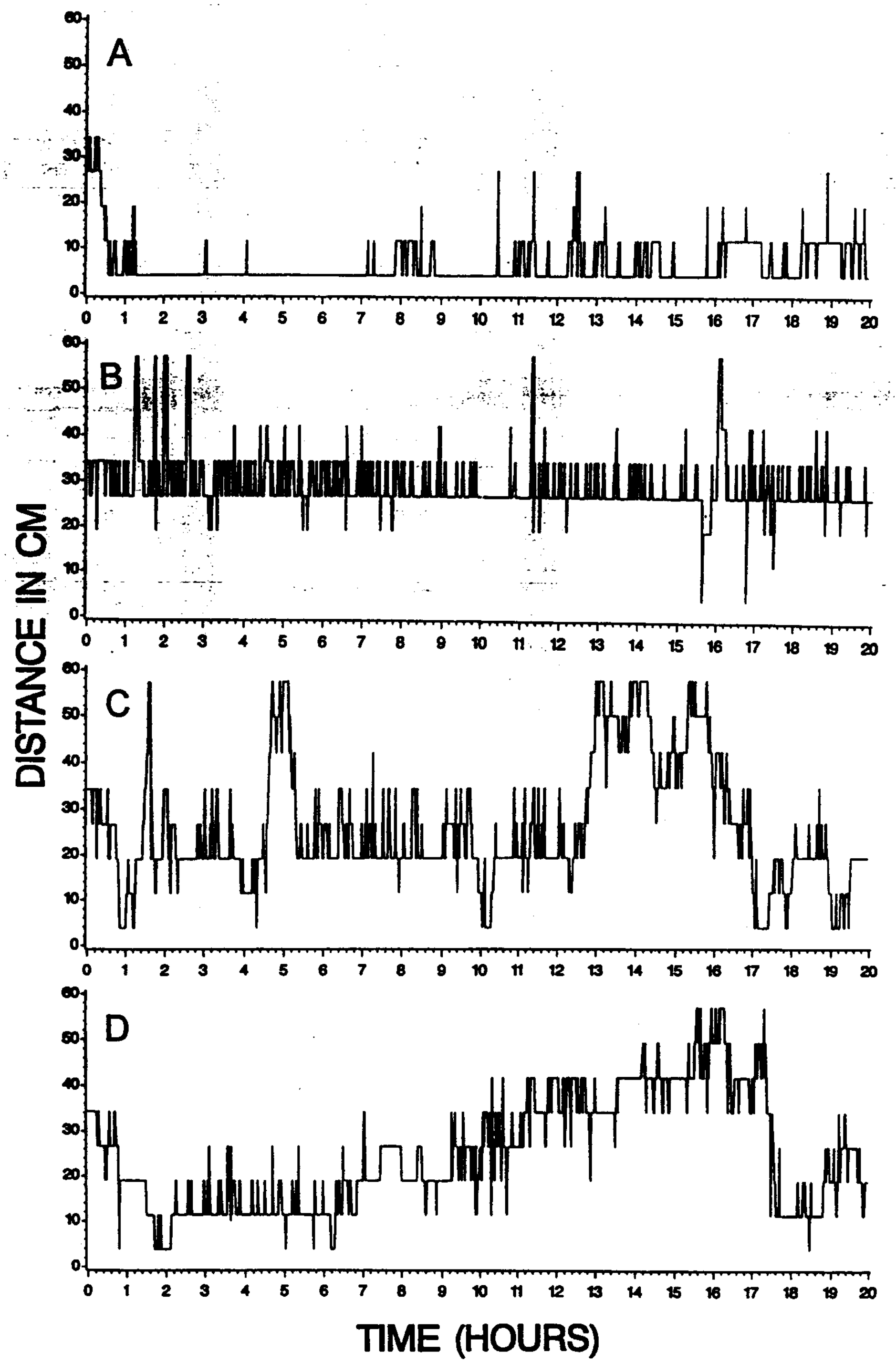

Fig. 3. Distances and directions moved during 80-s intervals plotted over the 20-h test period for single adult *T. castaneum* males (A and C) or females (B and D) with (A and B) and without (C and D) a temperature gradient. Adult locations are given as distances from the end of the trough with the heater (Fig. 1).

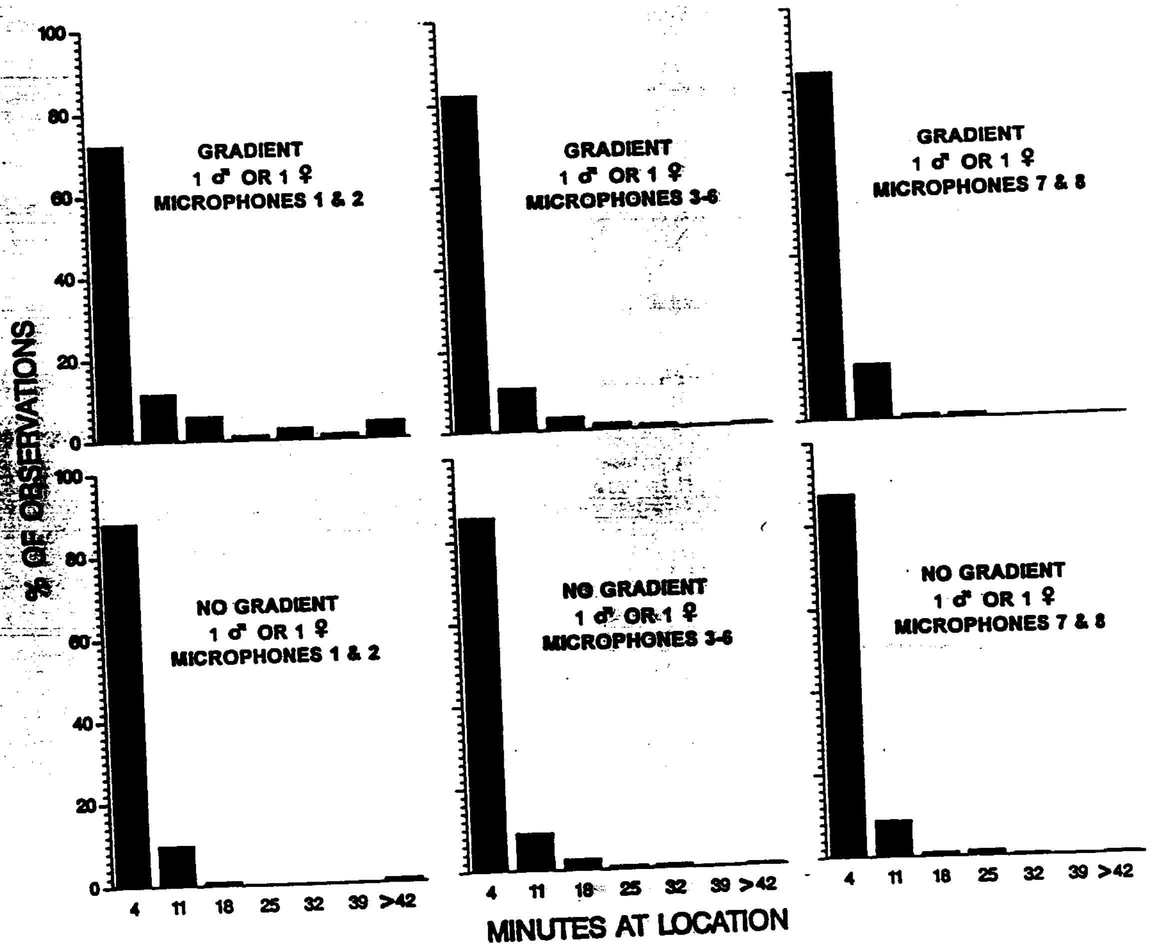

Fig. 4. Frequency distributions of the numbers of minutes spent near different groups of microphones with and without a temperature gradient derived from data for 20 adult *T. castaneum* similar to those shown in Fig. 3.

extreme temperatures rather than a response to an aggregation pheromone.

Grain moisture changes were small because moisture diffusion through grain was slow (Anderson et al. 1943, Pixton and Griffiths 1971). The decrease in grain moisture content near the warm end of the temperature gradient was probably too small to influence adult behavior, but if it had an influence it would have made that location less attractive.

Adults in the current study generally did not stay at one location very long, which was also observed by Amos et al. (1968). More frequent monitoring (80-s versus 5-min intervals) of adult dispersal in the current than in the earlier study by Amos et al. (1968) allows better resolution of the time spent at different temperatures and the distances moved. These observations improve our ability to predict the temperatures to which adults will be exposed in grain bins when they are given a choice. Even when adults remained close to the location where they were introduced, they were probably moving short distances, being detected alternately by adjacent microphones. In a grain mass, adults moved to locations >7.6 cm away from where they were previously detected in only 11.8% of the observations. Males tended to disperse more readily than females. With a temperature gradient, single males were detected less often near the point of introduction (microphone 4) than single females and more often near microphones 1–3. Also, without a temperature gradient, the percentages of sounds detected for single males were spread more evenly among microphone locations than those for single females. Further, several single males moved directly to the warm end of the temperature gradient but single females tended to spend more time near the location where they were introduced. The movement of males directly to the warm end may be explained by their ability to detect heat with the 3rd antennal segments (Holsapple and Florentine 1972). Differences in the dispersiveness of the 2 sexes will need to be considered in predicting adult dispersal in a grain bin during the autumn and spring.

Each of several earlier studies have investigated different aspects of insect response to a temperature gradient. Amos et al. (1968) photographically monitored individual females on the surface of a thin layer of flour but did not compare their behavior to that of males or groups of adults. Amos and Waterhouse (1969) examined the response of groups of males and groups of females to a temperature gradient but did not consider the response of individual adults. King and Dawson (1973) used a 2.5-cm layer of flour, but allowed the adults to move along the surface as well as through the flour mass. The current study is unique because it used a factorial design to consider the effects of the sex of an adult, the presence of other adults, and possible interactions, and because it confined adults so that they could only move within a grain mass.

A model for the dispersal of *T. castaneum* adults along temperature gradients within a grain mass during the autumn and spring will need to consider the effects of the sex of an adult and the presence of other adults. Males tended to move around more than females and temperature preferences were more evident when the densities were high enough that adults frequently found other adults. The current study provides information on the time spent at different temperatures, the distances moved, and the influence of the sex of an adult and the presence of other adults on temperature preference that is needed to develop a predictive model for adult distribution in stored grain during the autumn and spring.

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