OLFACTORY RESPONSE OF RED FLOUR BEETLES, TRIBOLIUM CASTANEUM (HERBST), TO VARIOUS FORMS OF WHEAT, MILLET AND A FUNGUS AS DETERMINED BY A LIGHT-SENSITIVE APPARATUS

by

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B. S., University of Khartoum, 1974
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A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1981

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INTRODUCTION

Tribolium spp. are known to attack a wide variety of foods, such as most prepared cereal products, grain and seeds, animal matter and dry insect specimens, yeast, nuts, dried fruits, chocolate, certain spices and plant products. However, whole wheat flour seems especially liable to infestation. They have difficulty feeding on entire, undamaged grain, seeds or in-shell nuts. Thus, the presence of a certain percentage of broken grain or nuts without shells greatly enhanced the ability of these beetles to develop and reproduce (Good, 1936).

The knowledge of the relative susceptibility of the cereal products is an important factor on which measures for their protection must be based; more susceptible products should be isolated, if possible, so they can be more carefully stored. The current investigation was to determine the attractiveness of wheat and millet, and of their fractions and solvent extracts, as well as moldy wheat and molds associated with grain. The results of this study may help storage and food processing personnel carry out a more effective preservation program.

REVIEW OF LITERATURE

By using characteristic behavior responses, as described by Dethier et al. (1960), it has been demonstrated by workers such as Willis and Roth (1950), Loschiavo (1959; 1965a; 1965b), Schoonhoven (1968), Honda et al. (1969), Tamaki et al. (1971) and others that several insect species are able to recognize the volatile odors of their preferred food substances. Willis and Roth (1950) reported that 2-day-starved adults of the red flour beetle (RFB) were strongly attracted to volatiles of flour kept below a wire screen, and Soliman (1975) found that under uniform dark conditions, female RFB are more responsive to volatiles of foods than males. However, the response of the RFB to flour volatiles in the case of Willis and Roth also could be optical since the test was under bright candle light. Extracts prepared from brewer's yeast, unenriched patent flour, bran or wheat germ elicited equally strong attraction, aggregation, and feeding responses of male and female RFB; however, extracts of wheat germ were the most active of all cereal extracts; this was attributed in part to certain fatty acids such as palmitic acid which elicited intense aggregation and feeding (Loschiavo, 1965a; 1965b). Loschiavo (1959) showed that among 6 cereal products, 30% of the RFB were attracted to patent wheat flour containing 5% wheat germ. In a 3-food test, 46% of the adult flat grain beetles were attracted to a food mixture consisting of 85% whole wheat flour, 10% wheat germ and 5% brewer's yeast; 27% were attracted to whole wheat flour and 24% to enriched second-patent flour. Similar results were reported by Cotton and Frankenfeld (1945), who suggested that the addition of vitamins of the B-group to the enriched flour increased its susceptibility to flour beetles. However, Oosthuizen (1945) recorded that wheat germ was the least attractive of the wheat fractions tested with RFB. In the above cases the attraction could also have been tactile since no barrier was used to prevent beetles from contacting the odor sources. The naturally occurring triglycerides extracted from wheat germ, such as 1-palmito-2; 3-diolein, 2-linoleo-1;3-dipalmitin, and 1-palmito-2-lino-3-diolein elicited attraction and aggregation of the confused and red flour beetles on the treated discs under darkness, but for the synthetic triglycerides, only the unsaturated ones caused aggregation (Tamaki et al. 1971; 1971). Levinson and Levinson (1978) found that the olfactory attraction of storage insects such as the granary weevil, Sitophilus granarius; confused flour beetle, Tribolium confusum; RFB, T. castaneum; and larvae of the Angoumois grain moth, Sitotroga cerealella, can be induced by blending food volatiles such as the aroma of wheat or other cereals, while aggregation and feeding may be stimulated by less volatile food components including salts, sugars and lipids. Olfactory orientation and attraction due to an odor of the host was also noticed among stored-product insects such as the rice weevil, Sitophilus oryzae. Honda et al. (1969) reported that both male and female rice weevils responded similarly to volatiles of rice grain extracts. Ohsawa et al. (1970) showed that the rice weevil, under complete darkness, is highly responsive to acidic and neutral fractions of rice, wheat and corn grains. A 7-fold increase over control in egg laying by cigarette beetles when subjected to volatiles from extracts of tobacco or wheat flour as ovipositional stimulants in a nonfood ovipositional site was found by Fletcher and Long (1971). The ability of the Ephestia cautella to locate and to oviposit in response to the source of grain odor was reported by Barrer and Jay (1979). Volatiles from polar and nonpolar extracts of wheat, corn, and peanuts, induced strong orientation and feeding responses in the larvae of the Indian meal moth, Plodia interpunctella (Baker and Mabie, 1973). They also reported that fatty acids and sterols alone had no effect on the larvae of the Indian meal moth, but when linoleic, oleic and palmitic acids were used in combination with subthreshold levels of sucrose, a definite synergistic feeding effect occurred. Spangler (1965), by using a venturi-type olfactometer, reported a strong attraction of khapra beetle larvae, Trogoderma granarium to volatiles from sesame seeds, fish meal, almonds and peanuts. Micha (1976) also reported that the khapra beetle larvae were strongly attracted to the same materials tested by Spangler (1965), and to their extracts; however, larvae in the free-choice test were free to come in contact with the test materials or extract-treated surfaces.

The cereal volatiles that might be responsible for olfactory responses of some of the stored-product insects were isolated and studied by McWilliams and Mackey (1969), Hougen et al. (1971), Maga (1978) and Nara et al. (1981). Their studies included volatiles from corn, oats, rye, wheat, triticale, barley and rice. These different cereal grains appeared to produce largely the same volatile components but in different relative amounts (Hougen et al., 1971).

Christensen (1957) and Sinha et al. (1962) reported that a close association exists between insects, mites and fungi. VanWyk et al. (1959) found that adult confused flour beetles, T. confusum, were attracted to and developed better in flour or grain contaminated with fungi than in fungus-free flour or grain. Sinha (1966) showed that the red and confused flour beetles fed, laid eggs and completed their life cycles on certain species of seed-borne fungi but the rate of larval development and survival were lower than on a wheat flour-yeast diet. In 1971, he reported that live mycelia of Aspergillus glaucus were isolated from the midgut of the granary weevil, S. granarius. Starratt and Loschiavo (1971; 1972) found that confused flour beetles were strongly attracted to and aggregated on discs treated with extracts of the fungus Nigrospora sphaerica. They reported that the monounsaturated triglycerides of which the major component was oleodipalmitin were the most active fractions. The analysis of extracts of certain fungi revealed the presence of ethyl acetate, ethanol, iso-butanol, n-butanol, iso-amylalcohol, 3-octanone, 1-octen-3-ol and octanol in the extracts of all species (Saito et al., 1979). Their study showed that 1-octen-3-ol was a strong fungal volatile and 3-octanone had a citrus-like odor. Further support for a fungus involvement was found by Thomas and Dicke (1972), who showed that the grain mite, Acarus siro, was attracted to discs treated with extracts of 8 fungi associated with stored-product commodities.

MATERIALS AND METHODS

Insect Cultures

Red flour beetles, Tribolium castaneum (Herbst), were reared on a medium composed of 95% whole wheat flour and 5% brewer's yeast. Quart jars of the medium were placed in a freezer at -17°C for at least one week to destroy any possible insect infestation, then stored at 5°C until needed. The insect cultures used were from stock cultures that have been maintained for several years in a laboratory of the Department of Entomology, Kansas State University, and were started from insects collected in Kansas.

After the rearing medium was transferred from the cold room to the rearing room (27$\pm$1^{o}C, 67$\pm$3%RH) and allowed to equilibrate for at least one week, two hundred (1.3 ml) 2- to 4-week-old adult beetles were placed in each of 2 quart jars containing about 175 g of the equilibrated medium. The jars were closed using filter paper inserts in the lid ring. After 3 days, the adults were screened out of the jars using a U.S. Standard No. 20 sieve. The adults were discarded and the medium, containing eggs, was returned to the jars which were kept in the rearing room. Female and male pupae (0-24 hr old) were separated and held in the rearing room so that adults of known age and sex could be obtained for tests. The sexes were determined in the pupal stage (Good, 1936). For each test, newly-emerged adults, 0 to 24 hr old, were collected and the sexes kept separate, except that 5 males and 5 females were placed in a third dish. All 3 dishes were provided with food and kept in the rearing room. When 48 to 72 hr old, the beetles were screened out of the medium and starved for one day, until 72 to 96 hr old. All except a preliminary experiment were conducted with beetles of this age.

Test Equipment

The equipment used for monitoring the response of red flour beetles (RFB) to food odors was composed of a light-sensitive event detector unit (Pinniger and Collins, 1976) and a Heath Model EUW-20A Servo Recorder. The sensory detector unit (Fig. 1) is composed of a rectangular Plexiglas base (35 x 20 cm), with two light dependent resistors recessed into the base, 15 cm apart. The test arena consisted of a Plexiglas

Fig. 1. An schematic diagram illustrating the sensory equipment and tools.

rp Rectangular Plexiglas plane (35 x 20 cm)

fp Filter paper

pr Plexiglas ring (15.2 cm diam., 2.6 cm high)

gl Glass lid

wf Wire frames

mp Modified insect pins

tm Test material or control object

os Object (styrofoam) for attaining light balance

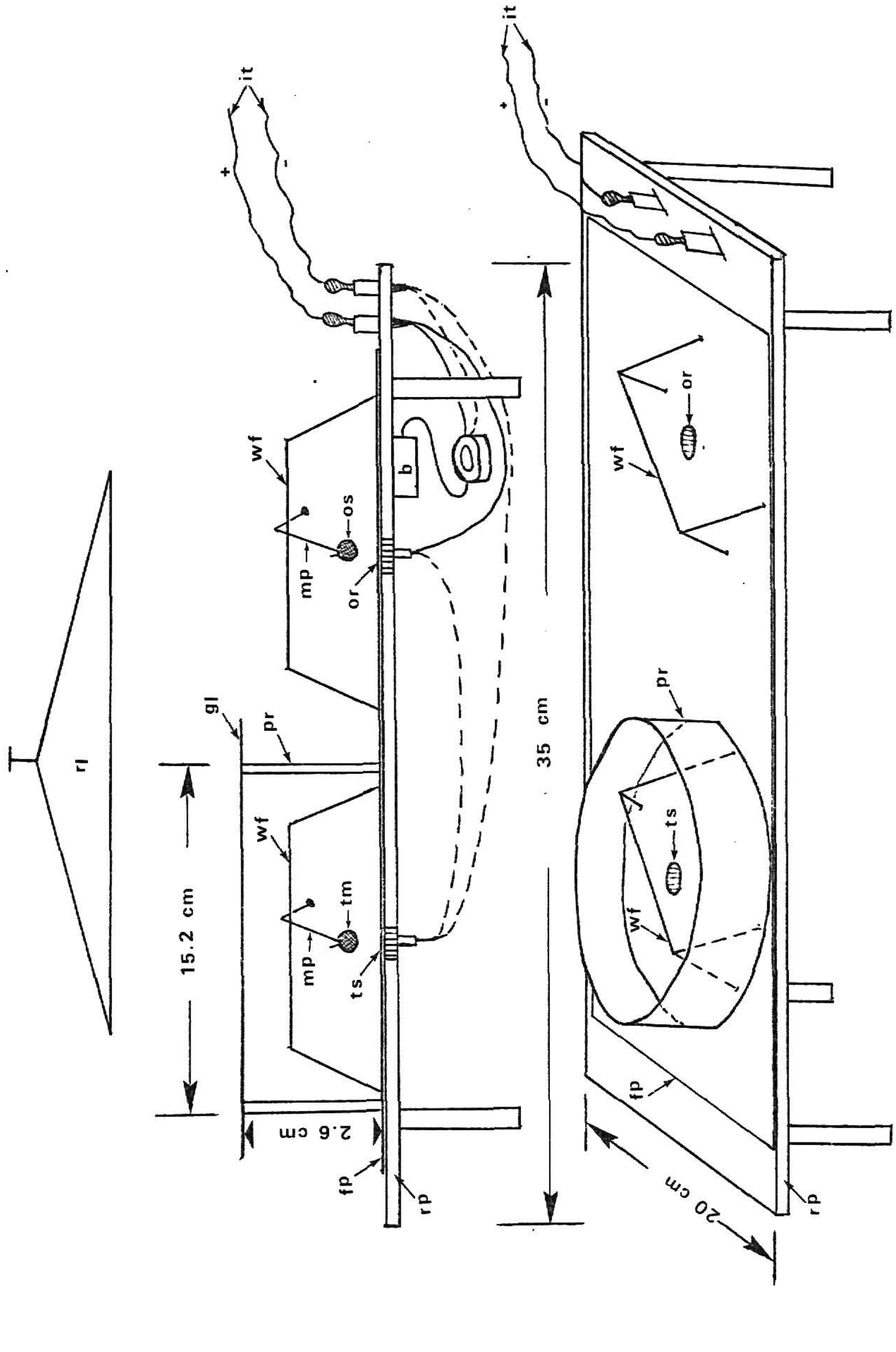
ts Test sensor

or Out-of-ring sensor

it Input terminals

rl Red light

b 1.5 volt battery



it
+
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15.2 cm
35 cm
tm
ts
2.6 cm
fp
rp
20 cm

ring 15.2 cm diam. and 2.6 cm high placed on a filter paper that covered the "floor" of the arena including the light dependent resistor (sensor); the arena was covered with a glass lid. The ring encircled the test sensor while the other sensor, also covered with filter paper, was outside the test area. A red light (15-watt incandescent light bulb behind a red Kodak Safelight Glass Filter, Series IA) suspended over the test equipment was adjusted so that the light intensity was the same on each sensor. The recorder pen was then at the zero point. It has been shown that the movements of RFB are not affected by red light (Loschiavo, 1959). During tests, as an insect passed onto the sensor in the arena, the light intensity imbalance between the sensors caused the recorder pen to leave the zero point and remain thus until the insect moved off the sensor. The chart paper of the recorder was moving at 1 inch per minute, which permitted calculation of the time each insect(s) spent on the sensor. All tests were conducted in a darkroom in complete darkness except for the red light.

Insect pins, 4 cm in length, were used to suspend the test and control materials from a wire frame (13.6 x 2 cm) directly above the sensor (Fig. 1), but high enough that beetles could not touch the test materials with their antennae. The pointed end of each pin was hooked and the center bent at about 60° to hang on a wire frame directly above the sensor. The hook at the pointed end was to hang a small cotton ball containing either bagged or impregnated test substance or control object. In some instances, pins without hooks were used to suspend whole kernels, such as of wheat, by inserting the pin point into the kernel crease.

Effect of Age of Adult Red Flour Beetles
on Response to Wheat Volatiles

Adult red flour beetles (RFB) of different ages and sexes were exposed to suspended wheat kernels in the test arena. Within each age-group, males and females were exposed separately, or mixed. The hard red winter wheat kernels, collected at harvest in June 1979 were stored in a sealed container in a freezer at -17°C. Prior to tests, a few kernels were moved into the rearing room at 27$\pm$1°C and 67$\pm$3%RH for at least one week for equilibration. The moisture content was not measured, but equilibrium to about 13.5% was expected.

Ten one-day-starved adult RFB of the same sex and age were placed in the test arena (Fig. 1) and left under the red light for at least 1 hour to adjust to the conditions before testing. A styrofoam pellet (control), similar in shape and size to a wheat kernel was carefully suspended with a modified insect pin (Fig. 1) above the sensor in the arena, and 3 successive, 10-min. periods of control observations were done. The styrofoam control pellet was carefully replaced by a whole wheat kernel with the pin inserted into the crease, and 3 more 10-min. observation periods were done. The number of times that insects crossed the sensor, and the time spent on it, were detected by the light-sensitive event detector and were recorded on the chart of the Servo Recorder. Ages ranging from 0-24 hr to 144-168 hr were tested, and within each age-group, virgin males, virgin females, or a mixture of both sexes were tested.

Attractiveness of Wheat, Its Fractions and Its Solvent Extracts to Red Flour Beetles

Whole Wheat Kernels and Their Fractions

The attractiveness of whole wheat kernels, whole wheat flour or wheat fractions (germ, bran, endosperm) to groups of 10 each of 72-96 hr old males, females or mixed sexes of RFB was tested using the light-sensitive event detector. These test materials, in closed plastic boxes were moved from the freezer (-17°C) into the rearing room (27±1°C, 67±3%RH) for about 24 hr equilibration prior to their use. A wheat kernel was suspended above the sensor on a modified insect pin inserted into the crease and suspended from a wire frame. Whole wheat flour and wheat fractions were enclosed in a small sack made of cotton stretched into a sheet, then suspended on the hooked point of the pin (Fig. 1). For whole wheat kernel tests, a styrofoam pellet, similar to a kernel in size and shape was used for the control test; for sacked whole wheat flour or for fractions, a small empty cotton ball was used as the control.

Solvent Extracts of Whole Wheat Kernels and Their Fractions

The response of the RFB to volatiles from extracts of whole wheat kernels, whole wheat flour, and wheat fractions was also tested. Two sets of experiments were designed using extracts of wheat; one set included only an extract of hard red winter wheat kernels (harvested in June, 1979) and was tested with males, females and a mixed group of RFB. The other set included another group of whole wheat kernels, whole wheat flour, and wheat fractions, all from the same lot of wheat of unknown source and age. These extracts were tested only with female

RFB since females proved to be the most responsive sex-group to wheat volatile materials in earlier experiments (Fig. 4 and 5), and it seemed unproductive to test males and the mixed-sex group. The extracts were prepared by placing the kernels in a funnel lined with glass wool and supported above a flask; and 250 ml of solvent (freshly opened diethyl ether) were poured directly on the kernels in 3 portions, so that the kernels were completely submerged with each portion of solvent. With whole wheat flour and wheat fractions, a 7-cm Buchner funnel with Whatman No. 1 filter paper was used. In the first set, 100 g of whole wheat kernels were extracted and concentrated by a rotary vacuum evaporator to a relatively small amount which was transferred into a volumetric tube and further concentrated to 1 ml under a flow of nitrogen gas directly on the extract and while hot air flowed around the tube. In the second set, 50 g of whole wheat kernels, whole wheat flour, or wheat fractions were extracted and concentrated to 2 ml by the above procedure. A capillary tube was used to impregnate small cotton balls with 1 µl, 5 µl, 10 µl or 15 µl of extract of wheat kernels of the first set, equivalent to 0.1 g, 0.5 g, 1.0 g and 1.5 g of grain; and 4 µl, 20 µl or 40 µl of each extract of the second set of tests which were also equivalent to 0.1 g, 0.5 g and 1.0 g of grain, flour or fraction, respectively. The solvent was allowed to evaporate from the impregnated cotton balls before they were tested and each concentration was tested separately. A cotton ball impregnated with solvent, then dried, was used as a control for each test. Each test was repeated at least 3 times.

Responses of the Red Flour Beetles to Fungal-Infected Wheat and Its Extracts and to *Aspergillus glaucus* and Its Extracts

Fungal-Infected Wheat Kernels

The attractiveness of fungal-infected wheat kernels to 72-96-hr-old males, females or a mixed group of RFB was tested using the light-sensitive event detector. The growth and development of the internal and/or external fungi was initiated by placing the wheat kernels in a potassium chloride humidity chamber in which 84.26% RH (=18% M.C.) was achieved, and they were kept there until fungal mycelia were visible on the kernels. An infected kernel was suspended on an insect pin above the test sensor (Fig. 1), and its attractancy to the 3 groups of the RFB was tested. A fungal-free wheat kernel for a control test was used, and each test was repeated at least 3 times.

Extract of Fungal-Infected Wheat Kernels

The response of the 3 groups of the RFB to volatiles from the extract of fungal-infected wheat kernels was also tested. The extract was prepared by the same procedures described before; however, with fungal-infected wheat, 50 g of the kernels were extracted with 125 ml of solvent and a rotary vacuum evaporator and a nitrogen gas were used to concentrate the extract to 2 ml. A capillary tube was used to impregnate small cotton balls with 4 μl, 20 μl or 40 μl of extract, equivalent to 0.1 g, 0.5 g and 1.0 g of grain, respectively. The solvent was allowed to evaporate from the impregnated cotton balls before they were tested and each concentration was tested with the 3 sex groups separately. A cotton ball impregnated with the solvent and dried, was used as a control for each test and each test was replicated at least 3 times.

Extract of *Aspergillus glaucus* Mycelium

The response of the 72-96-hr-old males, females and a mixed group of RFB to volatiles from a solvent extract of *A. glaucus* mycelium with spores or with no spores was tested. Wheat kernels were surface disinfected by shaking in a small amount of sodium hypochlorite (Chlorox) for 2 minutes and then rinsed with sterile, autoclaved water at least 3 times. These kernels were placed on malt, 4% salt agar medium and incubated in the rearing room for one week. The wheat was found to be invaded by *A. glaucus*. Spores were transferred into a liquid medium of Potato Dexteose (Potato Infusion 200 g and Bacto-Dextrose 20 g) and kept in the rearing room for growth and development. After an incubation period the fungal mycelia were separated from the medium by filtration in a funnel lined with a facial tissue on top of a coarsely-woven cloth. The fungal material was washed several times with sterile, autoclaved water, peeled off the facial tissue paper and dried overnight under a fume hood. A mortar and pestle were used to grind the fungal material and a Buchner funnel (7 cm) lined with Whatman No. 1 filter paper was used for extraction. Two sets of extracts were prepared; one set was from 0.5 g of A. glaucus mycelium with spores extracted with 30 ml of solvent and the other from a 3.3 g of A. glaucus mycelium with no spores extracted with 198 ml of solvent; both were concentrated to 2 ml by a rotary vacuum evaporator and a flow of nitrogen gas. A capillary tube was used to impregnate small cotton balls with 1 µl, 5 µl or 10 µl of extract of mycelium with no spores, equivalent to 0.0016 g, 0.0083 g and 0.0165 g of fungal material. To provide the same gram-equivalents of the extract of mycelium with spores, 7 µl, 33 µl or 66 µl were used in the tests. The solvent was evaporated from the impregnated cotton balls before testing. A solvent-impregnated cotton ball was used as a control test for testing an extract of A. glaucus mycelium with no spores, and a cotton ball impregnated with an extract of A. glaucus mycelium with no spores was used as a control test for the extract of A. glaucus mycelium with spores. Each concentration was tested with the 3 sex groups separately, and each test was repeated at least 3 times.

Live Culture of Aspergillus glaucus

The response of the 72-96-hr-old female RFB to volatiles from live A. glaucus mycelium was also tested. A. glaucus spores were transplanted into a small sterile cotton ball impregnated with autoclaved agar medium and were incubated in the rearing room for development. When the cotton ball was covered with mycelium, it was suspended over the sensor and the attractancy tested using the female RFB. A sterile, dry cotton ball, a wet cotton ball and a cotton ball impregnated with sterile agar medium were tested as controls, and each test was replicated at least 3 times.

Responses of the 72-96-hr-old Female Red Flour Beetles to Whole Wheat Flour and to Wheat Fractions in Free-Choice Tests

In this study, modifications were made in the method described by Gibson and Raina (1972), in fabricating free-choice chambers. A sheet of styrofoam about 2.5 cm thick, cut into a circular disc with a 25 cm diam. upper surface and 24 cm diam. lower surface, so as to fit firmly in the bottom of a cylindrical plastic container. On the disc's upper surface, 5 square cavities (2 x 2 x 2.5 cm) were cut equidistant from the center and from one another. The disc was covered with filter paper with an X-cut above each cavity so that small plastic boxes (2 x 2 x 2 cm) could be inserted into the cavities. The tops of the boxes were even with the styrofoam surface. The lid of each plastic box had a 1 cm diam. hole covered with wire screen. Either whole wheat flour, wheat germ, wheat bran or wheat endosperm was placed in each box and the 5th was an empty control box. Two chambers were used, and the 5 boxes for one replicate (4 with test material, 1 without) were placed randomly in the cavities of one chamber. The 5 boxes of the second replicate were placed so that test materials adjacent to each other in the first chamber were not adjacent in the second chamber (Fig. 2).

Forty, 72-96-hr-old, one-day starved female RFB were used to test the relative attractiveness of the wheat fractions. The beetles were

Fig. 2. An schematic diagram illustrating the test arena in the free-choice test.

A Center of test arena (insects released here from under an inverted vial, 2.5 cm)

C Control box (empty)

WG Box containing wheat germ

WE Box containing wheat endosperm

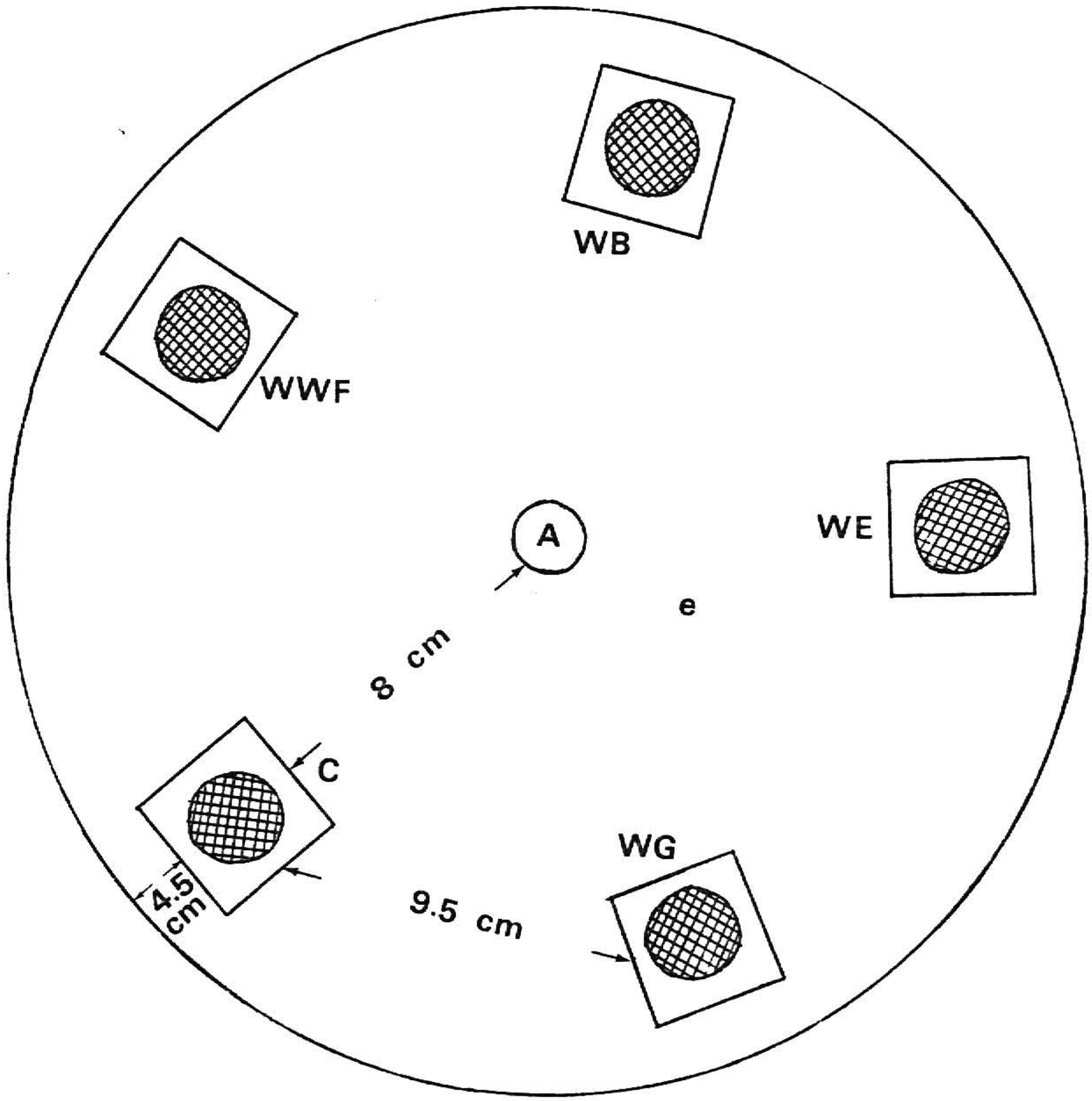
WB Box containing wheat bran

WWF Box containing wheat whole flour

Note. Order of placement.

Chamber I. C, WG, WE, WB, WWF

Chamber 2. C, WE, WWF, WG, WB


WB
WWF
A
WE
e
8 cm
C
4.5 cm
9.5 cm
WG

released at the center of the test arena by lifting an inverted glass vial under which the beetles were acclimated. The plastic chamber was covered with a lid fitted to the center with a glass window to allow viewing the inside of the chamber. Observations were made under red light. The number of beetles on each screen was counted at 10-min. intervals for 20 times in both test chambers.

Attractiveness of Millet and Millet Products, as Prepared for Food in the Sudan, to Red Flour Beetles

Whole Millet Kernels and Millet Products

Millet is consumed in various forms in parts of the Sudan and is usually prepared in the following way:

Whole millet kernels ----- whole millet flour ----- consumed

↓

Dampened with water

↓

Pounded with a mortar and pestle (wooden) to loosen the bran (pericarp)

↓

Sun-dried ----- winnowed ----- bran (fowl feed)

↓

Fermented (soaked in abundant water for 3-5 days or until the water turns deep yellow with bubbles and a sour acidic taste and odor)

↓

Sun-dried ----- winnowed ----- remaining bran, damaged kernels
and grain dust (fowl feed)

↓

Bran-free fermented kernels

↓

Ground to fine flour ----- consumed

↓

Mixed with water, stirred, and allowed to settle for 2-3 days

↓

Water discarded and starch sediment collected

↓

Sun-dried ----- consumed

For attractancy testing, millet (harvested, 1976) was processed in the laboratory as nearly as possible as above and 4 forms; whole millet kernels, whole millet flour, bran-free fermented millet flour and millet starch were selected. The test procedures were similar to those for whole wheat kernels and wheat fractions. However, millet kernels (3 kernels) were sacked in a small cotton ball and suspended on a hooked pin point, and for all, a small empty cotton ball was used as the control.

Solvent Extracts of Whole Millet Kernels

The extraction procedure was similar to that for whole wheat kernels, and the extract was tested using all 3 sex groups of 72-96-hr-old RFB. Small cotton balls were impregnated with 1 µl, 5 µl, 10 µl or 15 µl of extracts of millet kernels, equivalent to 0.1 g, 0.5 g, 1.0 g and 1.5 g of grain, respectively. A cotton ball impregnated only with solvent and then dried was used as a control for each test, and each test was replicated at least 3 times.

RESULTS

Effect of Beetle Age on Response to Food Volatiles

Within each sex group, the response, measured in total number of visits to the control object (styrofoam pellet) was generally less than that to the whole wheat kernel (Fig. 3a), and the total time spent on the sensor below the control was less than that below the test material (Fig. 3b). However, male and female beetles 0-24 hr old made more visits to the control than to the test material (Fig. 3a). The mixed-sex group beetles 0-24 hr old spent no time at the control while those 24-48 hr old spent more time at the control than at the test material (Fig. 3b). The analysis of variance showed no significant differences at the 5% level among the 3 sex groups; however, significant differences were found between the controls and the test materials for both the mean number of visits and mean time spent ($p = 0.05$). The response of the adult beetles to whole wheat kernel volatiles, measured in total number of visits to the kernel during three 10-min. periods, increased with an increase of age (Fig. 3a); however, their response measured in total time spent on the sensor during three 10-min. periods, increased as age increased to 72-96 hr, then decreased (Fig. 3b). The maximum time spent on the sensor under the test material (whole wheat kernel) was by beetles at 72-96 hr of age, and beetles at this age were considered to be the most responsive of those tested, to volatile food odors and were used in subsequent studies.

Fig. 3a. Responses of the different age groups of adult red flour beetles, *Tribolium castaneum*, to volatiles of whole wheat kernels, as measured by the total number of visits to the sensor below a suspended wheat kernel in the test arena. (Total of three, 10-min. replications of 10 insects each for wheat kernels or for control.)

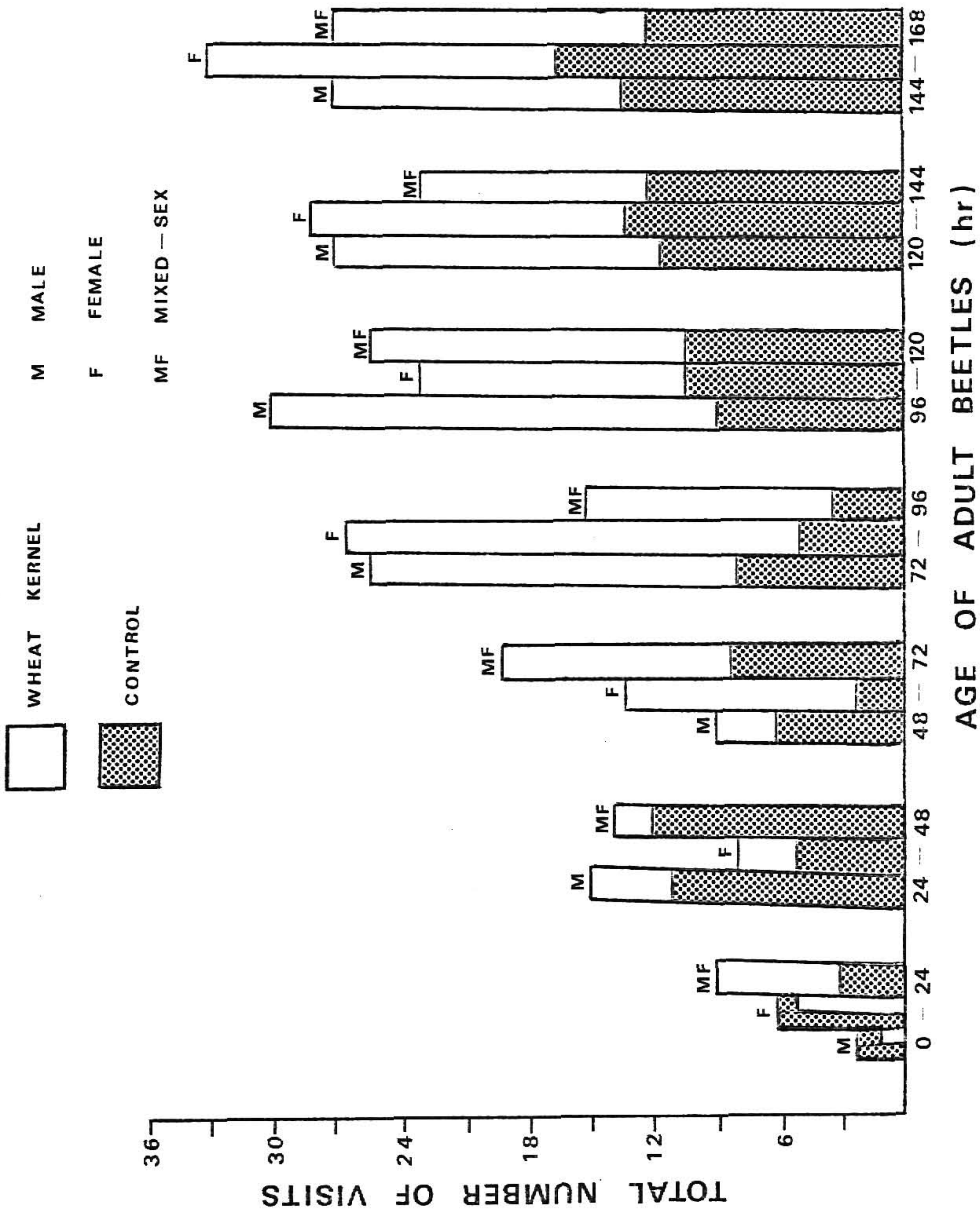
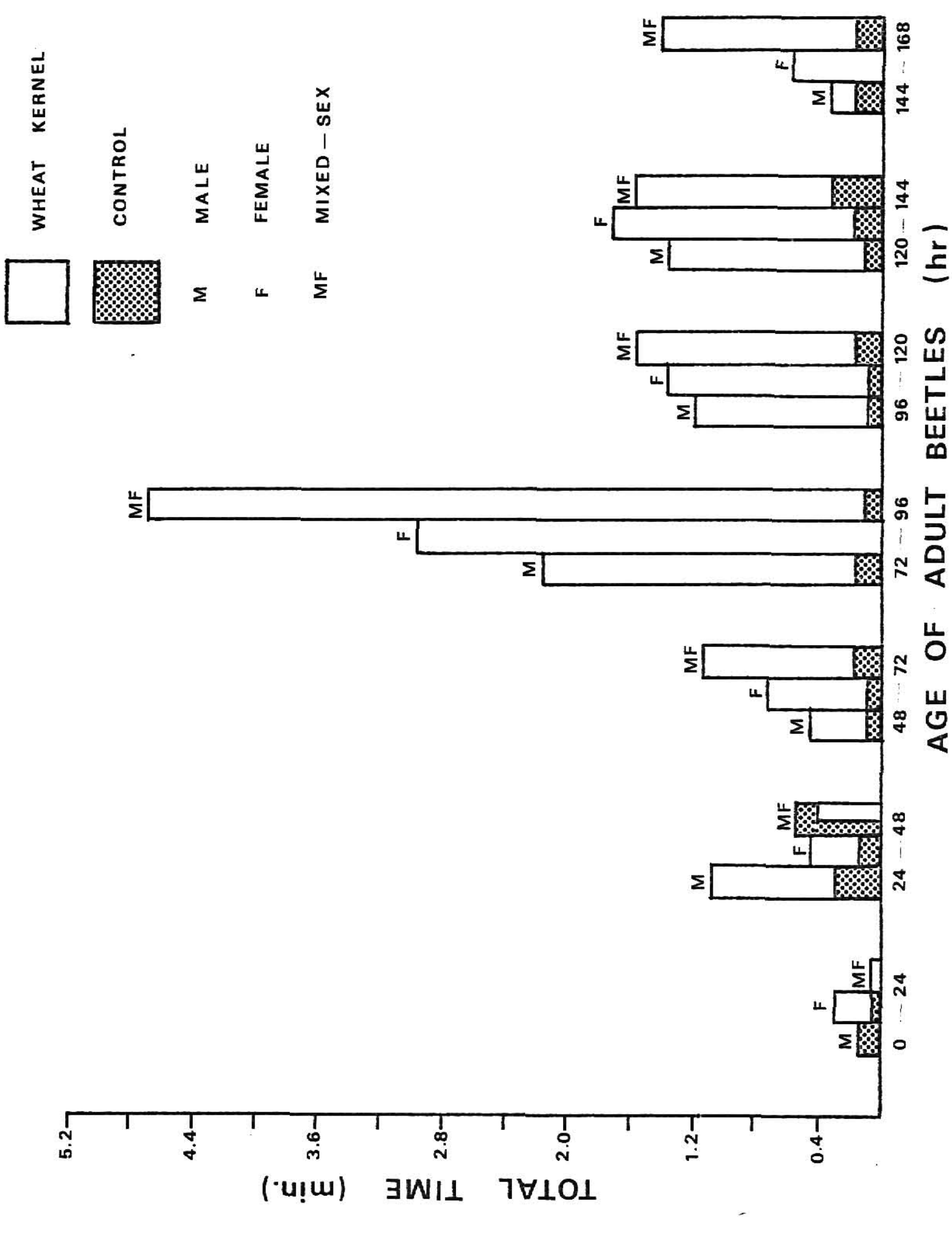

WHEAT KERNEL
CONTROL
M MALE
F FEMALE
MF MIXED — SEX
TOTAL NUMBER OF VISITS
36
30
24
18
12
6
M
F
MF
0 — 24
24 — 48
48 — 72
72 — 96
96 — 120
120 — 144
144 — 168
AGE OF ADULT BEETLES (hr)

Fig. 3b. Responses of the different age groups of adult red flour beetles, *Tribolium castaneum*, to volatiles of whole wheat kernels as measured by the total time spent (min.) on the sensor below a suspended wheat kernel in the test arena. (Total of three, 10-min. replications of 10 insects each for wheat kernel or for control.)


WHEAT KERNEL
CONTROL
M MALE
F FEMALE
MF MIXED — SEX
TOTAL TIME (min.)
0.4
1.2
2.0
2.8
3.6
4.4
5.2
AGE OF ADULT BEETLES (hr)
0 — 24
24 — 48
48 — 72
72 — 96
96 — 120
120 — 144
144 — 168
M
F
MF

Attractiveness of Volatiles of Whole Wheat Kernels and Wheat Fractions

Highly significant differences (5% level) were found in the responses of the 3 sex groups (males, females, mixed), considered together, to the volatiles between controls and test materials (whole wheat kernel, whole wheat flour, wheat germ, wheat bran, and wheat endosperm) (Table 1). The number of visits to, and the time spent on the sensor below the control objects, was much less than for the test substances. Females were more responsive than the other two groups to the volatiles of the wheat kernel and of the fractions as determined by the total number of visits and total time spent on the sensor below the test materials (Fig. 4). Males made more visits to whole wheat kernels, wheat germ or wheat endosperm than to whole wheat flour or wheat bran. They spent more time at wheat endosperm than did the mixed-sex group, which made more visits to whole wheat flour or wheat bran and fewer to whole wheat kernels, wheat germ or wheat endosperm. They spent more time at whole wheat kernels or wheat bran (Fig. 4). The difference in the mean number of visits to the whole wheat kernel or wheat fractions between the female group and the other two sex groups (males and mixed) was significant at the 5% level. The difference in the mean time spent on the sensor under whole kernel or wheat fractions between the female and the male groups and between the male and the mixed-sex groups was not significant ($P = 0.05$) (Table 1). The most visits made by the 3 sex groups, considered together, were to wheat germ followed by wheat endosperm, and the longest time spent was at whole wheat flour followed by whole wheat kernel (Fig. 4).

Table 1. Duncan's multiple range test for mean number of visits and mean time spent for the 3 sex groups of 72-96-hr-old red flour beetles (mean for all fractions) and for whole wheat kernels or wheat fractions (mean for all sex groups).

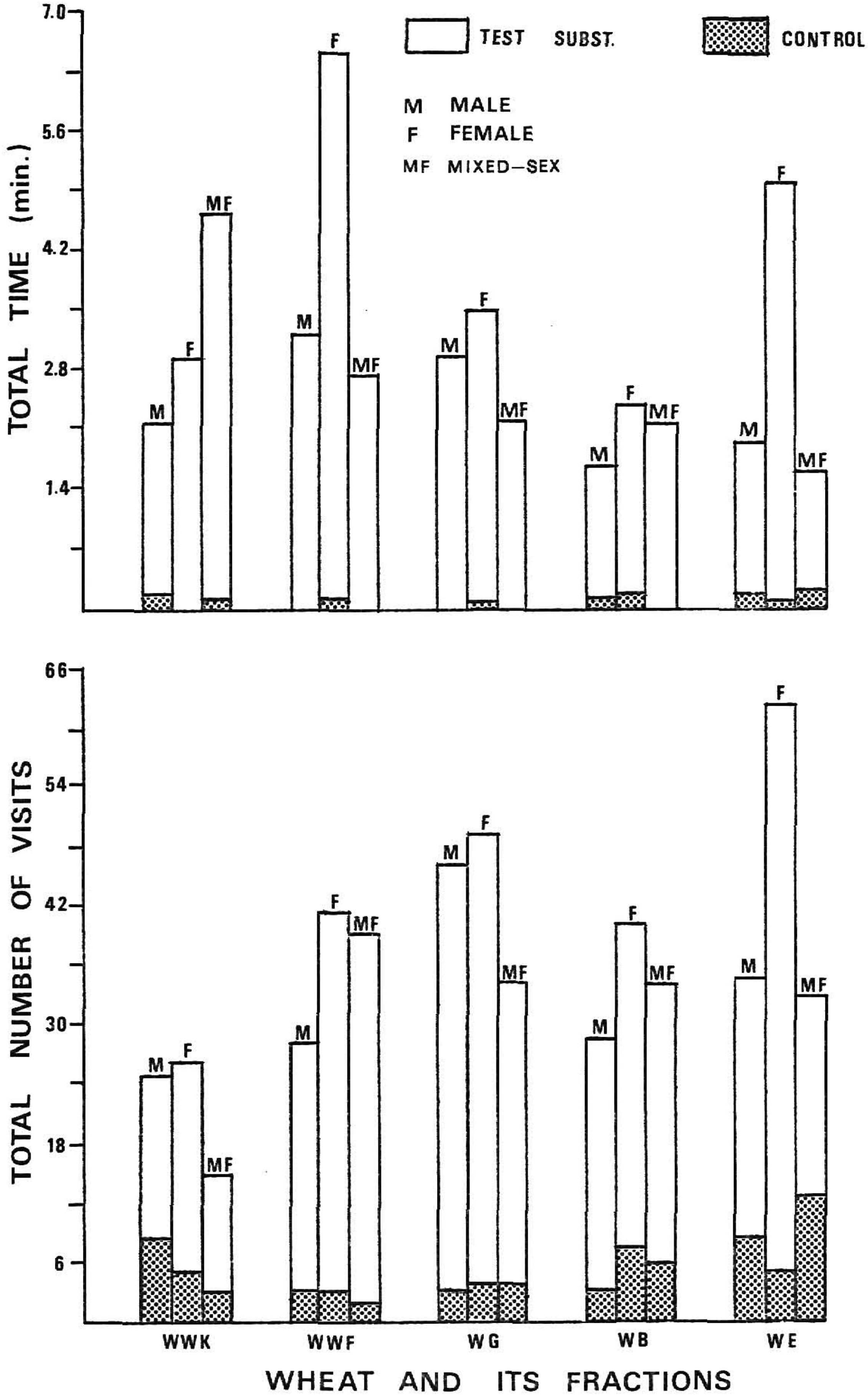
	Mean number of visits[1]	Mean time spent (min.)[1]
A. All fractions		
Females	8.033 a	0.687 a
Males	6.233 b	0.457 ab
Mixed	6.000 b	0.404 b
B. All sex groups		
Whole wheat kernel	7.333 c	1.091 ab
Whole wheat flour	12.000 ab	1.374 a
Wheat germ	14.333 a	0.957 ab
Wheat bran	11.444 b	0.689 b
Wheat endosperm	14.444 a	0.949 ab
Control	1.600 d	0.020 c

[1]Means followed by the same letter are not significantly different at the 5% level.

Fig. 4. Attraction responses of male, female, or mixed-sex groups of 72-96-hr-old red flour beetles to whole wheat kernels (WWK), whole wheat flour (WWF) or wheat fractions /germ (WG), bran (WB), endosperm (WE)/. (Three 10-min. reps.)

Upper: Total time spent on sensor under test or control materials.

Lower: Total number of visits to sensor under test or control materials.


TEST SUBST.
CONTROL
M MALE
F FEMALE
MF MIXED-SEX
TOTAL TIME (min.)
7.0
5.6
4.2
2.8
1.4
TOTAL NUMBER OF VISITS
66
54
42
30
18
6
WWK
WWF
WG
WB
WE
WHEAT AND ITS FRACTIONS

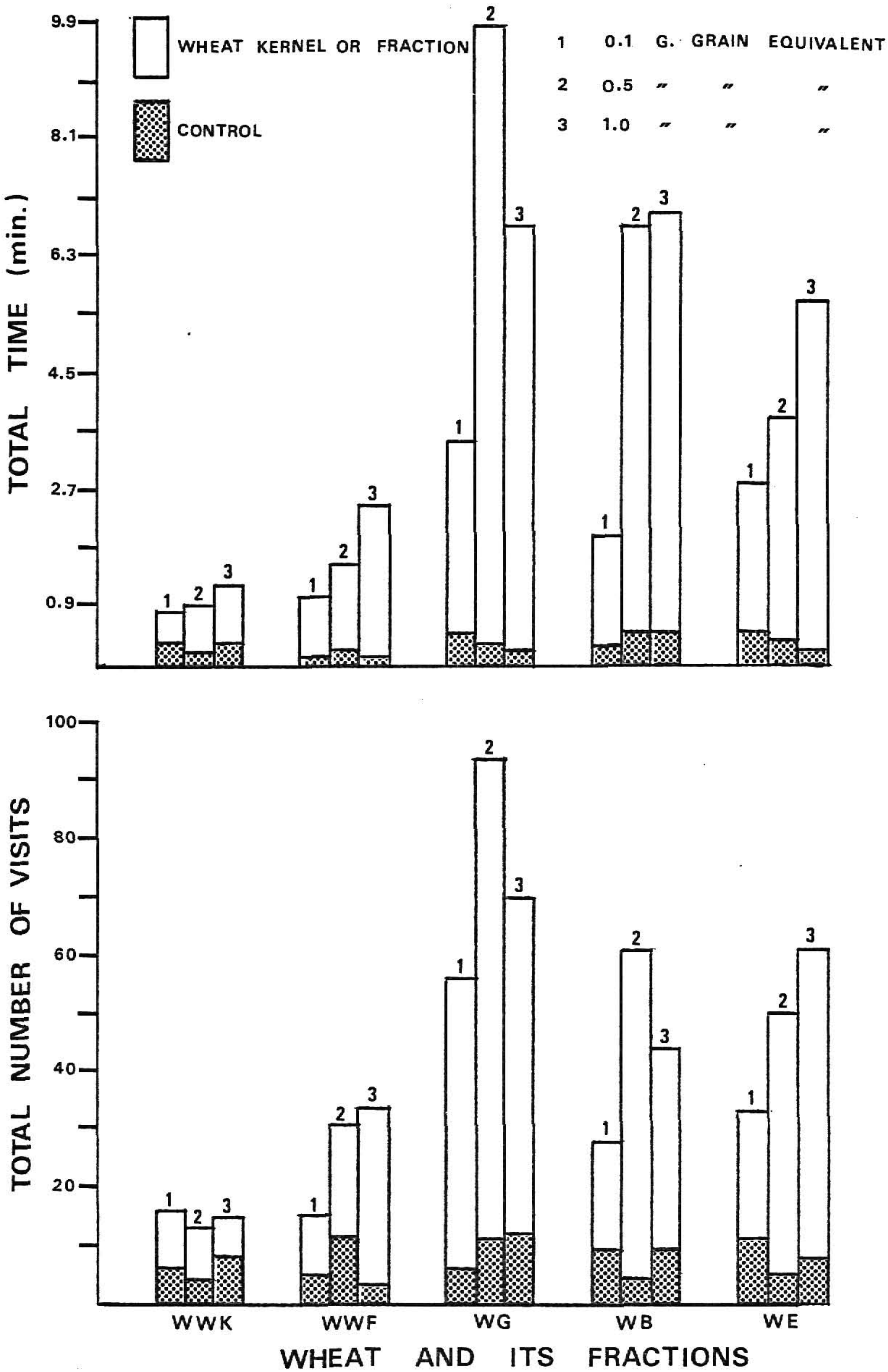
Attractiveness of Volatiles from Extracts of Whole Wheat Kernels and Wheat Fractions

The responses of the 3 sex groups of the adult RFB, taken together, to the volatiles of extracts of the hard red winter wheat kernels were erratic and low in magnitude (Appendix Table 3) compared with the response of the female group to the extracts of the same-lot, unknown-age whole wheat kernels, its whole wheat flour or its fractions (Fig. 5). Highly significant differences were found between responses to control and test substances (P = 0.05). The significant differences were also noticed among the different extract concentrations (P = 0.05), measured by the mean number of visits and mean time spent on the sensor below the test materials. Each of the 3 sex groups made more visits to and spent more time at the 0.5 g grain equivalent concentration of extract of the hard red winter wheat. The least attraction was to 0.1 g and an intermediate attraction was noticed for 1.0 g and 1.5 g grain equivalent of extract concentrations (Appendix Table 3). The female beetles responded with more visits and more time spent at extracts of wheat germ, wheat bran, or wheat endosperm, than at extracts of whole wheat kernels or of whole wheat flour (Fig. 5), and compared with the responses of any of the 3 sex geoups to hard red winter wheat kernel extracts. Significant differences (P = 0.05) in RFB responses were found between controls and test materials (3 extract concentrations). Among the wheat fractions, wheat germ was significantly more attractive than the rest and among the concentrations within each fraction, 0.5 g and 1.0 g grain equivalent concentrations were significantly different

Fig. 5. Attraction responses of 72-96-hr-old female red flour beetles to 3 extract concentrations of whole wheat kernels (WWK), whole wheat flour (WWF) or wheat fractions /germ (WG), bran (WB), endosperm (WE)/. (Three 10-min. reps.)

Upper: Total time spent (min.) on sensor under controls or test materials.

Lower: Total number of visits to sensor under controls or test materials.


WHEAT KERNEL OR FRACTION
CONTROL
1 0.1 G. GRAIN EQUIVALENT
2 0.5 " " "
3 1.0 " " "
TOTAL TIME (min.)
9.9
8.1
6.3
4.5
2.7
0.9
TOTAL NUMBER OF VISITS
100
80
60
40
20
WWK
WWF
WG
WB
WE
WHEAT AND ITS FRACTIONS

from 0.1 g grain equivalent concentration (P = 0.05) (Table 2). Wheat germ extract elicited a greater response from the female beetles, followed by wheat bran and whole wheat kernels (Fig. 5). Responses due to 0.5 g and 1.0 g grain equivalent concentrations were not significantly different (P = 0.05); however, the response to 0.1 g grain equivalent concentration was significantly different from the other two. The response to 0.5 g grain equivalent concentration was greater for wheat germ or wheat bran but 1.0 g of extract was more attractive for wheat endosperm, whole wheat flour or whole wheat kernels (Fig. 5).

Attractiveness of Fungal-Infected Wheat Kernels and Their Extracts

The analysis of variance for the mean number of visits and mean time spent by the 3 sex groups of RFB, considered together, indicated significant differences (P = 0.05) in responses to extract volatiles of fungal-free wheat kernels (control) and fungal-infected wheat kernels. The volatiles from fungal-infected wheat kernels elicited greater response in the number of visits and time spent than the volatiles from fungal-free wheat kernels, and females were significantly more responsive than males, or males and females together (Fig. 6). The response of males to the fungal-infected wheat kernels was not significantly different (P = 0.05) from that of the mixed-sex group. Females were also more responsive than males or mixed-sex group to extract concentrations of fungal-free or fungal-infected wheat kernels. The extract concentration of 1.0 g grain equivalent elicited

Table 2. Duncan's multiple range test for mean number of visits and mean time spent (min.) by the 72-96-hr-old female red flour beetles near the 3 concentrations of all fractions combined or near the whole wheat kernel or wheat fractions of all the concentrations combined.

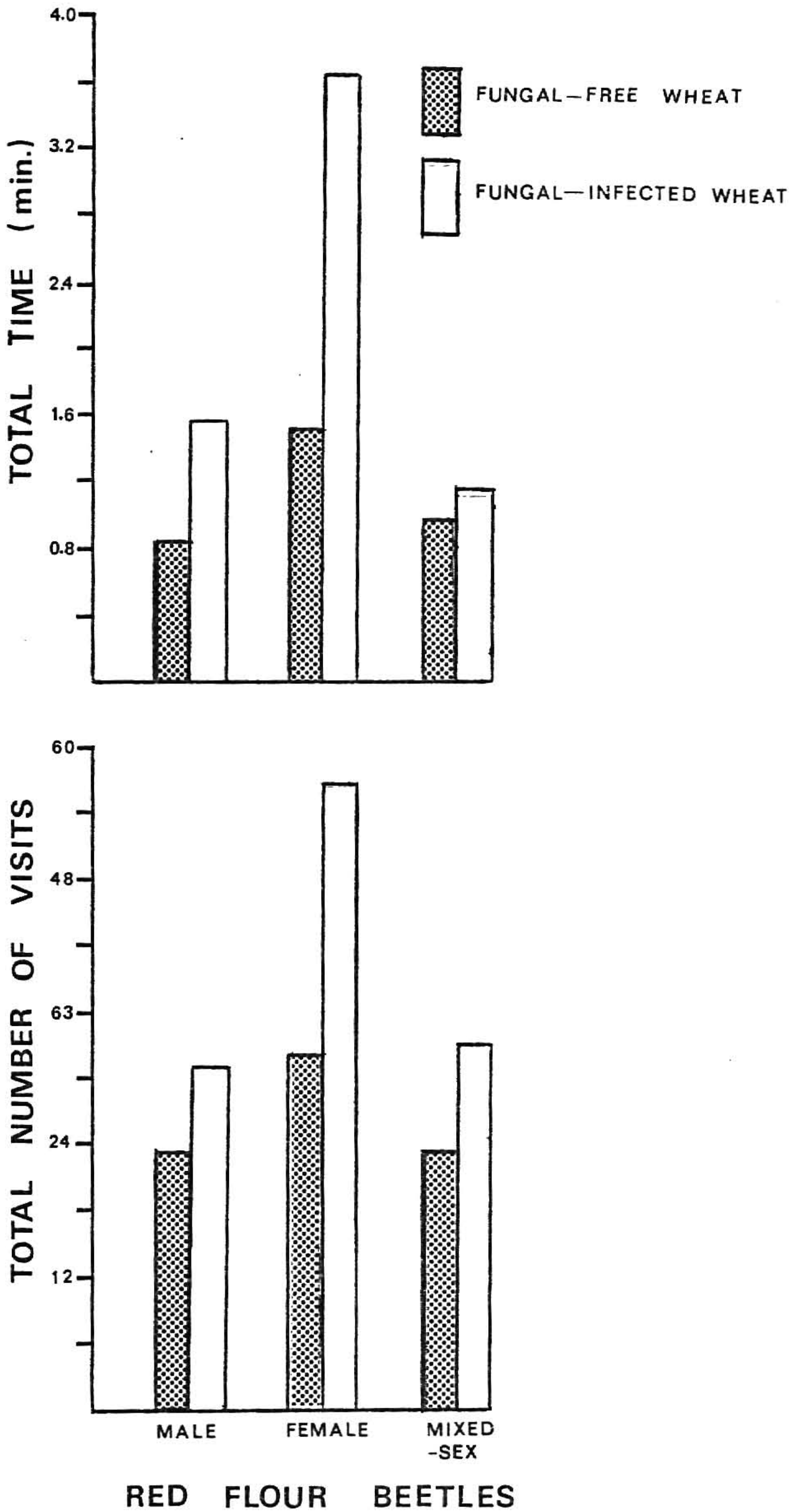
	Mean number of visits[1]	Mean time spent (min.)[1]
A. Extract concentration (g. equivalent)		
0.1	6.133 b	0.369 b
0.5	9.300 a	0.802 a
1.0	8.800 a	0.793 a
B. Fraction		
Whole wheat kernel	4.889 d	0.317 cd
Whole wheat flour	8.889 c	0.550 c
Wheat germ	24.444 a	2.247 a
Wheat bran	14.778 b	1.727 b
Wheat endosperm	16.000 b	1.334 b
Control	2.356 e	0.074 d

[1]Means followed by the same letter are not significantly different at the 5% level.

Fig. 6. Attraction responses of male, female, or mixed-sex groups of 72-96-hr-old red flour beetles to fungal-free or fungal-infected wheat kernels. (Three 10-min. replications.)

Upper: Total time spent on sensor under kernels.

Lower: Total number of visits to sensor under kernels.


FUNGAL—FREE WHEAT
FUNGAL—INFECTED WHEAT
TOTAL TIME (min.)
4.0
3.2
2.4
1.6
0.8
TOTAL NUMBER OF VISITS
60
48
63
24
12
MALE
FEMALE
MIXED -SEX
RED FLOUR BEETLES

greater responses than 0.1 g or 0.5 g grain equivalents, especially from females or mixed-sex groups (Fig. 7). However, Duncan's multiple range test for means separation indicated no significant differences (P = 0.05) among the 3 concentrations in the mean number of visits or mean time spent on the sensor, but a significant difference was found between the responses of the beetles to the extracts of fungal-infected wheat and the extracts of fungal-free wheat (P = 0.05).

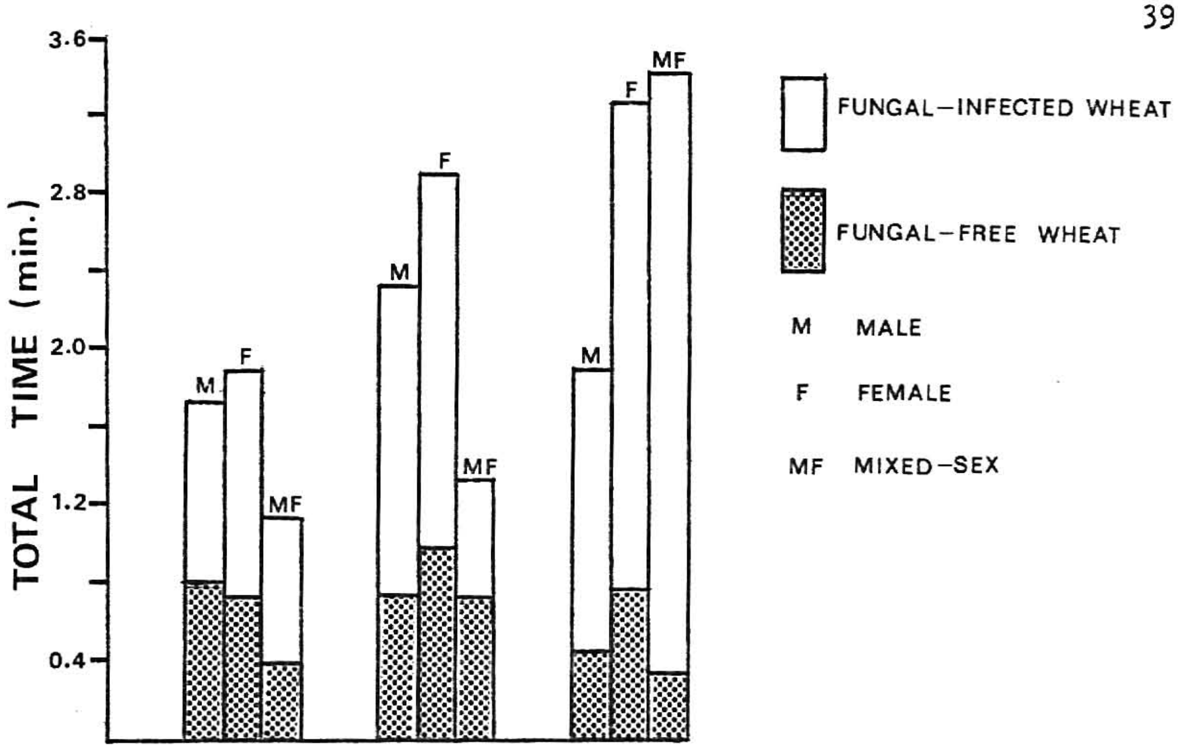
Responses to *Aspergillus glaucus* Mycelium Extracts

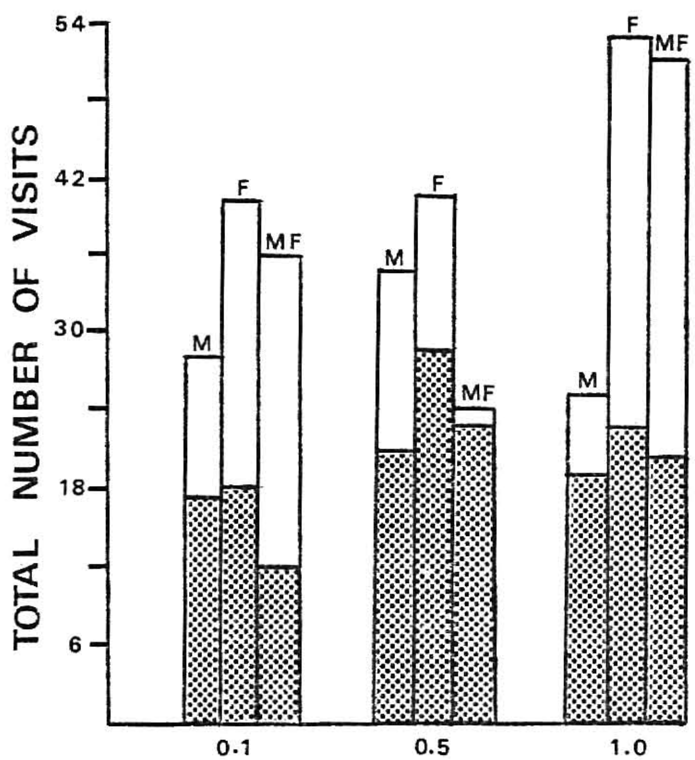
The 3 extract concentrations of *A. glaucus* mycelium with no spores were more attractive to the female beetles than to males or mixed-sex groups. The attractiveness of the extracts to male or to mixed-sex groups was erratic, with slightly greater attractancy in the mixed-sex group (Appendix Table 8). Means separated by Duncan's multiple range test indicated significant differences between controls (solvent on cotton balls) and test materials (P = 0.05). Females were significantly (P = 0.05) more responsive than the males or mixed-sex groups, but there were no significant differences among the 3 extract concentrations as measured by the mean number of visits and mean time spent by RFB at the test materials (P = 0.05). Extracts of fungal mycelium with spores were significantly (P = 0.05) more attractive than extracts of mycelium with no spores, as determined by either the number of visits or the mean time spent on the sensor. The extract of *A. glaucus* mycelium with spores elicited greater responses than did the extract of mycelium with no spores, except that the 0.0016 g

Fig. 7. Attraction responses of male, female or mixed-sex groups of 72-96-hr-old red flour beetles to 3 extract concentrations of fungal-infected wheat kernels or fungal-free wheat kernels. (Three 10-min. reps.)

Upper: Total time spent on sensor under test or control materials.

Lower: Total number of visits to sensor under test or control materials.





EXTRACT CONC. OF FUNGAL-INFECTED AND FUNGAL-FREE WHEAT (G. GRAIN EQUIVALENT) mycelium equivalent treatment was less attractive than the control (Fig. 8). According to the total time spent by RFB on the sensor, the mixed-sex group was equally attracted to 0.0083 g mycelium equivalent extracts of mycelium with or without spores. The presence of spores increased the response of the RFB, indicating additional attractive volatile substances and/or different concentrations in the extract. The 3 RFB sex groups spent significantly (P = 0.05) more time at the 0.0083 g mycelium equivalent concentration than at the 0.0016 g, but the number of visits were not different.

Responses of Female Red Flour Beetles to Volatiles of Live *Aspergillus glaucus*

The 72-96-hr-old female RFB made significantly (P = 0.05) more visits and spent more time on the sensor under the live *A. glaucus* culture on a cotton ball than under the controls. An average of 23.00 visits were made by the beetles to the live *A. glaucus* culture, compared with an average of 13.33, 3.67 and 3.00 visits made to agar-in-cotton, wet-cotton or dry-cotton balls, respectively. An average of 1.41 min. were spent on the sensor below the live culture of *A. glaucus*, compared with an average of 0.97, 0.09 and 0.07 min. spent on the sensor below agar-in-cotton, wet-cotton and dry-cotton balls, respectively (Table 3).

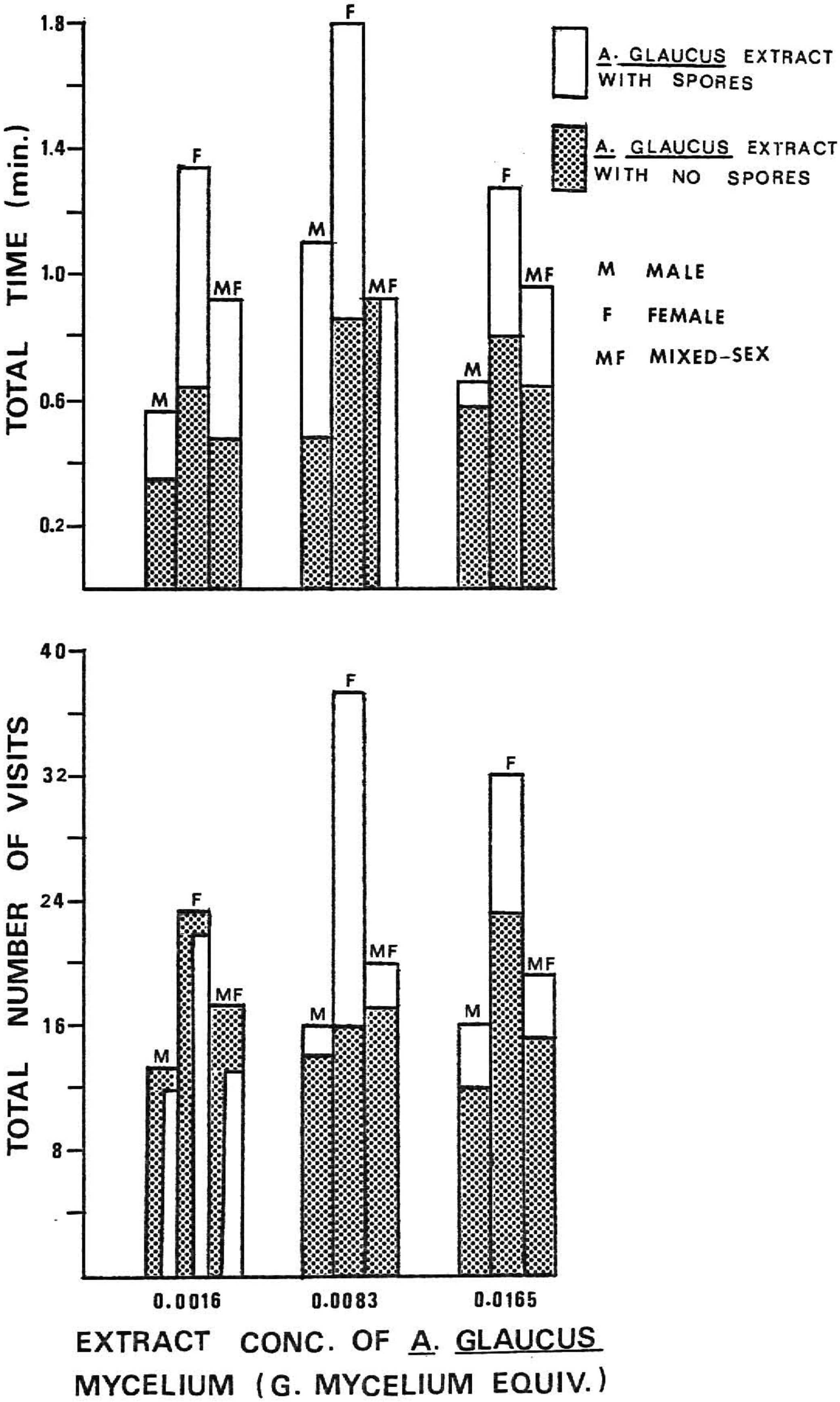
Responses of Female Red Flour Beetles to Volatiles of Wheat Fractions in Free-Choice Tests

Responses of 72-96-hr-old female RFB to various wheat fractions and controls differed significantly (P = 0.05). The differences

Fig. 8. Attraction responses of male, female or mixed-sex groups of 72-96-hr-old red flour beetles to 3 extract concentrations of *Aspergillus glaucus* mycelium with spores or without spores. (Three 10-min. reps.)

Upper: Total time spent on sensor under extracts.

Lower: Total number of visits to sensor under extracts.



TOTAL TIME (min.)
1.8
1.4
1.0
0.6
0.2
M
F
MF
A. GLAUCUS EXTRACT WITH SPORES
A. GLAUCUS EXTRACT WITH NO SPORES
M MALE
F FEMALE
MF MIXED-SEX
TOTAL NUMBER OF VISITS
40
32
24
16
8
0.0016
0.0083
0.0165
EXTRACT CONC. OF A. GLAUCUS MYCELIUM (G. MYCELIUM EQUIV.)

Table 3. Attraction responses in number of visits (V) and time spent (T, in min.) by 72-96-hr-old female red flour beetles under a suspended live culture of A. glaucus or "control" objects. (Three 10-min. reps.)

Test Subs.	Cotton Dry		Cotton Wet		Cotton + Agar		Cotton + Agar + A. glaucus	
	V	T	V	T	V	T	V	T
	3	.12	4	.06	9	.51	15	1.16
	1	.02	4	.22	18	1.31	31	1.63
	5	.06	3	.00	13	1.09	23	1.44
Total	9	.20	11	.28	40	2.91	69	4.23
Mean[1]	3.00	.07	3.67	.09	13.33	.97	23.00	1.41
	c_2	c_3	c_2	c_3	b_2	b_3	a_2	a_3

[1]Means (for V and T) followed by the same letter and number, respectively, are not significantly different at the 5% level.

among the fractions in the attractancy to the female beetles also were significant (P = 0.01). Whole wheat flour in both chambers elicited the greatest response from the beetles, followed by wheat germ, wheat endosperm, wheat bran and control. The response to each fraction in chamber 2 was slightly less, for unknown reason(s), than that to the same fraction in Chamber 1; these differences were not significant (P = 0.05) (Fig. 9). It is likely that the fractions were not "pure" due to less than perfect separation during the milling process.

Attractiveness of Millet, Its Fractions and Extracts

The solvent extracts of whole millet kernels were significantly more attractive at all concentrations than the controls (P = 0.05). The 1.0 g grain equivalent was the most attractive for all 3 sex groups considered together, followed by 0.5 g, 0.1 g and 1.5 g grain equivalents, respectively. Males were more responsive than the other groups to the 1.0 g grain equivalent concentration, females to the 0.5 g and the mixed-sex group to the 0.1 g. The volatiles of the 1.5 g grain equivalent concentration, in addition to being less attractive than the other 3 extract concentrations, induced more erratic responses; males made the most visits but the mixed-sex group spent more time, while the female group responded least (Fig. 10).

The difference between controls and the whole millet kernel or its processed products in the responses of the 3 sex groups was significant (P = 0.01). The volatiles of the processed millet products (as consumed in the Sudan) were more attractive to the RFB than those

Fig. 9. Attraction responses of 72-96-hr-old female red flour beetles to whole wheat flour (WWF), wheat germ (WG), wheat endosperm (WE), wheat bran (WB) and control (C). (Twenty 10-min. replications.)

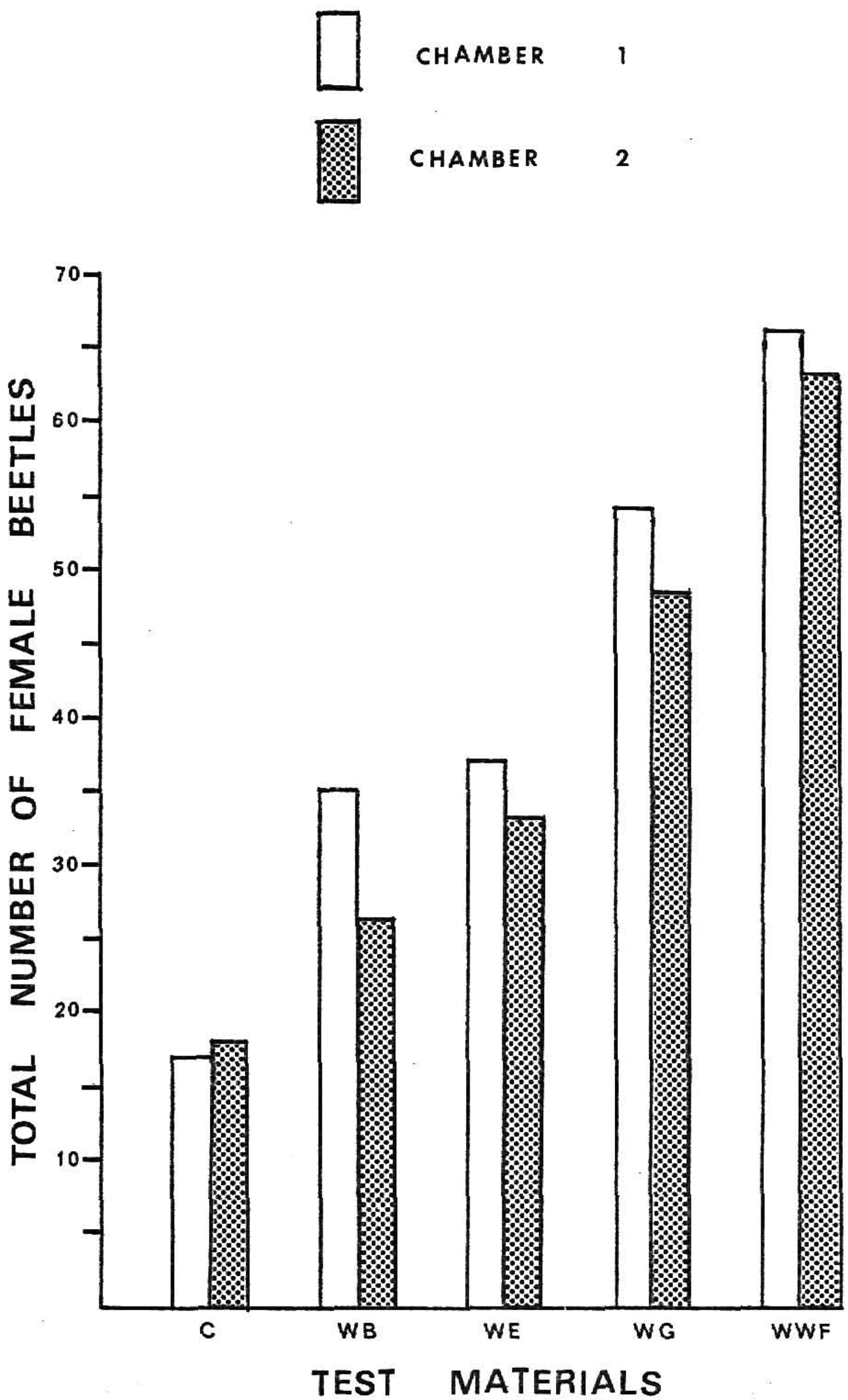
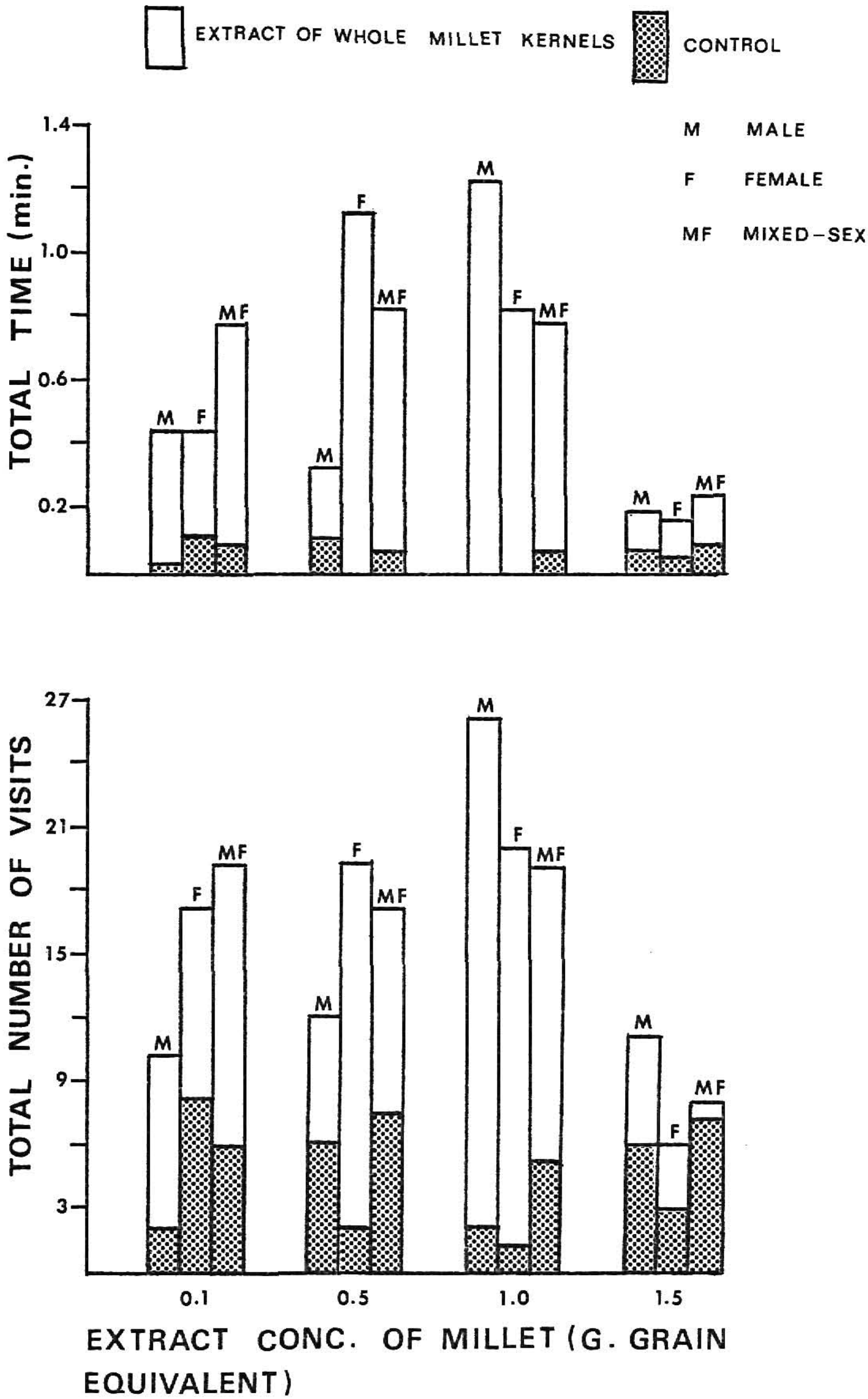

CHAMBER 1
CHAMBER 2
TOTAL NUMBER OF FEMALE BEETLES
70
60
50
40
30
20
10
C
WB
WE
WG
WWF
TEST MATERIALS

Fig. 10. Attraction responses of male, female, or mixed-sex groups of 72-96-hr-old red flour beetles to 4 extract concentrations of whole millet kernels. (Three 10-min. reps.)

Upper: Total time spent on sensor under controls or test materials.

Lower: Total number of visits to sensor under controls or test materials.



EXTRACT OF WHOLE MILLET KERNELS
CONTROL
M MALE
F FEMALE
MF MIXED-SEX
TOTAL TIME (min.)
1.4
1.0
0.6
0.2
TOTAL NUMBER OF VISITS
27
21
15
9
3
0.1
0.5
1.0
1.5
EXTRACT CONC. OF MILLET (G. GRAIN EQUIVALENT)

of the whole millet kernel extracts (Fig. 10). Volatiles of whole millet kernels or of millet starch elicited more responses from female beetles both in the number of visits and the time spent on the sensor, while those of whole millet flour or of bran-free fermented millet flour elicited more responses from the mixed-sex group, as measured by the number of visits only (Fig. 11). However, remale beetles spent more time on the sensor below the bran-free fermented millet flour than the other sex groups (Fig. 11). The greatest response for the 3 sex groups considered together, indicated by the total number of visits and total time spent, was for the bran-free fermented millet flour, followed by the whole millet flour. Millet starch elicited relatively more visits than whole millet kernels, and whole millet kernels elicited more time spent than millet starch from the 3 sex groups considered together (Fig. 11).

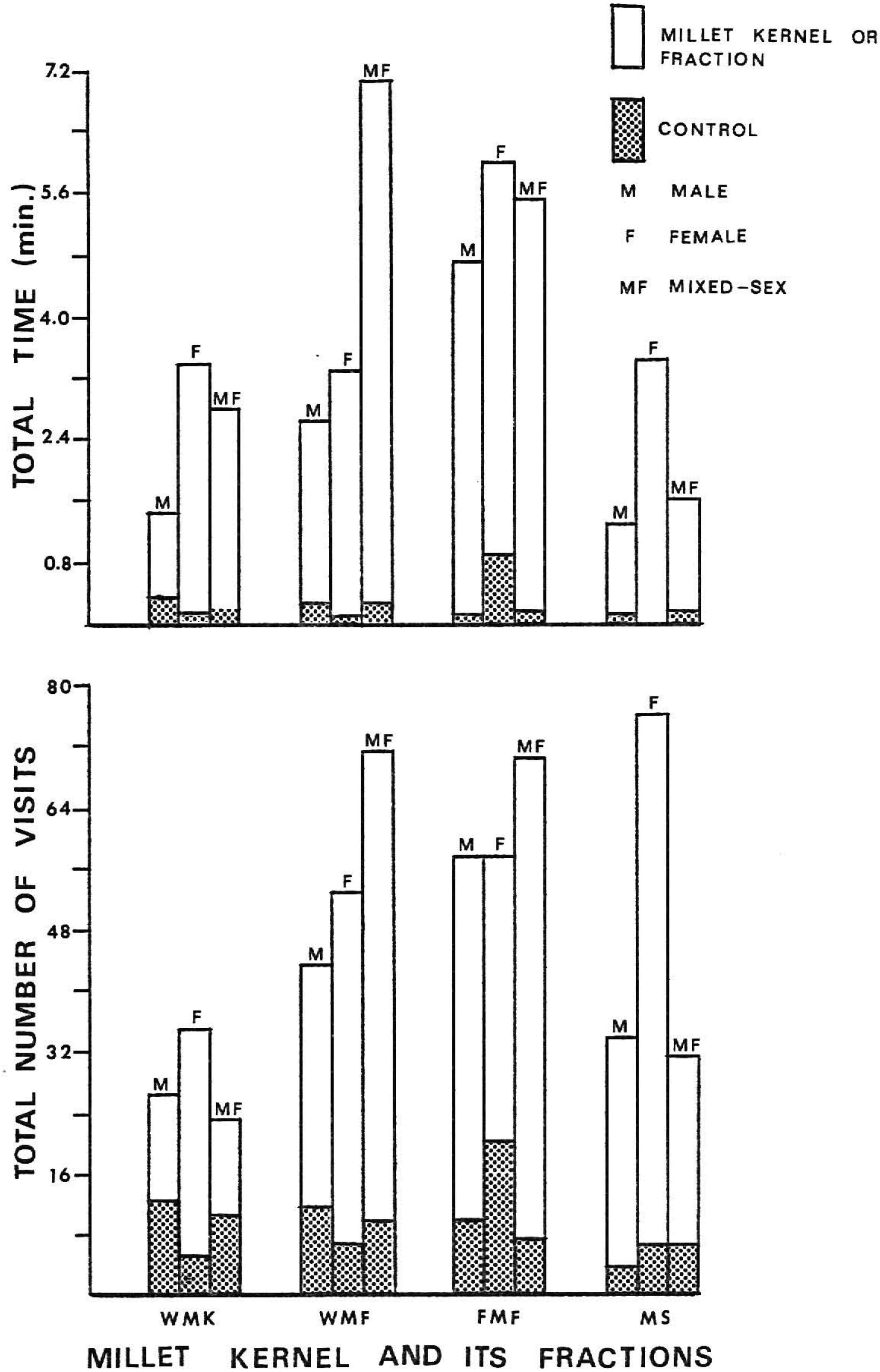
DISCUSSION AND CONCLUSIONS

The primary objective of the study was to compare laboratory techniques for testing the attraction of the red flour beetles (RFB) to volatile substances from kernels, fractions or extracts of wheat and millet, and of moldy wheat and molds. The principal criterion on which the study was conducted was that both the optical and mechanical factors of response were excluded and only the olfactory aspect of food selection was used. All experiments were conducted in the dark except for the red light, and all test substances were suspended above the sensor out of reach of the insects' antennae. The exclusion of the optical

Fig. 11. Attraction responses of male, female, or mixed-sex groups of 72-96-hr-old red flour beetles to whole millet kernel (WMK), whole millet flour (WMF), bran-free fermented millet flour (FMF) or millet starch (MS). (Three 10-min. reps.)

Upper: Total time spent on sensor under controls or test materials.

Lower: Total numbers of visits to sensor under controls or test materials.


MILLET KERNEL OR FRACTION
CONTROL
M MALE
F FEMALE
MF MIXED-SEX
TOTAL TIME (min.)
7.2
5.6
4.0
2.4
0.8
TOTAL NUMBER OF VISITS
80
64
48
32
16
M
F
MF
WMK
WMF
FMF
MS
MILLET KERNEL AND ITS FRACTIONS

factor of response in such olfactory studies were also reported by Oosthuizen (1945), Honda et al. (1969), Ohsawa et al. (1970) and Soliman (1975); however, the beetles tested had the opportunity to contact the test material (mechanical response) except for Honda et al. (1969) who used a modified Chamberlain cage-type olfactometer.

The frequency of olfactory attraction of the RFB to wheat kernel volatiles was found to increase with the age; however, the duration of time spent at the source of the volatiles increased with age to 72-96 hr, then decreased. Thus, beetles at this age were considered to be most responsive to food volatile substances and were used in subsequent experiments. The female RFB were more responsive to volatile food odors than male or mixed-sex groups in most experiments, and this confirms the report of Soliman (1975) who studied confused flour beetles (CFB). However, Loschiavo (1965a) stated that male and female CFB did not differ in their responses to the volatile odors from the same source. The wheat germ volatiles, from germ or germ extract, were most attractive to the RFB tested, except that in the free-choice test, whole wheat flour was more attractive to the females. Good (1936), Loschiavo (1959; 1965b), Tamaki et al. (1971) and Nara et al. (1981) reported similar results; however, Oosthuizen (1945) stated that of the wheat fractions, the germ appeared to be the least attractive to adult RFB. The strong attraction of the flour beetles to volatiles from either the extracts or the fractions of wheat such as enriched patent flour, bran, or germ, was also reported by Cotton and Frankenfeld (1945), Willis and Roth (1950) and Levinson and Levinson (1978). Tamaki et al.

(1971) attributed such attractiveness of wheat germ volatiles to triglycerides such as 1-palmito-2;3-diolein, 2-linoleo-1;3-dipalmitin and 1-palmito-2-linoleo-3-olein.

The RFB were attracted to volatiles of the fungus, Aspergillus glaucus, associated with wheat, and they preferred fungal-infected wheat volatiles over those of fungal-free wheat volatiles. According to Starratt and Loschiavo (1971; 1972), the monounsaturated triglycerides of the fungus Nigrospora sphaerica, of which the major component was oleodipalmitin elicited strong attraction and aggregation of the CFB. Sinha (1966; 1971) noted that the RFB could feed, lay eggs and develop on fungi associated with seeds. 1-octen-3-ol which is a strong volatile and 3-octanone, having a citrus-like volatile, in addition to n-butanol, iso-amylalcohol, ethyl acetate, ethanol and iso-butanol are volatiles, perhaps responsible for attraction of RFB, were isolated by Saito et al. (1979) from fungal species associated with grain.

The RFB were also attracted to volatiles of millet kernels, millet fractions and extracts. The most preferred attractant(s) for the 3 sex groups of RFB tested were in the fermented millet flour, to which the most visits were made and longer times spent. The attractive components of the millet volatiles were not known; Hougen (1971) indicated that the different species and varieties of cereal grains appeared to produce largely the same volatile components but in different relative amounts. This may suggest that the volatiles of wheat and millet may be the same but in different relative amounts. The fermented millet no doubt contained volatiles of microorganisms responsible for the fermentation.

Maga (1978) listed 24 identified volatiles of wheat, including methanol, ethanol, acetaldehyde, acetone, butanal, isobutanal, butanone and 3-methyl-2-butanone.

The data on attractiveness of volatiles of wheat, millet and molds to RFB, clearly indicated that these beetles show a definite predilection for these products, and that the olfactory responses lead the RFB toward the food source. However, further studies might supply more needed data on the olfactory mechanisms and behavior involved in food location, and ways to utilize this information in their control.

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ACKNOWLEDGMENTS

The author wishes to acknowledge his indebtedness to his major professor, Dr. Robert B. Mills, for his supervision, encouragement, patience, and above all, friendship during the course of this graduate study. He would like also to express his sincere gratitude to Dr. Theodore L. Hopkins for serving on the supervisory committee and for the keen interest he has shown, for many invaluable suggestions he has made, and for providing the equipment necessary for the study; to Dr. William A. Ramoska and Dr. Clifton E. Meloan for serving on the supervisory committee, providing suggestions on the research plan and thesis; and to Dr. Valerie F. Wright for her helpful suggestions on certain portions of this study.

Due thanks are also extended to Dr. George A. Milliken for his help in statistical analysis, and to Mrs. Helyn Marshall for typing the manuscript.

A P P E N D I X

Append. Table 1. Attraction responses in number of visits and time spent (min.) by adult red flour beetles of different age and sex groups under a suspended control object or wheat kernel as the test material.

AGE (hr)	MALES				FEMALES				MIXED				MEAN			
	Visits		Time		Visits		Time		Visits		Time		Visits		Time	
	C	Wk	C	Wk	C	Wk	C	Wk	C	Wk	C	Wk	C	Wk	C	Wk
0 - 24	2	1	.09	.00	6	5	.01	.25	3	9	.00	.03	3.67	5.00	.03	.09
24 - 48	11	15	.26	1.06	5	8	.13	.44	12	14	.52	.38	9.33	12.33	.30	.63
48 - 72	6	9	.06	.41	2	13	.06	.73	8	19	.09	1.09	5.33	13.67	.07	.74
72 - 96	8	25	.13	2.16	5	26	.00	2.97	3	15	.09	4.69	5.33	22.00	.07	3.27
96 - 120	9	30	.09	1.22	10	23	.09	1.34	10	25	.13	1.56	9.67	26.00	.10	1.37
120 - 244	11	27	.09	1.34	13	28	.22	1.72	12	23	.28	1.59	12.00	26.00	.20	1.55
144 - 168	13	27	.13	.31	16	33	.00	.56	12	27	.13	1.41	13.67	29.00	.08	.76

C = Control

Wk = Wheat kernel

Append. Table 2. Attraction responses of male, female or mixed-sex groups of 72-96-hr-old red flour beetles, in number of visits (V) and time spent (T, in min.) to each of whole wheat kernels (WWK), whole wheat flour (WWF) and wheat fractions [germ (WG), bran (WB), endosperm (WE)] (Three 10-min. reps.)

SEX	MALES				FEMALES				MIXED			
	Control		Test Subs.		Control		Test Subs.		Control		Test Subs.	
FRACTION	V	T	V	T	V	T	V	T	V	T	V	T
	6	.13	9	.47	3	.00	8	.38	1	.06	3	.03
	1	.00	10	1.47	1	.00	7	.30	2	.03	6	.16
WWK	1	.00	6	.22	1	.00	11	2.29	0	.00	6	4.50
Total	8	.13	25	2.16	5	.00	26	2.97	3	.09	15	4.69
Mean	2.67	.04	8.30	.72	1.67	.00	8.67	.99	1.00	.03	5.00	1.56
	3	.00	12	1.09	3	.06	7	1.78	0	.00	11	.50
WWF	0	.00	8	1.09	0	.00	18	2.22	0	.00	15	1.44
	0	.00	8	.97	0	.00	16	2.50	1	.00	13	.84
Total	3	.00	28	3.15	3	.06	41	6.50	1	.00	39	2.78
Mean	1.00	.00	9.33	1.05	1.00	.02	13.67	2.17	.33	.00	13.00	.93
	1	.00	16	1.13	0	.00	10	.69	1	.00	13	.72
WG	0	.00	15	.97	1	.00	11	.66	2	.00	11	.81
	1	.00	15	.84	2	.03	28	2.16	0	.00	10	.63
Total	2	.00	46	2.94	3	.03	49	3.51	3	.00	34	2.16
Mean	.67	.00	15.33	.98	1.00	.01	16.33	1.17	1.00	.00	11.33	.72
	0	.00	13	.53	3	.00	11	.69	2	.00	17	1.09
	2	.00	4	.50	3	.09	14	.91	1	.00	9	.38
WB	1	.03	12	.63	1	.00	15	.78	3	.00	8	.69
Total	3	.03	29	1.66	7	.09	40	2.38	6	.00	34	2.16
Mean	1.00	.01	9.67	.56	2.33	.03	13.33	.79	2.00	.00	11.33	.72
	3	.06	10	.56	1	.03	23	1.53	5	.09	11	.61
	4	.09	15	.84	0	.00	19	1.61	2	.03	9	.53
WE	1	.02	10	.53	4	.06	20	1.84	5	.09	13	.49
Total	8	.17	35	1.93	5	.09	62	4.98	12	.21	33	1.63
Mean	2.67	.06	11.67	.64	1.67	.03	20.67	1.66	4.00	.07	11.00	.54

Append. Table 3. Attraction responses of males, females or mixed-sex groups of 72-96-hr-old red flour beetles, in number of visits (V) and time spent (T, in min.) to each of 4 extract concentrations of the hard red winter wheat. (Three 10 min. reps.)

SEX	MALES				FEMALES				MIXED			
Conc.	Control		Test Subs.		Control		Test Subs.		Control		Test Subs.	
(g. equi.)	V	T	V	T	V	T	V	T	V	T	V	T
	3	.06	0	.00	3	.13	9	.56	2	.03	5	.08
0.1	1	.00	4	.11	0	.00	7	.34	2	.01	2	.06
	1	.00	5	.19	0	.00	2	.12	0	.00	4	.16
Total	5	.06	9	.30	3	.13	18	1.02	4	.04	11	.30
Mean	1.67	.02	3.00	.10	1.0	.04	6.00	.34	1.33	.01	3.67	.11
	4	.06	9	.34	2	.03	12	.43	2	.00	11	.41
0.5	2	.19	4	.13	3	.16	8	.31	4	.06	8	.28
	2	.09	5	.22	0	.00	6	.28	1	.03	12	.28
Total	8	.34	18	.69	5	.19	26	1.01	7	.09	31	.97
Mean	2.67	.11	6.00	.23	1.67	.06	8.67	.34	2.33	.03	10.33	.32
	2	.00	5	.19	0	.00	2	.06	1	.00	4	.03
1.0	1	.00	3	.03	1	.00	2	.09	0	.00	2	.00
	3	.00	7	.47	1	.04	8	.35	2	.06	12	.23
Total	6	.00	15	.69	2	.04	12	.50	3	.06	18	.26
Mean	2.00	.00	5.00	.23	.67	.01	4.00	.17	1.00	.02	6.00	.09
	4	.00	1	.06	0	.00	1	.00	1	.00	1	.02
1.5	0	.00	3	.19	2	.08	9	.38	3	.09	4	.16
	3	.06	13	.34	0	.00	0	.00	0	.00	12	.16
Total	7	.06	17	.59	2	.08	10	.38	4	.09	17	.34
Mean	2.33	.02	5.67	.20	.67	.03	3.33	.13	1.33	.03	5.67	.11

Append. Table 4. Attraction responses of 72-96-hr-old female red flour beetles in number of visits (V) and time spent (T, in min.) to each of 3 extract concentrations of whole wheat kernels (WWK), whole wheat flour (WWF) and wheat fractions [wheat germ (WG), wheat bran (WB), wheat endosperm (WE)]. (Three 10-min. reps.).

Conc. (g. equi.)	0.1				0.5				1.0			
	Control		Test Subs.		Control		Test Subs.		Control		Test Subs.	
Fraction	V	T	V	T	V	T	V	T	V	T	V	T
	0	.00	7	.31	1	.00	8	.47	2	.08	3	.34
WWK	2	.12	5	.28	0	.00	1	.13	2	.06	5	.29
	4	.11	4	.22	3	.16	4	.25	4	.14	7	.56
Total	6	.23	16	.81	4	.16	13	.85	8	.28	15	1.19
Mean	2.00	.08	5.33	.27	1.33	.05	4.33	.28	2.67	.09	5.00	.40
	0	.00	7	.31	4	.08	14	.78	0	.00	22	1.38
WWF	0	.00	5	.42	1	.00	7	.19	3	.08	5	.50
	4	.09	3	.25	1	.05	10	.56	0	.00	7	.56
Total	4	.09	15	.98	6	.13	31	1.53	3	.08	34	2.44
Mean	1.33	.03	5.00	.33	2.00	.04	10.33	.51	1.00	.03	11.33	.81
	5	.09	19	.63	5	.13	32	2.34	7	.13	25	.81
WG	0	.00	21	1.75	2	.03	33	4.75	3	.00	21	1.88
	1	.00	16	1.09	4	.06	29	2.88	2	.00	24	4.09
Total	6	.09	56	3.47	11	.22	94	9.97	12	.13	70	6.78
Mean	2.00	.03	18.67	1.16	3.67	.07	31.33	3.32	4.00	.04	23.33	2.26
	4	.11	13	.88	0	.00	26	2.34	2	.08	16	3.03
WB	2	.06	10	.63	3	.25	23	3.44	1	.06	9	1.25
	3	.12	5	.41	1	.09	12	1.00	6	.22	19	2.56
Total	9	.29	28	1.92	4	.34	61	6.78	9	.36	44	6.84
Mean	3.00	.10	9.33	.64	1.33	.11	20.33	2.26	3.00	.12	14.67	2.28
	6	.19	9	.72	2	.13	15	1.25	4	.09	11	1.25
WE	1	.03	13	1.13	0	.00	18	1.31	3	.09	21	2.06
	4	.22	11	.91	3	.19	17	1.19	1	.00	29	2.19
Total	11	.44	33	2.76	5	.32	50	3.75	8	.18	61	5.50
Mean	3.67	.15	11.00	.92	1.67	.11	16.67	1.25	2.67	.06	20.33	1.83

Append. Table 5. Attraction responses of male, female and mixed-sex groups of 72-96-hr-old red flour beetles, in number of visits (V) and time spent (T, in min.) to sensor below each of a suspended fungal-free wheat kernel (FFW) (control) and fungal-infected wheat kernel (FIW) (test substance). (Three 10-min. reps.).

SEX	MALES				FEMALES				MIXED			
Test subs.	FFW		FIW		FFW		FIW		FFW		FIW	
	V	T	V	T	V	T	V	T	V	T	V	T
	8	.36	11	.54	11	.72	17	.65	6	.21	14	.46
	7	.23	8	.28	14	.52	20	1.38	8	.29	10	.29
	8	.23	12	.76	7	.30	19	1.61	9	.44	9	.36
Total	23	.82	31	1.58	32	1.54	56	3.64	23	.94	33	1.11
Mean	7.67	.27	10.33	.53	10.67	.51	18.67	1.21	7.67	.31	11.00	.37

Append. Table 6. Attraction responses of male, female and mixed-sex groups of 72-96-hr-Old red flour beetles, in number of visits (V) and time spent (T, in min.) to each of 3 extract concentrations of fungal-free wheat kernels (FFW) (control) and fungal-infected wheat kernels (FIW) (test substance). (Three 10-min. reps.).

SEX	MALES				FEMALES				MIXED			
Test subs.	FFW		FIW		FFW		FIW		FFW		FIW	
Conc. (g. equi)	V	T	V	T	V	T	V	T	V	T	V	T
	7	.31	9	.47	6	.12	20	1.03	2	.06	22	.80
0.1	9	.42	13	.99	7	.56	15	.66	7	.27	2	.06
	1	.06	6	.25	5	.08	5	.17	3	.06	6	.29
Total	17	.79	28	1.71	18	.76	40	1.86	12	.39	30	1.15
Mean	5.67	.26	9.33	.57	6.00	.25	13.33	.62	4.00	.13	10.00	.38
	11	.36	10	.75	5	.18	18	1.47	8	.19	7	.34
0.5	6	.18	10	.56	14	.46	11	.50	6	.24	7	.36
	4	.16	15	1.03	10	.34	12	.91	9	.26	10	.63
Total	21	.70	35	2.34	29	.98	41	2.88	23	.69	24	1.33
Mean	7.00	.23	11.67	.78	9.67	.33	13.67	.96	7.67	.23	8.00	.44
	7	.14	8	.39	5	.09	20	1.21	7	.07	24	1.66
1.0	2	.09	5	.47	11	.35	18	1.13	10	.24	9	.55
	10	.18	12	.97	7	.31	15	.94	3	.02	18	1.13
Total	19	.41	25	1.83	23	.75	53	3.28	20	.33	51	3.34
Mean	6.33	.14	8.33	.61	7.67	.25	17.67	1.09	6.67	.11	17.00	1.11

Append. Table 7. Attraction responses of males, females and mixed-sex groups of 72-96-hr-old red flour beetles, in number of visits (V) and time spent (T, in min.) to each of 3 extract concentrations of A. glaucus mycelium with no spores (AGNS) (control) and A. glaucus mycelium with spores (AGWS) (test substance). (Three 10-min.reps.).

SEX	MALES				FEMALES				MIXED			
Test. subs	AGNS		AGWS		AGNS		AGWS		AGNS		AGWS	
Conc. (g. equi.)	V	T	V	T	V	T	V	T	V	T	V	T
	4	.13	5	.29	6	.10	4	.28	6	.21	1	.18
0.0016	4	.06	1	.00	8	.24	11	.64	7	.20	8	.43
	5	.09	6	.28	9	.30	7	.43	4	.08	4	.31
Total	13	.28	12	.57	23	.64	22	1.35	17	.49	13	.92
Mean	4.33	.09	4.00	.19	7.67	.21	7.33	.45	5.67	.16	4.33	.31
	5	.22	4	.24	4	.21	10	.33	9	.54	7	.35
0.0083	6	.13	5	.26	8	.33	13	.64	3	.12	7	.36
	3	.12	7	.60	4	.31	14	.83	5	.26	6	.21
Total	14	.47	16	1.10	16	.85	37	1.80	17	.92	20	.92
Mean	4.67	.16	5.33	.37	5.33	.28	12.33	.60	5.67	.31	6.67	.31
	4	.16	7	.31	10	.31	15	.56	6	.26	4	.20
0.0165	5	.28	5	.16	6	.26	12	.44	7	.29	9	.53
	3	.14	4	.19	7	.23	5	.28	2	.09	6	.22
Total	12	.58	16	.66	23	.80	32	1.28	15	.64	19	.95
Mean	4.00	.19	5.33	.22	7.67	.27	10.67	.43	5.00	.21	6.33	.32

Append. Table 8. Attraction responses of male, female and mixed-sex groups of 72-96-hr-old red flour beetles, in number of visits (V) and time spent (T, in min.) to each of 3 extract concentrations of A. glaucus mycelium with no spores (AGNS) (test subs.) and solvent-treated cotton ball as a control. (Three 10-min. reps.).

SEX	MALES				FEMALES				MIXED			
Test Subs.	Control		Test Subs.		Control		Test Subs.		Control		Test Subs.	
Conc. (g. equi.)	V	T	V	T	V	T	V	T	V	T	V	T
	4	.03	2	.28	2	.06	12	.54	1	.00	1	.00
0.0016	0	.00	1	.00	6	.05	11	.44	5	.13	1	.06
	1	.00	6	.13	2	.04	2	.19	0	.00	14	.88
Total	5	.03	9	.41	10	.15	25	1.17	6	.13	16	.94
Mean	1.67	.01	3.00	.14	3.33	.05	8.33	.39	2.00	.04	5.33	.31
	1	.06	4	.15	4	.19	11	.84	0	.00	5	.08
0.0083	2	.00	2	.09	0	.00	5	.09	1	.00	4	.13
	2	.05	1	.03	3	.09	9	.46	1	.00	6	.45
Total	5	.11	7	.27	7	.28	25	1.39	2	.00	15	.66
Mean	1.67	.04	2.33	.09	2.33	.09	8.33	.46	.67	.00	5.00	.22
	2	.01	3	.07	3	.06	8	.33	0	.00	3	.19
0.0165	2	.02	4	.13	1	.00	5	.25	4	.06	6	.38
	5	.18	8	.18	1	.00	8	.41	1	.00	4	.22
Total	9	.21	15	.38	5	.06	21	.99	5	.06	13	.79
Mean	3.00	.07	5.00	.13	1.67	.02	7.00	.33	1.67	.02	4.33	.26

Append. Table 9. Attraction responses of 72-96-hr-old female red flour beetles, in number of visits to each of whole wheat flour (WWF), wheat bran (WB), wheat endosperm (WE), wheat germ (WG) and control (C). (Twenty 15-min reps.)

Rep.	Test Material Placement Order in the Arena									
	Chamber #1					Chamber #2				
	C	WG	WE	WB	WWF	C	WE	WWF	WG	WB
1	0	1	1	0	3	1	3	0	2	1
2	1	2	1	0	1	0	1	3	3	2
3	0	4	5	3	1	0	0	3	0	2
4	0	2	2	1	2	0	1	2	1	1
5	1	2	2	2	3	1	1	4	3	0
6	1	3	1	4	3	2	4	3	5	1
7	2	4	1	2	5	3	0	2	4	3
8	2	3	2	1	4	0	1	6	2	0
9	0	1	2	0	3	0	3	0	1	1
10	3	1	6	5	4	2	3	2	4	1
11	1	4	1	2	1	1	1	5	3	2
12	1	3	8	1	6	1	2	3	2	0
13	2	2	1	2	3	1	1	4	2	3
14	0	2	1	1	4	3	2	7	3	0
15	1	4	0	2	7	0	0	4	1	2
16	0	6	2	0	3	1	4	4	2	0
17	0	2	1	3	3	1	1	5	3	0
18	2	3	0	4	6	0	0	3	1	0
19	0	4	0	0	3	0	5	1	2	2
20	0	1	0	2	1	1	0	2	4	5
Total	17	54	37	35	66	18	33	63	48	26
MEAN	.85	2.70	1.85	1.75	3.30	.90	1.65	3.15	2.40	1.30

C = Control

WG = Wheat Germ

WE = Wheat Endosperm

WB = Wheat Bran

WWF = Whole Wheat Flour

Append. Table 10. Attraction responses of male, female and mixed-sex groups of red flour beetles, in number of visits (V) and time spent (T, in min.) to each of 4 extract concentrations of whole millet kernels (WMK) and solvent-treated cotton ball as a control. (Three 10-min. reps.)

SEX	MALES				FEMALES				MIXED			
	Control		WMK		Control		WMK		Control		WMK	
Conc. (g. equi)	V	T	V	T	V	T	V	T	V	T	V	T
	0	.00	3	.13	2	.01	8	.21	2	.00	8	.21
0.1	1	.02	4	.19	1	.00	7	.14	4	.08	3	.16
	1	.00	3	.12	5	.09	2	.09	0	.00	8	.42
Total	2	.02	10	.44	8	.10	17	.44	6	.08	19	.79
Mean	.67	.01	3.33	.15	2.67	.03	5.67	.15	2.00	.03	6.33	.26
	0	.00	6	.13	0	.00	10	.74	4	.00	5	.16
0.5	4	.06	2	.03	0	.00	5	.13	1	.06	6	.23
	2	.03	4	.16	2	.00	4	.26	2	.00	6	.43
Total	6	.09	12	.32	2	.00	19	1.13	7	.06	17	.82
Mean	2.00	.03	4.00	.11	.67	.00	6.33	.38	2.33	.02	5.67	.27
	2	.00	13	.59	0	.00	8	.13	2	.00	8	.19
1.0	0	.00	11	.50	0	.00	9	.47	0	.00	7	.38
	0	.00	2	.13	1	.00	3	.22	3	.06	4	.22
Total	2	.00	26	1.22	1	.00	20	.82	5	.06	19	.79
Mean	.67	.00	8.67	.41	.33	.00	6.67	.27	1.67	.02	6.33	.26
	1	.01	4	.06	1	.01	2	.01	2	.00	1	.00
1.5	1	.03	0	.00	0	.00	2	.09	4	.08	2	.04
	4	.02	7	.13	2	.02	2	.06	1	.00	5	.19
Total	6	.06	11	.19	3	.03	6	.16	7	.08	8	.23
Mean	2.00	.02	3.67	.06	1.00	.01	2.00	.05	2.33	.03	2.67	.08

Append. Table 11. Attraction responses of male, female and mixed-sex groups of 72-96-hr-old red flour beetles, in number of visits (V) and time spent (T, in min.) to each of whole millet kernels (WMK), whole millet flour (WMF), bran-free fermented millet flour (FMF) and millet starch (MS). (Three 10-min. reps.).

SEX	MALES				FEMALES				MIXED			
	Control		Test Subs.		Control		Test Subs.		Control		Test Subs.	
Fract.	V	T	V	T	V	T	V	T	V	T	V	T
	7	.13	9	.25	1	.03	10	.84	5	.06	9	2.09
WMK	3	.03	5	.25	4	.03	14	1.03	3	.00	5	.06
	2	.13	12	.94	0	.00	11	1.44	2	.09	9	.59
Total	12	.29	26	1.44	5	.06	35	3.31	10	.15	23	2.74
Mean	4.00	.10	8.67	.48	1.67	.02	11.67	1.10	3.33	.05	7.67	.91
	5	.13	12	.69	2	.00	12	.50	2	.00	18	.25
WMF	2	.00	11	.47	4	.06	20	.91	2	.09	33	1.50
	4	.09	20	1.44	0	.00	21	1.88	5	.09	20	5.28
Total	11	.22	43	2.60	6	.06	53	3.29	9	.18	71	7.03
Mean	3.67	.07	14.33	.87	2.00	.02	17.67	1.11	3.00	.06	23.67	2.34
	3	.00	25	1.97	4	.13	22	1.25	1	.06	19	1.44
FMF	1	.00	13	1.22	9	.19	15	2.13	4	.09	14	.72
	5	.09	19	1.56	7	.56	20	2.63	2	.00	37	3.34
Total	9	.09	57	4.75	20	.88	57	6.01	7	.15	70	5.50
Mean	3.00	.03	19.00	1.58	6.67	.29	19.00	2.00	2.33	.05	23.33	1.83
	0	.00	9	.28	2	.00	30	1.25	3	.06	19	1.00
MS	2	.06	10	.16	0	.00	20	.94	3	.05	6	.28
	1	.00	14	.81	4	.04	26	1.28	0	.00	6	.34
Total	3	.06	33	1.25	6	.04	76	3.47	6	.11	31	1.62
Mean	1.00	.02	11.00	.42	2.00	.01	25.33	1.16	2.00	.04	10.33	.54

OLFACTORY RESPONSE OF RED FLOUR BEETLES, _TRIBOLIUM CASTANEUM_ (HERBST), TO VARIOUS FORMS OF WHEAT, MILLET AND A FUNGUS AS DETERMINED BY A LIGHT-SENSITIVE APPARATUS

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
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1981

A light-sensitive event detector unit, in conjunction with a Heath Model EUW-20A Servo Recorder were used to monitor olfactory responses of red flour beetles to volatiles of fungal-free or fungal-infected wheat kernels, wheat fractions (germ, bran, endosperm), millet and millet products, or to solvent extracts of all these materials. Sight and tactile responses were excluded by conducting all tests in darkness, except for a red light, and by suspending all substances to be tested above the sensor, out of reach of the beetles' antennae. Males, females, or mixed-sex groups of 72-96-hr-old, one-day starved beetles were tested separately. The number of visits made to the test substance suspended over the sensor and the length of time spent on the sensor were used to measure attractancy. A preliminary experiment showed that the frequency of olfactory attraction to wheat kernel volatiles increased with the age up to 144-168 hr; however, the duration of time spent near the source of the volatile substance increased with age to 72-96 hr, then decreased. Thus, beetles of this age were used in subsequent tests. Females were more responsive than males or the mixed-sex groups. Wheat germ volatiles, either from untreated germ material or from extracts, appeared to be most attractive. Wheat endosperm and wheat bran were statistically equally attractive and the whole wheat kernels were least attractive. Fungal-infected wheat volatiles were more attractive than those of fungal-free wheat. Volatiles from extracts of the processed millet products were more attractive than those from whole millet kernel extracts, and among the products, volatiles of fermented millet flour were most attractive. Volatiles of millet starch elicited more visits than those of whole millet kernels, and those of whole millet kernels elicited more time spent on the sensor by the 3 sex groups considered together. These findings may constitute a basis for further experimentation with regard to the olfactory mechanisms and behavior involved in food location in flour beetles.