THE ISOLATION AND IDENTIFICATION OF VOLATILE COMPOUNDS IN WHEAT WHICH INDUCE AN AGGREGATION RESPONSE TO THE RED FLOUR BEETLE, TRIBOLIUM CASTANEUM (Herbst.)

by

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INTRODUCTION

It is estimated there are about one million species of insects in the world (1). Most of them are of no concern to us. Some, like the honeybee, are beneficial with only about 0.1% of the insect population being harmful to us by destroying our food, property, and spreading disease.

Growing food shortages in the world today are forcing countries to invest more effort and money to increase food production by reducing the damage and losses of food and food products caused by pests during storage and transportation. It is estimated that the total annual losses to the world's supply of stored grains range from 5 to 10 percent of the world's production. This is equal to the amount of grain required to provide 130 million people with a subsistence level of food grains. In hot and humid tropical areas, the damage usually is much greater. If Kansas wheat in storage were damaged by insects at the low estimate of 0.5 percent, a 200 million bushel crop would pay one million bushels in tribute to the insects (1).

A lot of work has been done to control stored grain products insects and to prevent food from infestation. This includes the use of inert gases, radiation, pathogens, growth regulators (such as the juvenile hormone), pheromones, pesticides, sanitation, inspection, good packaging and providing good storage facilities (2).

The use of pesticides to control insects has several disadvantages in that; (1) they may be toxic not only to the target insects but also to

the harmless, beneficial insects and other creatures, (2) after many exposures, insect populations may build up resistance, (3) the high cost of production and application, and (4) the persistence of the residues may pollute the environment. For these reasons, the use of insecticides has been decreased in pest control (3). Recently more effort has been placed on developing alternatives to combat insect infestation.

In order for every animal to be aware of its surroundings, such as transmitting and receiving vital information, finding foods, avoiding predators and meeting mates, insects, fish, and many mammals depend extensively on odor for communication (4). For many creatures other than humans, the chemical senses, e. g., taste and smell, play much more important roles. The sense of smell or olfaction is more sensitive and more discriminating than the sense of taste. As a rule of thumb, olfactory sense is roughly 10,000 times more sensitive than the sense of taste (5).

Based on these phenomena, there has been a widespread search for natural attractants and repellents for different insects. Sex pheromones and food attractants for luring insects into traps or contact with insecticides or pathogens provide a few alternative ways to control stored-product insect infestations (2).

The purpose of this project was to analyze the volatile components emanating from whole wheat kernels and ground wheat kernels, to determine which of them might be responsible for the aggregation response of the red flour beetle, Tribolium castaneum (Herbst.). The red flour beetle and the confused flour beetle, Tribolium confusm (Jacquelin du Val), are common

and destructive insects for wheat flour and other prepared products. These insects are able to exist in a wide variety of foodstuffs with strong vital adaptive activity, and through world-wide commerce have become cosmopolitan. Thus, there are many records and reports about their occurrence and destructiveness around the world (6).

DESCRIPTION OF THE RED FLOUR BEETLE, T. CASTANEUM, (Herbst).

The red flour beetle is almost identical in appearance with the confused flour beetle to which it is closely related (Figures 1 & 2). It is a shiny, reddish-brown beetle about 0.36 cm long, flattened and oval, with the head and upper parts of the thorax densely covered with minute punctures and with wing covers ridged lengthwise and sparsely punctured between the ridges.

The red flour beetle can be distinguished from the confused flour beetle only with the aid of a magnifying glass. The segments of the antennae of the confused flour beetle increase in size gradually from the base to the tip, whereas in the red flour beetle the outer three segments of the antennae are abruptly much larger than the preceding ones, giving the antennae the appearance of being suddenly enlarged at the tip. In addition, the margins of the head of the confused flour beetle are expanded and notched at the eyes, whereas the margins of the head of the the red flour beetle are nearly continuous at the eyes. It is a general feeder on farinaceous material and is one of the most abundant and injurious insect pest of grain products in the United States, especially in the South. It is found in granaries, mills, warehouses and wherever grain or grain products are stored.

Figure 1; Tribolium castaneum. Adult, X28

Figure 2; Tribolium confusum. Adult, X28

(Reprint from Good, N. E. U. S. Dept. Agric., Washington, D. C. Tech. Bulletin No. 498)

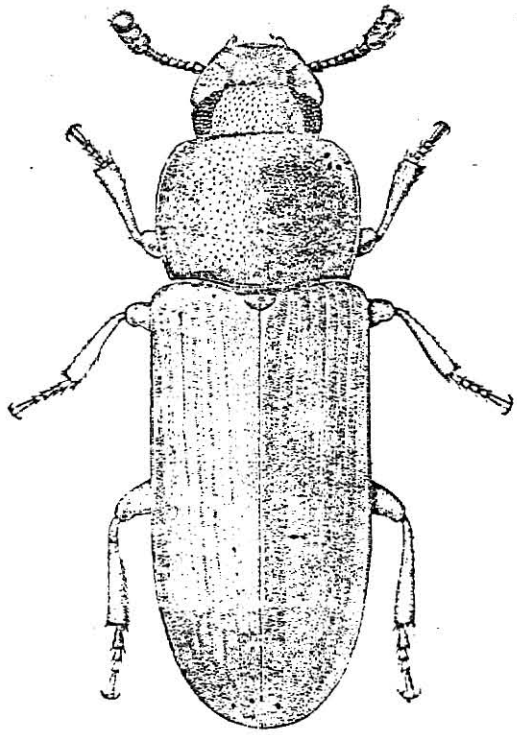


Figure 1; Tribolium castaneum. Adult, X28

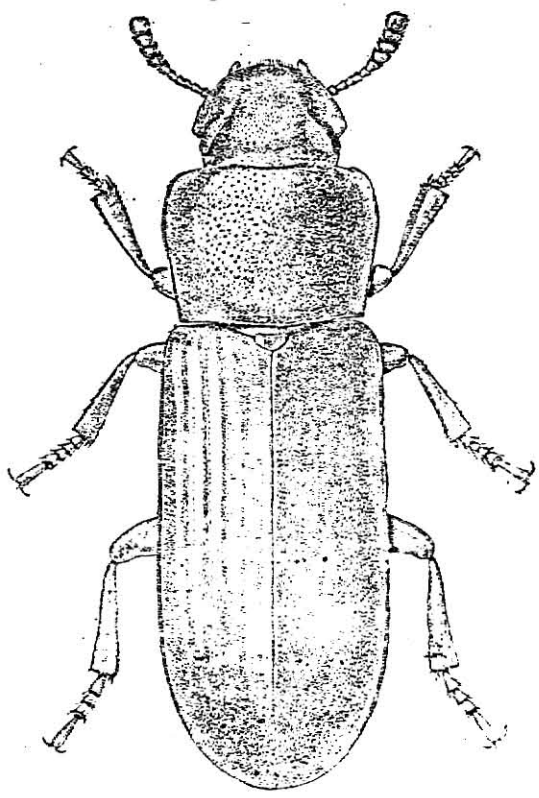


Figure 2; Tribolium confusum. Adult, X28

The average life of the beetle is about one year, but some have been known to live as long as three years nine months. The females lay an average of about 320 eggs during one oviposition period which lasts an average of 160 days. The small white eggs are laid loosely in flour or other food material in which the adults are living. The eggs hatch in from 5 to 12 days into small wormlike larvae, slender, cylindrical, and wiry in appearance, which when fully grown are about 0.48 cm long, and are white, tinged with yellow. These larvae feed on flour or other material such as grain dust and the broken surfaces of grain kernels. When fully grown they transform into small naked pupae. At first the pupa is white, then it gradually changes to yellow and then brown. Shortly afterward it transforms into a beetle. While being fed in the whole-wheat flour, the development period from egg to adult for the red flour beetle requires an average of 32 days at a temperature of 30°C, but the period for the confused flour beetle is somewhat longer under the same conditions. The life cycle is greatly prolonged by cold weather, as is true of all grain pests (7).

OBJECTIVE

The objective of this research was to isolate and identify as many as possible of the volatile compounds of wheat that attract the red flour beetles, Tribolium castaneum. Bioassays were later conducted to confirm the attractive properties of many of these volatile components.

LITERATURE REVIEW

Cereal grains play an important role in the diets of most people, yet comparatively little is known about the volatile components of the cereal grains in the raw state. These volatile components are important because they may act as attractants or repellents to insects.

To date, the published investigations relative to the volatile components of the three primary cereals in the raw state (rice, corn, and wheat) number less than a dozen (8). McWilliams and Mackey (9) separated and identified 18 compounds in the headspace vapor of a lightly milled, whole-grain soft wheat. All the components were low molecular weight aldehydes, ketones, and alcohols. Hougen et al. (10) investigated different species and varieties of cereal grains such as maize, rye, wheat, and triticale and found that these species produced largely the same volatile components but in different and characteristic relative amounts. Those compounds identified include aldehydes and ketones. The insect infestation of wheat was also studied. Three major volatile compounds associated with the isolated weevils were found also associated with the volatiles from wheat. Lorenz and Maga (11) identified carbonyl compounds and fatty acids in several wheat flour varieties. They found no qualitative differences but only small quantitative differences among the components. The carbonyl compounds in the various wheat varieties are quite similar. Ivanova and Popov (12) investigated wheat germ oil and found fatty acids and steroids. Lercker et al. (13) analyzed wheat germ and found high molecular weight fatty acids, hydrocarbons, sterols, and lipids. Buttery et al. (14) examined the wheat flour sample and found the presence of naphthalene, alkylnaphthalene, aldehydes and alcohols.

Maga (8) reviewed the volatiles found in cereals and 24 components were summarized (Table 1). Burkholder et al. (15) investigated an aggregating response of Trogoderma glabrum larvae to volatile compounds in wheat germ oil. They tested several synthetic compounds individually in selected combinations and found octanoic acid, cis-3-hexenal, octanal, and r-octalactone were the most attractive synthetic compounds. It was concluded that insects are able to find preferred food from olfactory stimulus emitted from stored food products.

Fraenkel and Blewett (16) showed that T. confusum was attracted by the odor of flour in an olfactometer. Loschiavo et al. (17), based an olfactory response of the smaller European Elm Bark Beetle, Scolytus multistriatus, to test feeding stimulants on extracts of Elm Bark. Yamamoto et al. (18, 19) tested an attractant for rice weevils from rice grains according to an olfactory response and also found several insects, including T. confusum and T. castaneum, responded to attractants in rice, wheat, barley and rye. Kennedy (41) proposed that the arrestant represented by scarcely volatile substances on which insects settle following contact gained by undirected movements, and the attractant comprising fairly volatile compounds, to which insects orient by directed movements. Willis and Roth (20) showed that the olfactory receptors of T. castaneum were the sensilla basiconica on the antennae. In the test arena the orientation of T. castaneum toward the odor of food was a direct response. Levinson and Levinson (21) stated that a blend of food volatiles from wheat or other cereals could induce the olfactory attraction of storage insects such as T. confusum, T. castaneum, and Sitophilus granarius (granary weevil). The less volatile food components

TABLE 1

VOLATILES ASSOCIATED WITH WHEAT (7)

METHANOL	PENTANAL
ETHANOL	ISOPENTANAL
ACETALDEHYDE	PENTANONE
ACETONE	2,3-DIMETHYL-3-PENTANONE
BUTANAL	HEXANAL
ISOBUTANAL	HEPTANAL
BUTANONE	OCTANAL
3-METHYL-2-BUTANONE	ETHYL ACETATE
BUTENAL	ISOAMYL ALCOHOL
DIACETYL	CYCLOPENTANONE
PROPANAL	PHENYLACETALDEHYDE
PROPANONE	AMYL ALCOHOL

including salts, sugars, and lipids, may also stimulate aggregation and feeding responses. Willis and Roth (22) demonstrated that the volatiles of the whole wheat flour and the moisture content play an important factor for the attraction of the T. castaneum. By placing the attractant beneath a screen, two days or several days starved T. castaneum were found to exhibit a strong attraction response. This was an olfactory response to flour. Loschiavo (23) found that with a free choice of food, the greatest numbers of insects were attracted to those foods containing the largest amounts of wheat germ. The food preferences of T. castaneum are similar to those of T. confusum. Later, Loschiavo (24) tested adult T. castaneum with six of the wheat products, i.e., enriched second-patent flour, second-patent flour plus 5% wheat germ, wheat middlings, coarse bran, fine bran, first tailings stock, and found that second-patent flour containing 5% wheat germ attracted more insects than any of the others. Other experiments showed a high response of the flat grain beetle to the high wheat-germ content foods. He also found that the adults of T. confusum and T. castaneum moved short distances toward preferred foods and suggested that these insects were responding to olfactory stimuli. This was in agreement with both Magis (25) and Leclercq (26). They showed that the food preferences of insects were independent of humidity but strongly induced by a particular chemical sense. Loschiavo (27, 28) demonstrated that the extracts of wheat germ contained highly active substances which elicited a strong chemosensory aggregation and feeding response by the T. castaneum and the T. confusum beetles. Tamaki et al. (29) identified 1-palmito-2,3-diolein, 2-linoleo-1,3-dipalmitin, and 1-palmito-2-linoleo-3-olein as major triglycerides in three active fractions from wheat germ that elicit the aggregation of T. confusum.

These triglycerides were synthesized and their biological activities verified. Tamaki et al. (30) also investigated the aggregation behaviour of T. confusum on 38 synthesized triglycerides. The beetles responded positively to nearly all of the unsaturated triglycerides but were little affected by saturated ones. Yinon et al. (31) found T. castaneum were attracted to saturated fatty acids and repelled by unsaturated ones. Cohen et al. (32) stated that certain lower fatty acids, viz. valeric, caproic, heptanoic, caprylic, pelargonic, capric and undecanoic acid strongly repel adult T. castaneum. Thus, it would be highly desirable to synthesize these repellents of T. castaneum as an alternative to the control of stored-product insects. Soliman (33) showed that phenyl-thio-carbamide (PTC) is toxic to T. castaneum as well as to animals and found the PTC chemoreceptor was localized in the antennae (34). Soliman (35) pointed out that PTC is a repellent to T. castaneum and older insects are more sensitive to this compound. Guy et al. (36) tested ninety synthetic compounds for the repellency of T. castaneum, and the most aversive one was N-butylsulfenyl-N,N-dimethyldithiocarbamate. This compound has some of the same radical groups as PTC.

Based on all of these papers, it can be concluded that insects are dependent on their olfactory organs to find preferred food or a host to complete their life cycle. There is limited information on the low molecular weight volatile components in raw wheat and wheat products that attract insects. This project was designed to provide more information about which volatiles attract Tribolium castaneum and to determine their chemical structures.

EXPERIMENTAL

A. GC SEPARATION AND GC/MS IDENTIFICATION OF CHEMICAL COMPOUNDS

1. EQUIPMENT

A Bendix model 2200 gas chromatograph equipped with both flame ionization and thermal conductivity detectors was used throughout the course of this study (Figure 3). A 1/8 inch by 6-feet stainless steel column, packed with Porapak QS 80-100 mesh (Waters Associates) was used for all separations. Porapak QS is a porous copolymer of styrene and divinyl benzene which can be used directly in gas chromatographic columns without further coating with a liquid phase.

A solid sampler (Figure 4) was used for the collection of the volatile compounds. The solid sampler is made of stainless steel and consists of five sections. The first section is threaded so that it can be attached to the injection port of the gas chromatograph. The connection is sealed by means of an O-ring. The second section is screwed into the first and again sealed by an O-ring. The third section has a hole drilled through its diameter. This allows for the addition of sample through the top and removal of spent sample through the bottom. The fourth and fifth sections are combined into a single unit, and consist of a plunger and an end cap which prevents the plunger from being ejected due to carrier gas back pressure. The plunger has a short channel into which the sample is placed by means of aligning this channel with the sample loading hole in the above mentioned third section. The sample is then inserted into the heated injection port by simply pushing in the plunger.

Figure 3; Gas chromatograph equipment.

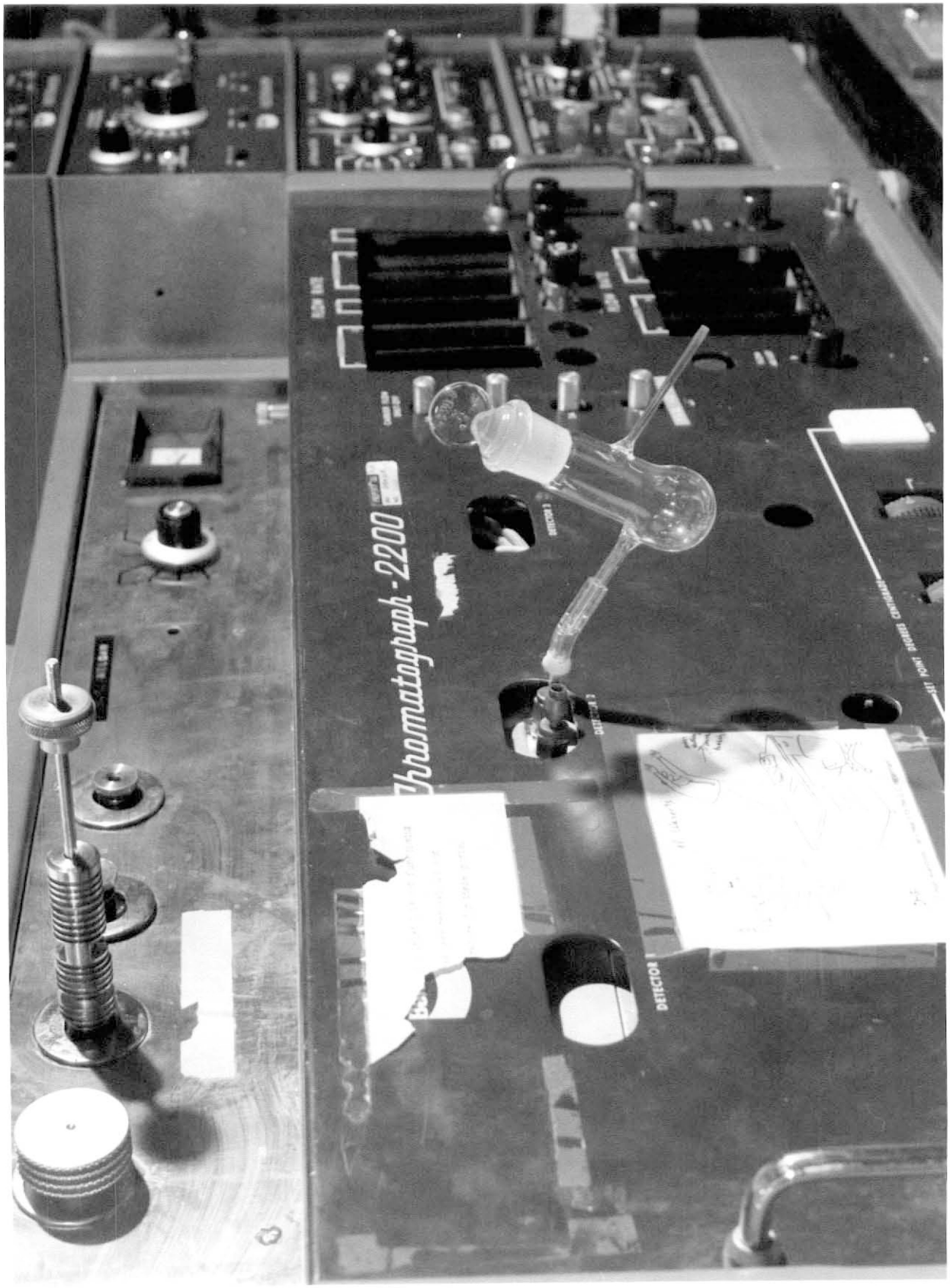

Chromatograph -2200
DETECTOR

Figure 4; Solid sampler

A. Facing view of part 1 and part 2.

B. O-ring.

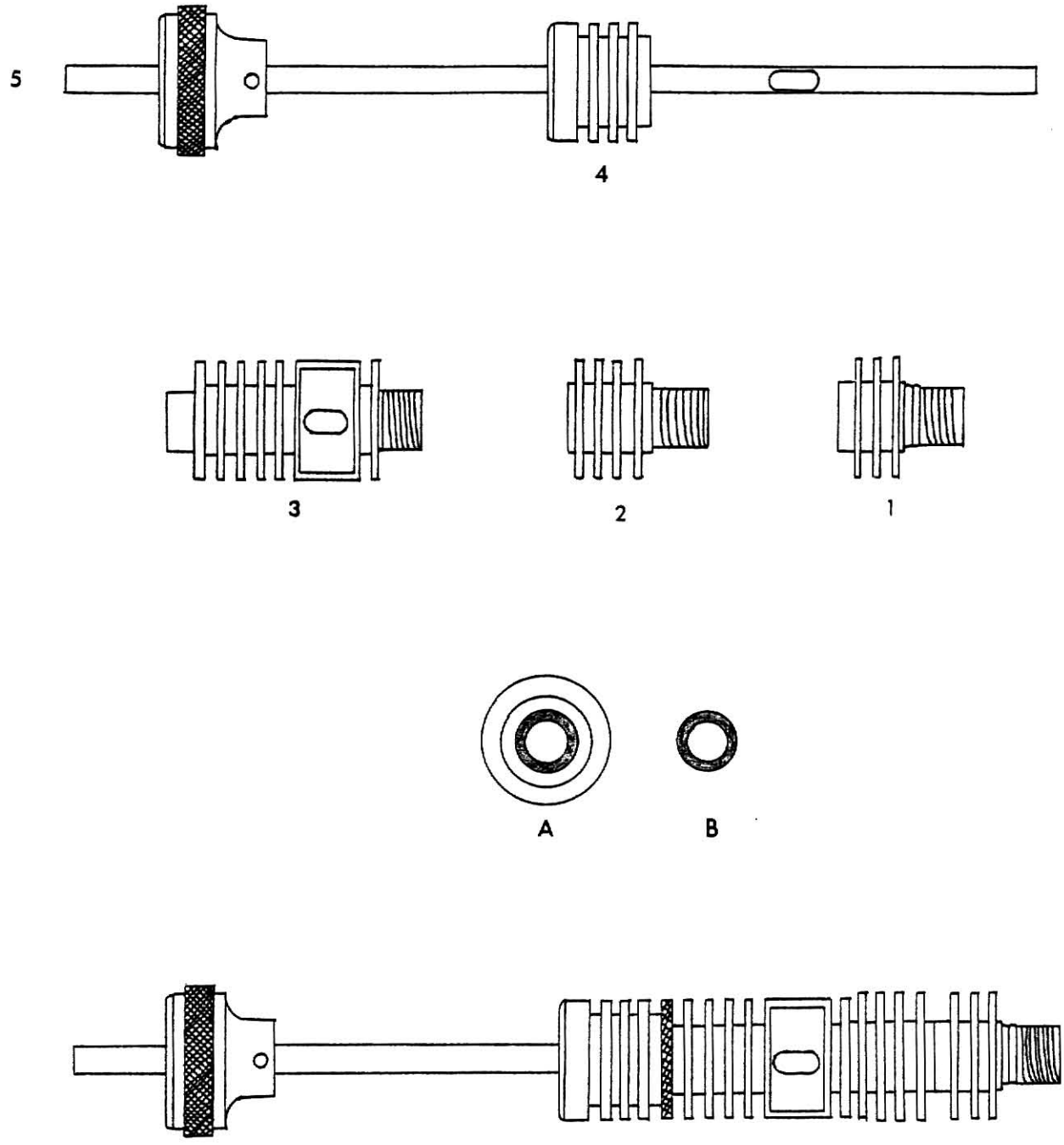


Figure 4

Figure 5; U-shaped stainless steel trap.

Figure 6; Glass collection vessel.

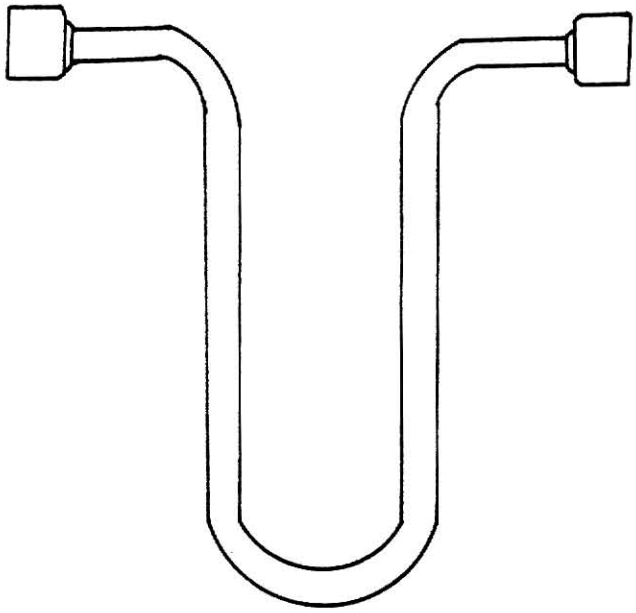


Figure 5

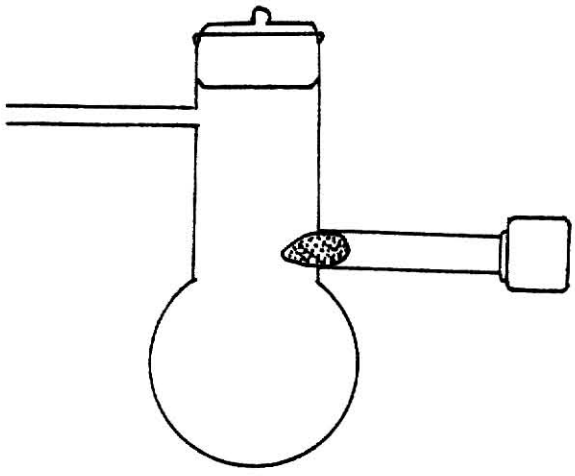


Figure 6

A U-shaped stainless steel trap (Figure 5), 1/8 inch by 6 inches is used to concentrate the volatile compounds prior to injection. One end of this trap is connected to the inlet of the gas chromatograph, the other end of the trap is either connected to the separation column or is capped off for later GC/MS analysis. Liquid nitrogen is used to cool the trap.

Zlatkis,et al. (37) studied three types of chromatographic packing material : Porapak P, carbon molecular sieve, and TENAX-GC. The excessive bleeding of Porpak P and the need to use temperatures in excess of 400°C in order to desorb organic volatiles from the carbon molecular sieves eliminated these materials as suitable adsorbants. TENAX-GC, a porous polymer of 2,6-diphenyl p-phenylene oxide, appeared to fulfill both requirements of efficient adsorptivity and easy desorptivity. TENAX-GC has been used previously in our laboratory by James (38) who isolated the fly attractant in cow manure and by Verma (39) who isolated and identified a cockroach repellent present in bay leaves. Consequently TENAX-GC, 60-80 mesh, (Applied Science Laboratories, Inc., State College, Pennsylvania) was used as the absorbent, with approximately 0.40 g being used in the U-tube trap.

The GC/MS identification work was done at the Midwest Center for Mass Spectrometry (MCMS) at Lincoln Nebraska. A Kratos-MS 50 Mass Spectrometer interfaced to a Perkin-Elmer Sigma II gas chromatograph and a Kratos DS-55 data system was used initially. All further analyses were performed on a Kratos-MS 80 Mass Spectrometer interfaced to a Carlo-Erba gas chromatograph and an Incos data system.

2. CHEMICALS

All chemicals were reagent grade unless specified otherwise.

Methanol; Lot no. 711871 from Fisher Scientific Company.

Acetaldehyde; Lot no. CVJ, from Mallinckrodt, Inc. St. Louis, Missouri.

Acetone; Lot no. 703173 from Fisher Scientific Company.

Ethanol; from Midwest Solvents, Atchison, Kansas.

2-Butanone; Lot no. 790317 from Fisher Scientific Company.

3-Methyl-2-Butanone; Lot no. A6B, from Eastman Kodak Company.

The standard addition method was used to aid in compound identification. Each standard material was diluted with deionized water.

3. PROCEDURE

Whole wheat kernels and whole wheat flour were obtained from the Department of Grain Science and Industry at Kansas State University. All samples were stored at room temperature. A whole wheat kernel or an equivalent amount of whole wheat flour was placed into the solid sampler. By means of the plunger the sample was inserted into the heated injection port of the gas chromatograph for 3 minutes. The temperature of the heating block was maintained at 240°C. The carrier gas (Helium) aids in driving the volatile components into the cold trap. This procedure was repeated 10 times in order to obtain a detectable quantity of the volatile compounds. The liquid nitrogen bath was removed and the system allowed to warm up to room temperature. The volatile components were desorbed from

the TENAX-GC as the oven was heated. The carrier gas flow rate was 20 ml/min, the injector port temperature was 240°C, the detector temperature was 245°C, and the oven temperature was programmed at 8°C/min from 60°C to 220°C. The attenuation was set at 10 and the chart speed was 1 cm/min.

Prior to collecting a sample, a blank run was performed in order to confirm the absence of any contaminants which may have been adsorbed on the TENAX-GC. Regenerating the TENAX-GC or Porpak-QS removed most of the contaminants which had been absorbed on them. A background chromatogram was obtained and appropriate corrections made on the sample chromatogram.

For the GC/MS identification, the sample collection was done in our laboratory. The volatile compounds were collected and trapped in the U-tube as described previously. The U-tube trap was then sealed with Swagelock unions and plugs and stored in liquid nitrogen until the GC/MS analyses were performed.

The conditions used for the GC attached to the mass spectrometer were the same as described above. The mass spectrometer conditions were as follows:

Temperature of ion source	250°C
Voltage	4000 V
Scanning rate	1 sec/decade
Beam current	100 μA
Band width	3000 Hz

The ionization was accomplished by means of electron impact.

The mass spectra were recorded and these data were compared with spectra reported in the literature (40). The retention times of the identified compounds were then checked and matched with the standards by the method of standard addition.

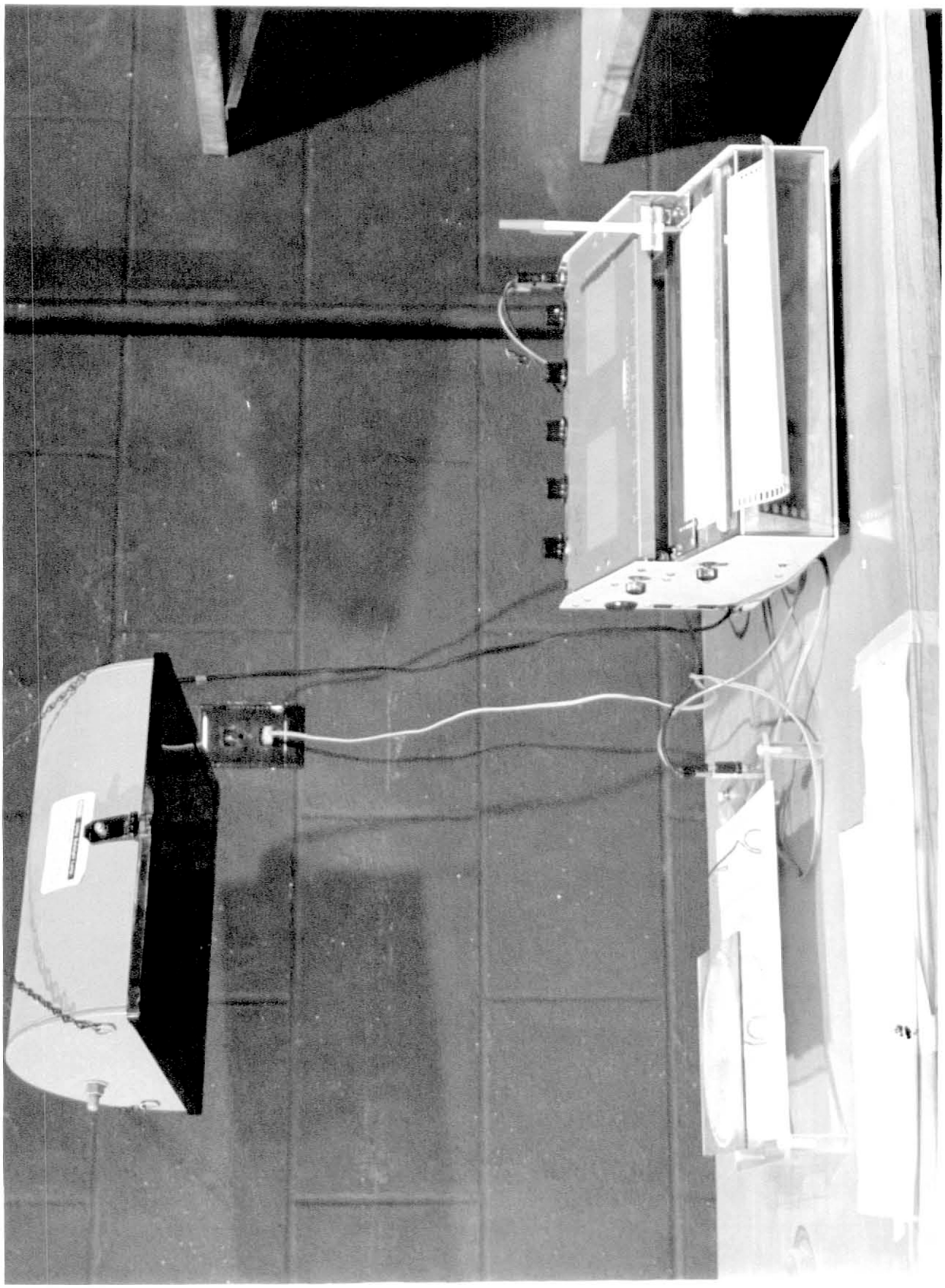
B. BIOASSAY TEST

1. EQUIPMENT

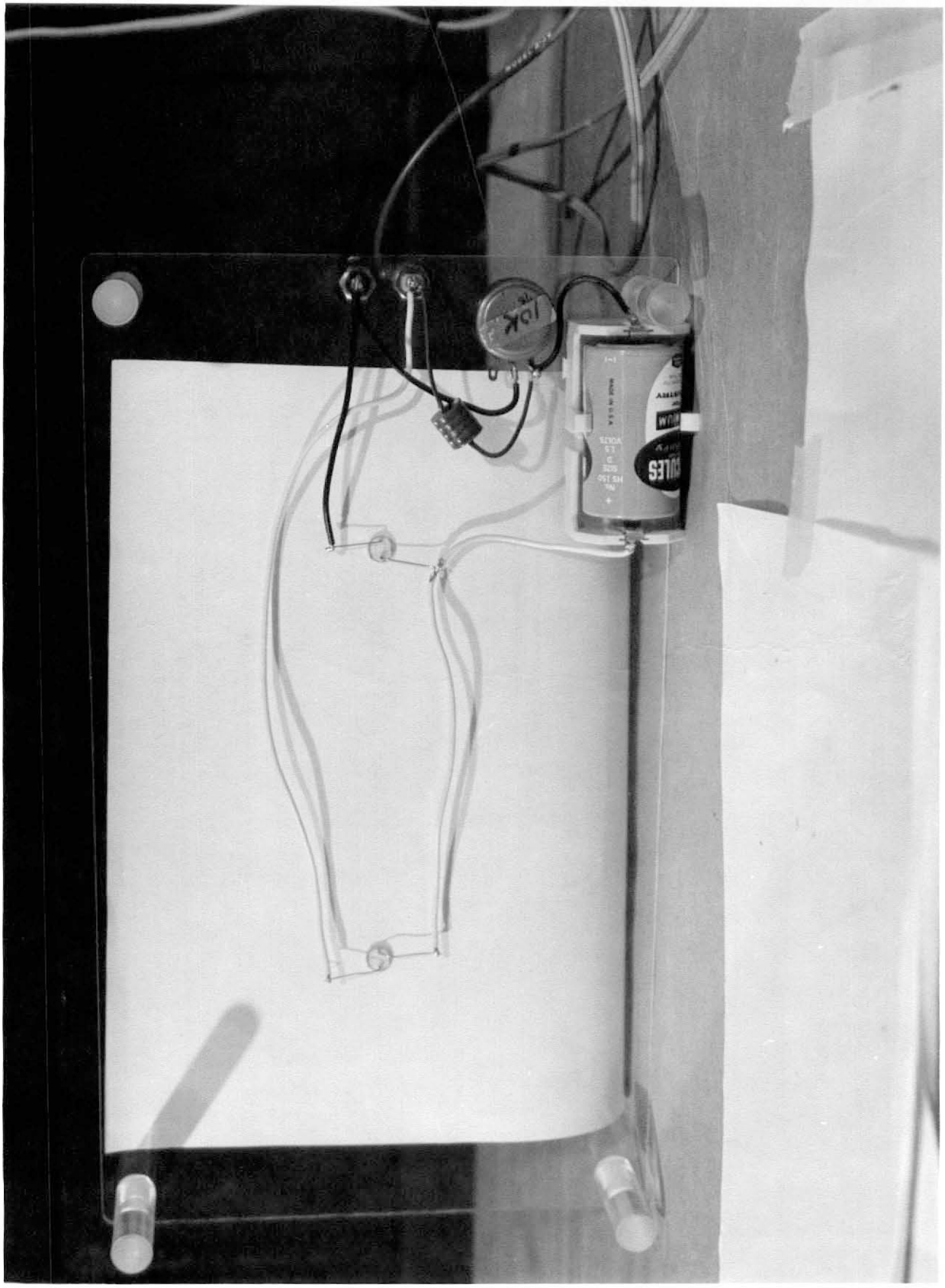
The equipment used for detecting the aggregate response of red flour beetles (RFB) to volatile flour compounds was described by Pinniger and Collins (42). Some modifications were made by Seifelnasr (43), Department of Entomology, Kansas State University. The equipment consists of a light sensitive event detector and a Heath Model EUW-20A servo Recorder (Figure 7). All of the bioassays were conducted with this equipment which is located in the Department of Entomology. The detector unit was used in conjunction with a Plexiglas^{TM} cage consisting of a rectangular base (35 by 20 cm) and a Plexiglas^{TM} ring 15.2 cm in diameter and 2.6 cm high. Two light sensitive resistors (sensor) were inserted in the base and were 15 cm apart. Filter paper was used to cover the base including the light sensitive resistors. The cage was covered with a glass lid. The test sensor in the cage was covered while the other one was outside the test cage. A red light (15-watt incandescent light bulb behind a red Kodak Safelight Glass Filter Series IA) suspended right above the test equipment was adjusted so that the light intensity was the same on each sensor. Insect pins, 4 cm in length, were used to suspend the test and control materials on a wire frame (13.6 by 2 cm) right above the sensor (Figure

Figure 7; A. Light sensitive event detector.

B. Bottom view of light sensitive event detector.



A.



B.

7). It was high enough so that insects could not touch the test materials with their antennae. This design should exclude factors other than olfactory. The pin was bent about 60° at the center in order to hang on the wire frame right above the sensor. The pointed end of the pin was inserted into a ball shaped test or control material. When an insect passed over or remained on a sensor an imbalance in light intensity between the reference and test sensors was effected causing the recorder pen to deflect from the zero point and remain there until the insect moved away from the sensor. The chart speed was 1 inch per minute, which permitted calculation of the time that the insects spent on the sensor.

2. INSECT REARING AND HANDLING

The red flour beetles were reared and provided by The Department of Entomology. To produce test insects, mature adults were placed in a mixture of 95% whole wheat flour and 5% brewer's yeast medium. After 30 to 35 days, progeny had become pupae which were separated by sex and placed in clean plastic boxes. Only females were used for tests. On each consecutive day, some of pupae would become adults. Those adults were removed, placed in the medium and dated. This process was continued for several days. Both pupae and adults were kept in a rearing room which was maintained at 27±1°C and 67±3% (R.H.). The 2-3-day-old adult insects were removed from the medium, and starved for 24 hours prior to testing in order to ensure a uniform nutritional condition. The insects used for testing the aggregate response to the wheat volatiles were 3-4 days old.

3. PROCEDURE

a. Sample Preparation

The volatile components of the wheat separated by gas chromatography were tested using the above apparatus and insects. All of the volatile compounds were collected with the solid sampler described in Part A. Because of the limited amount of sample, the use of a splitter to collect a portion of the separated volatile components prior to the destructive FID detector proved to be unsatisfactory. Therefore, a nondestructive thermal conductivity detector was used; the individual components could then be collected at the outlet of the T.C. detector. A small glass apparatus (Figure 6) was used for this purpose. During component collection, a liquid nitrogen bath was used as an aid in trapping the sample on the absorbent. TENAX-GC, 60-80 mesh was again used as the absorbent, and was heated to 320°C for at least 5 hours prior to use.

Approximately 0.1 g TENAX-GC was wrapped in a 2 cm square piece of Kimwipe tissue and formed into a ball while wearing a pair of clean plastic gloves to prevent contaminating odors. This material was then inserted into the glass collection vessel (Figure 6), which was connected to the T.C. outlet by means of Swagelock fittings. The volatile components were collected according to their retention times. After the component was trapped in the ball-shaped mass of TENAX-GC, it was removed from the collection vessel and stored in another bottle under liquid nitrogen until used for the bioassay. Only two bioassay tests were carried out on any set of insects of the same age. For any chromatographic separations only two specific volatiles were collected. This was due to the close proximity of the eluting peaks. There was insufficient time to collect two consecutive components due to the necessity of removing and replacing the TENAX-GC trapping material. The

two components collected on the same run were every other peak, such as peak #4 and peak #6 collected on the same run.

b. Aggregate Response Test

All of the bioassay tests were carried out under red light only, since red flour beetles are unaffected by either red or blue light (24). The room temperature was 75±5°F. Ten 3-4-day-old adult female RFB starved for one day were placed in the test cage (Figure 7), and left under red light for at least 1 hour to acclimate prior to testing. A control test was run on a blank consisting of a clean TENAX-GC without any chemical absorbed on it wrapped in a Kimwipe tissue, and shaped into a ball resembling the shape of the specific test material which had been stored in liquid nitrogen. This material was placed on a pin and suspended on a wire frame above the sensor in the cage. The material was warmed to room temperature. Three successive 10 minute periods of observations were conducted. The blank material was carefully replaced by the volatile compound absorbed on a similarly shaped TENAX-GC ball. Another three successive 10 minute periods of observations were done. The response to the compound was compared with the control in both time and number of visits. The same set of insects was tested with one control and two different compounds. Each set of insects was tested between 1 P.M. and 2 P.M..

RESULTS AND DISCUSSION

A. Identification of volatile compounds from the wheat kernel and whole wheat flour

After evaluating several column packing materials to separate the different volatile components present in wheat, Porapak-QS, 80-100 mesh seemed to work the best. It was found that the compounds emerge from a Porapak-QS column in a sequence based upon their polarities and molecular weight. This compared well with previous results (39). TENAX-GC, a porous polymer, which fulfills both the requirements of efficient adsorptivity and desorptivity, was selected as an absorbent for collecting the sample in a U-shaped cold trap. The solid sampler was used for the introduction of the volatile compounds absorbed on TENAX-GC for further driving into the separating column. By using this assembly, the typical gas chromatograms of whole wheat kernel and whole wheat flour are shown in Figures 9 and 10. Compared to the blank, Figure 8, we can see that peaks #1 through #3 came from the background, such as H_2O, CO_2, O_2, etc.. The unnumbered peak preceding the peak #1 is due to the pressure change of the carrier gas.

The information obtained from the gas chromatogram shows that there are at least nine different components present in the volatile fraction of wheat products. The volatile fractions of the wheat kernel and wheat flour are the same as compared using the two chromatograms in Figures 9 and 10. The milling process did not cause any appreciable loss of volatiles from the wheat. The wheat kernel and the whole wheat flour emanate the same volatiles into air which attract the insect.

Figure 8; Background chromatogram of blank check running on Porapak QS column.

Figure 8

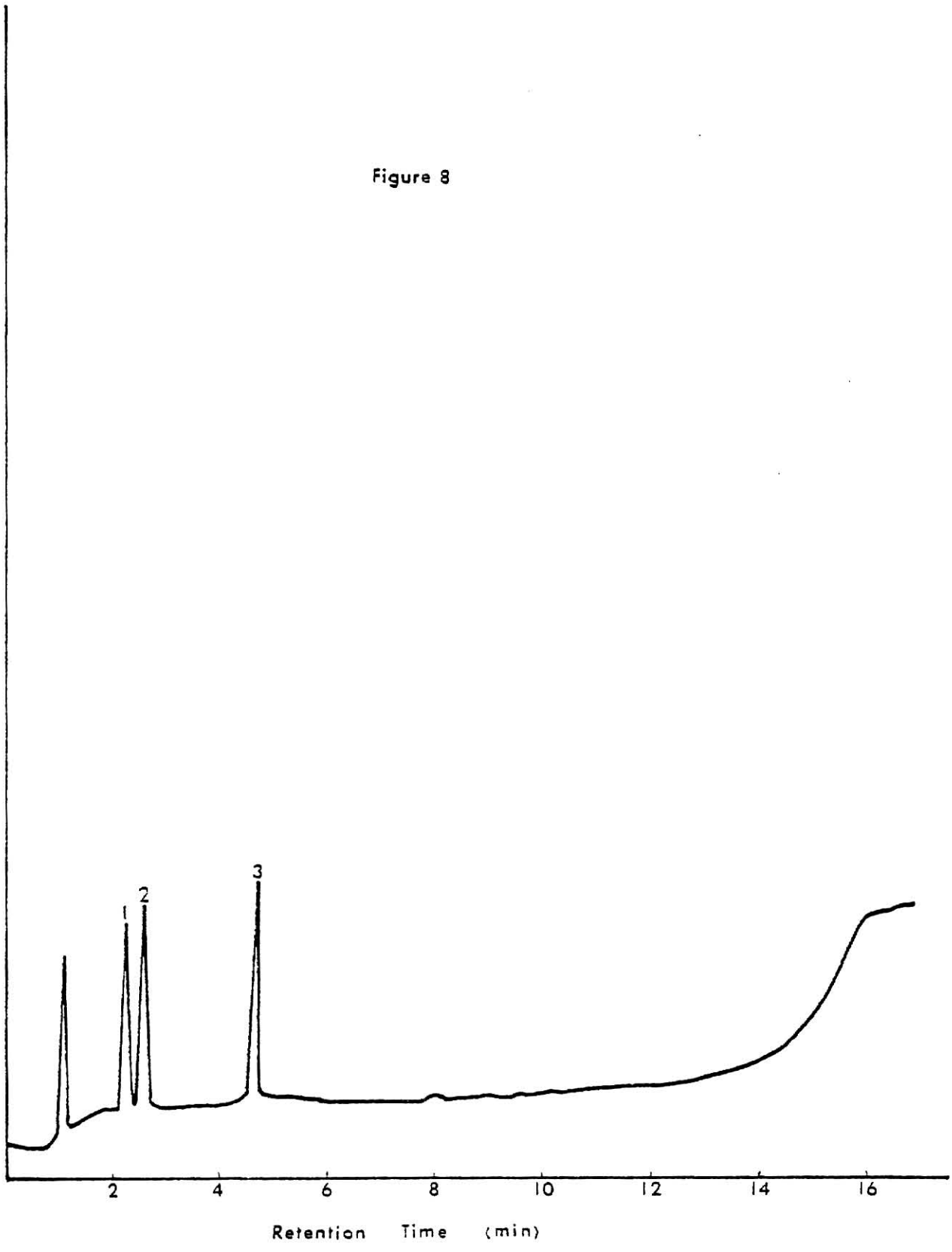


Figure 9; Chromatogram of volatile compounds of whole wheat kernel.

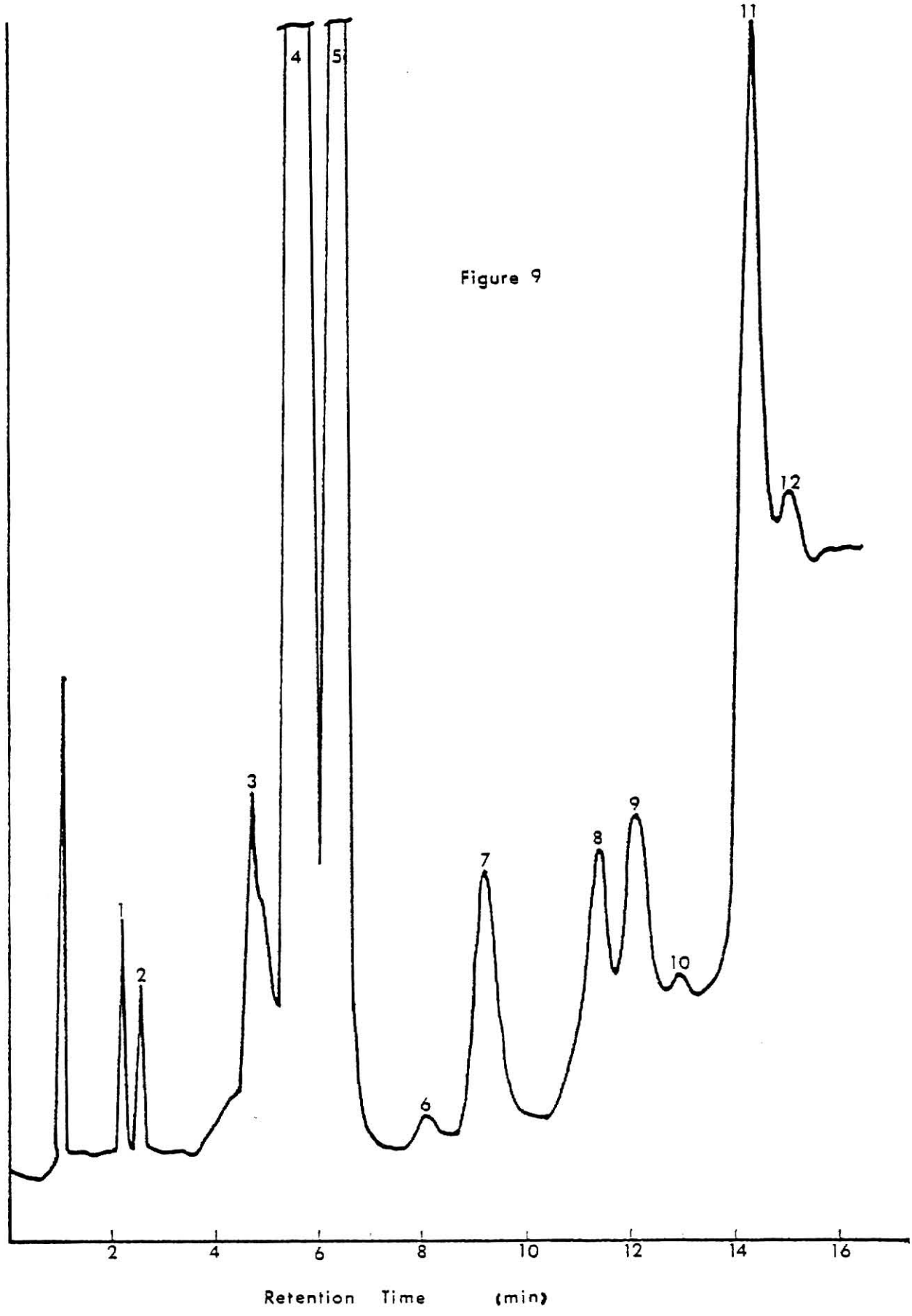


Figure 9

Figure 10; Chromatogram of volatile compounds of whole wheat flour.

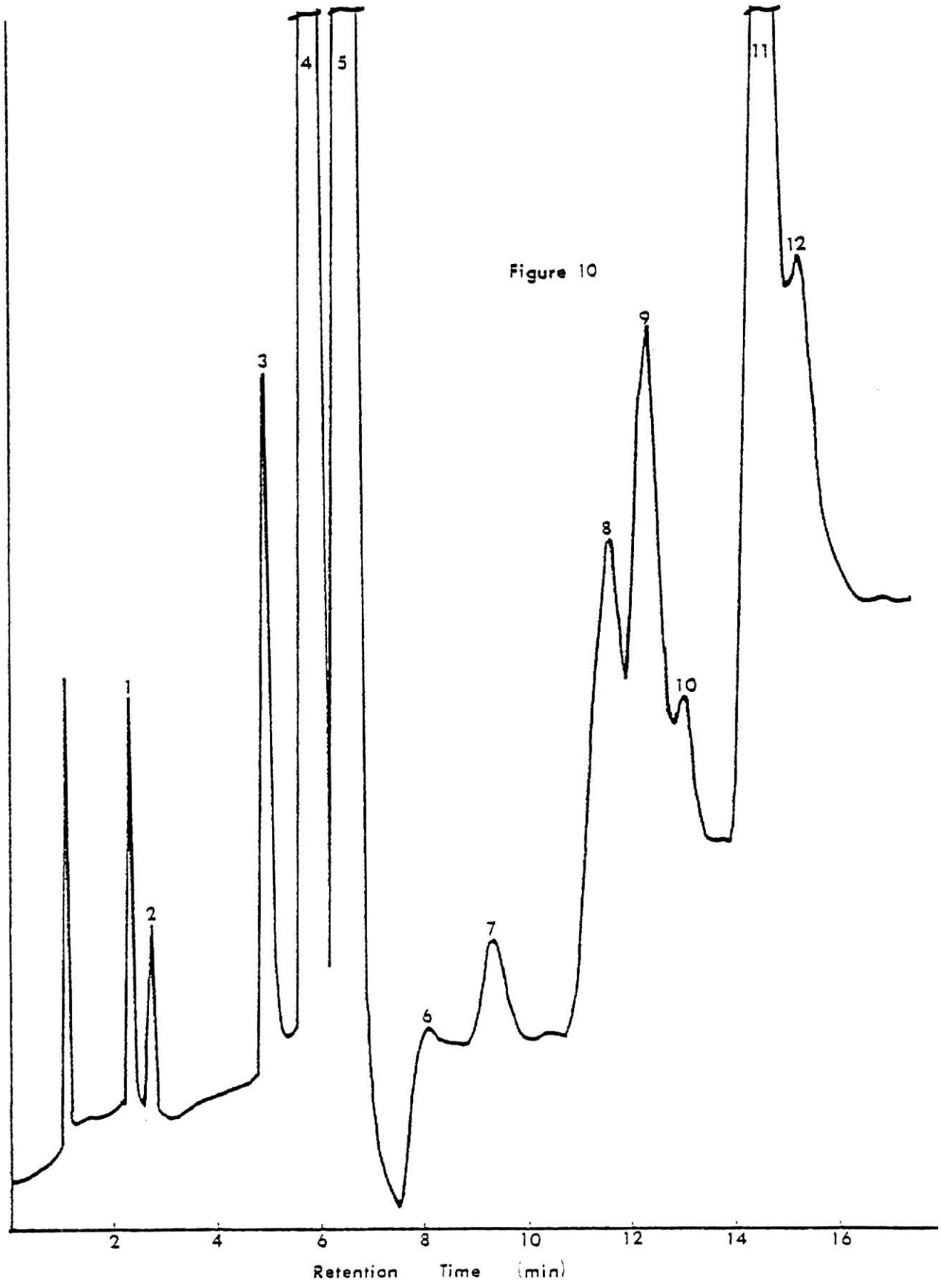


Figure 10

To identify the components, mass spectral data were obtained. Six compounds were identified by using the mass spectral data and confirmed by a standard addition GC method. For the specific compound, the retention time was matched and the peak area was increased. The identified compounds are listed in Table 2 along with the retention times on the Porapak QS column. The mass spectral fragmentation patterns of these compounds are presented in Figures 11 through 16. The analysis of the patterns is presented in Tables 3 through 8. Peaks #4 and #5 gave stronger insect responses than the others. The temperature of the gas chromatograph was programmed from 60°-220°C. The boiling point of each volatile compound separated and identified was within this region,i.e. low molecular weight compounds.

B. Bioassay test on RFB

The bioassay test was performed initially with the mixture of the first half and then the second half of all the volatiles from the wheat. Both responses were positive. This was followed by testing the mixture of the first quarter and the second quarter of the first half and the second half of the volatile compounds separately. All of the positive responses were shown on Tables 9 through 11. Because of the positive results, possibly all of the volatiles could be attractants to the insect and not just some of them. Each compound separated by the gas chromatograph was used to test the individual olfactory response. The results are shown on Tables 12 through 17.

All the test materials were prepared in our laboratory and were handled with clean plastic gloves to prevent as much as possible

TABLE 2

IDENTIFICATION OF VOLATILE COMPOUNDS IN WHEAT

Peak #	Compound	Retention time
4	METHANOL	5 Mins. 46 Secs.
5	ACETALDEHYDE	6 Mins. 28 Secs.
7	ETHANOL	9 Mins. 19 Secs.
8	ACETONE	11 Mins. 30 Secs.
11	METHYL ETHYL KETONE	14 Mins. 30 Secs.
12	3-METHYL-2-BUTANONE	15 Mins. 10 Secs.

Figure 11; Mass spectral fragmentation pattern of peak #4.

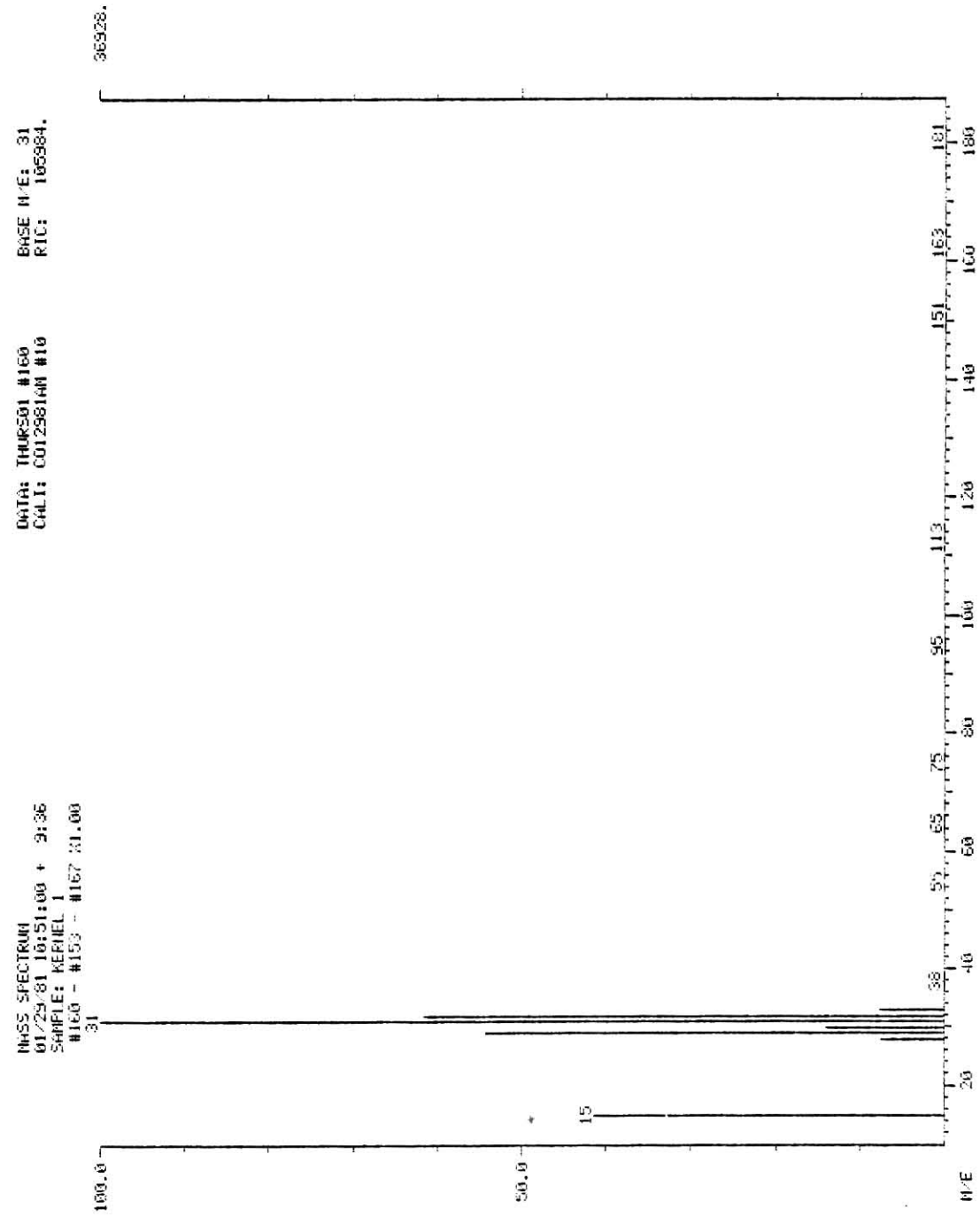

MASS SPECTRUM
01/29/81 10:51:00 + 3:36
SAMPLE: KEFINEL 1
#160 - #153 - #167 31.00
31
DATA: THURS01 #160
CALI: C012981HM #10
BASE M/E: 31
RIC: 165984.
36928.
100.0
50.0
15
38
M/E

TABLE 3

Mass Spec. Analysis of Peak #4

Mass	Intensity/Base
31	100%
32	61%
29	54%
15	42%
30	14%

Proposed Structure

31 CH_2OH^+

32 $CH_3OH^{+\cdot}$

29 CHO^+

15 CH_3^+

30 $CHOH^{+\cdot}$ or $CH_2O^{+\cdot}$

Chemical Name: METHANOL

Figure 12; Mass spectral fragmentation pattern of peak #5.

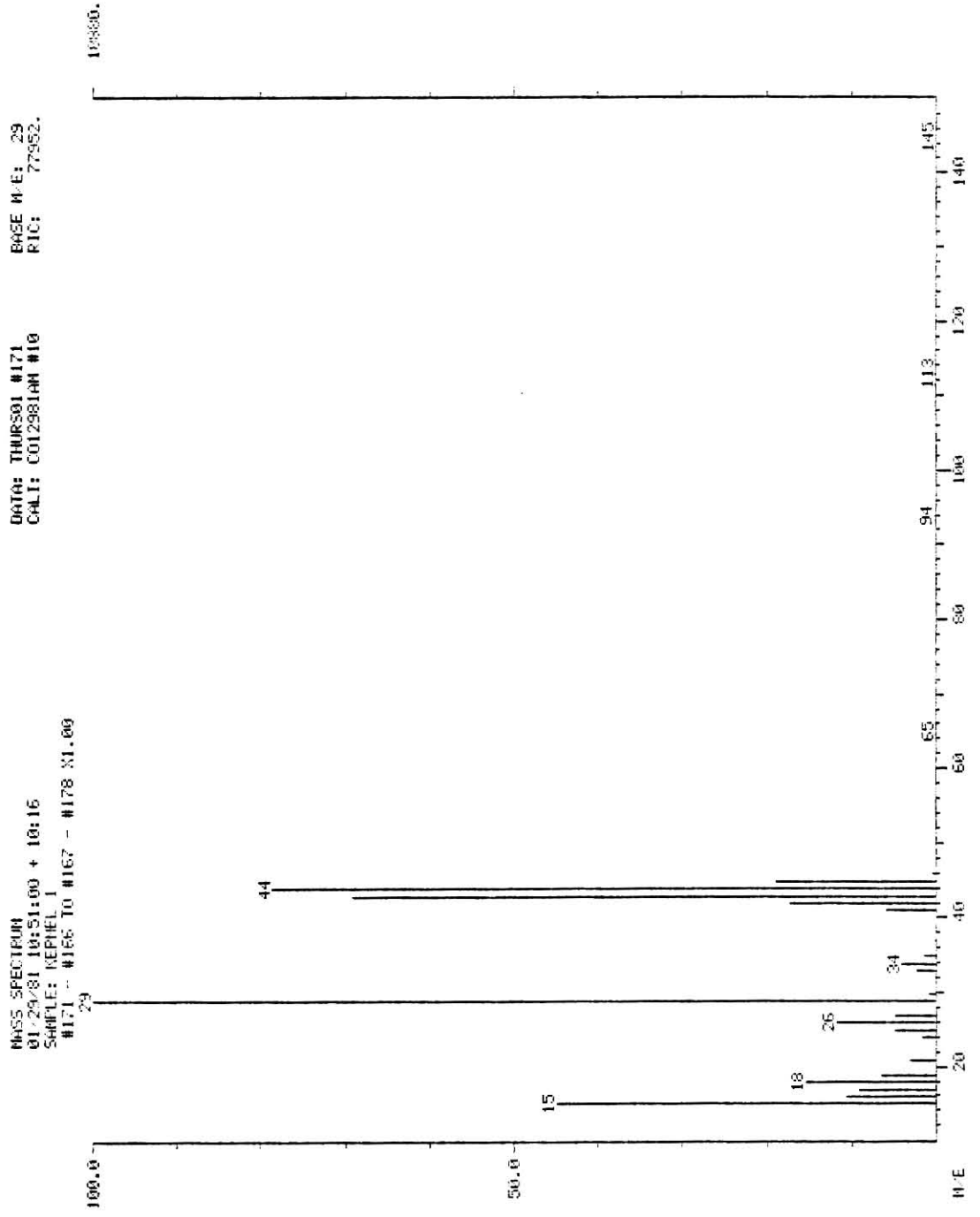

MASS SPECTRUM
01-29-81 10:51:00 + 10:16
SAMPLE: KEPHEL 1
#171 - #166 TO #167 - #178 X1.00
DATA: THURS01 #171
CAL1: C012981AM #10
BASE M/E: 29
RIC: 77952.
10000.
100.0
50.0
M/E
29
44
15
18
26
34
20
40
55
60
80
94
100
113
120
140
145

TABLE 4

Mass Spec. Analysis of Peak #5

Mass	Intensity/Base
29	100%
44	78%
43	68%
15	45%
45	18%
42	16%

	Proposed Structure
29	CHO^{+}
44	$CH_3CHO^{+\cdot}$
43	CH_3CO^{+}
15	CH_3^{+}
45	$CH_3CHO^{+\cdot}$
42	$CH_2CO^{+\cdot}$

Chemical Name: ACETALDEHYDE

Figure 13; Mass spectral fragmentation pattern of peak #7.

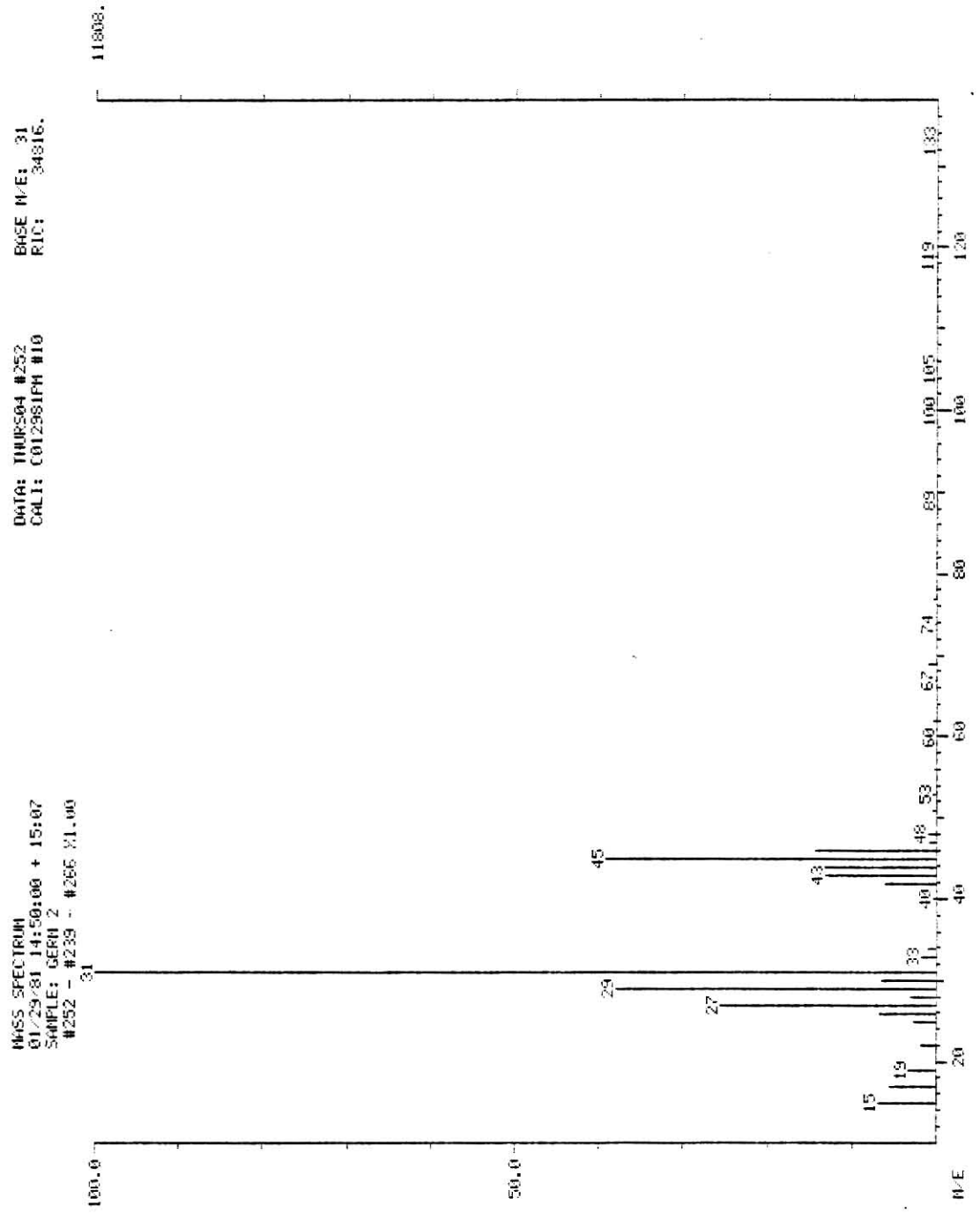

MASS SPECTRUM
01/29/81 14:50:00 + 15:07
SAMPLE: GERM 2
#252 - #239 - #266 X1.00
DATA: THURS04 #252
CAL1: C01298IPH #10
BASE M-E: 31
RIC: 34816.
11808.
100.0
50.0
M-E
15
19
20
27
29
31
33
40
43
45
48
53
60
67
74
80
89
99
105
106
119
120
133

TABLE 5

Mass Spec. Analysis of Peak #7

Mass	Intensity/Base
31	100%
45	39%
29	38%
27	26%
46	14%

	Proposed Structure
46	$CH_3CH_2OH^{+\cdot}$
45	CH_3CHOH^{+}
31	CH_2OH^{+}
29	$CH_3CH_2^{+}$

Chemical Name: ETHANOL

Figure 14; Mass spectral fragmentation pattern of peak #8.

DS-55 MASS INTENSITY REPORT:
17570Z.128 [TIC=6406, 100%=4070] EI

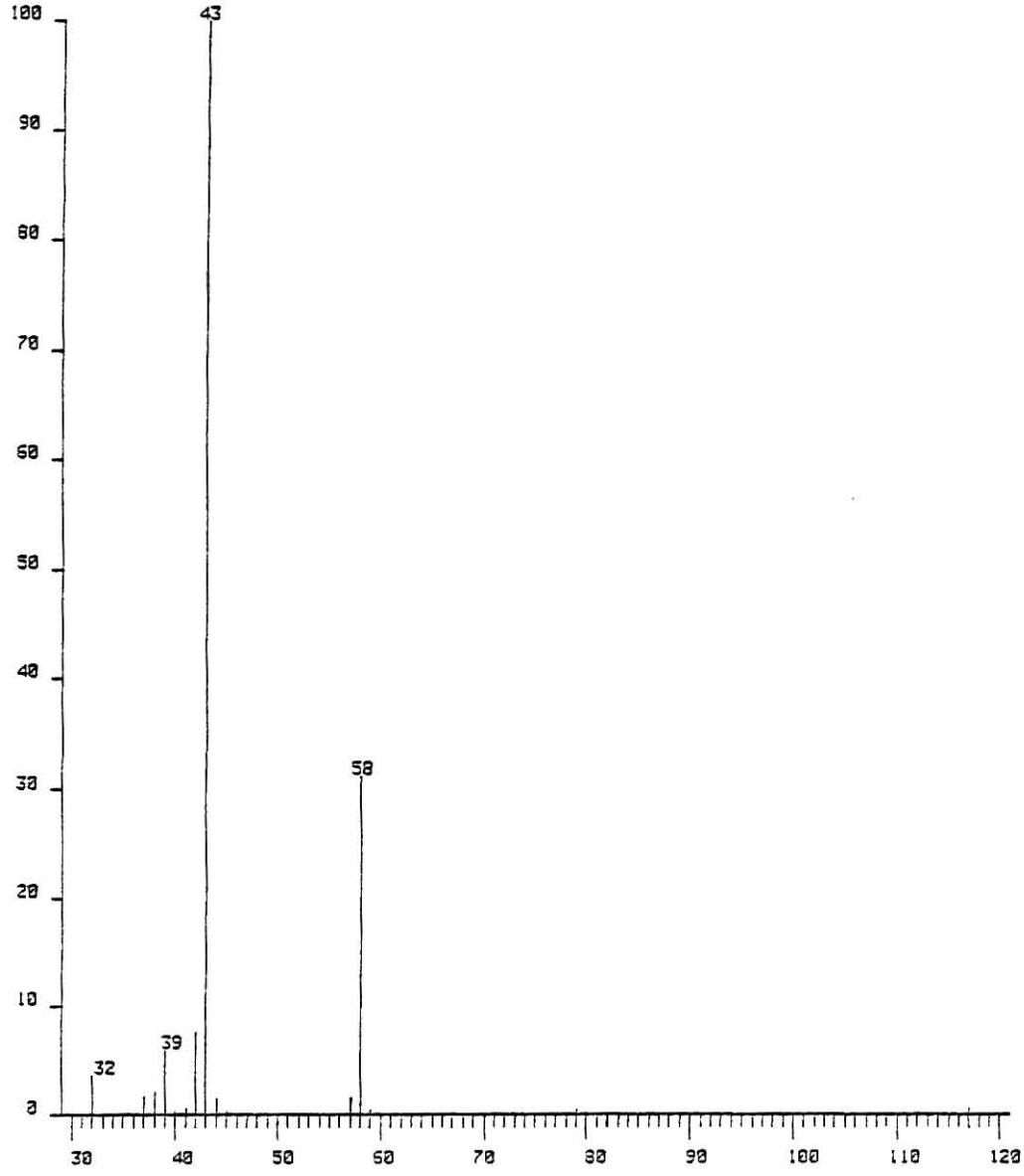


TABLE 6

Mass Spec. Analysis of Peak #8

Mass	Intensity/Base
43	100%
58	31%
42	8%

Proposed Structure

43 CH_3CO^+

58 $(CH_3)_2CO^{+\cdot}$

Chemical Name: ACETONE

Figure 15; Mass spectral fragmentation pattern of peak #11.

DS-55 MASS INTENSITY REPORT:
1757MZ.147 [TIC=43592, 100%=16436] EI

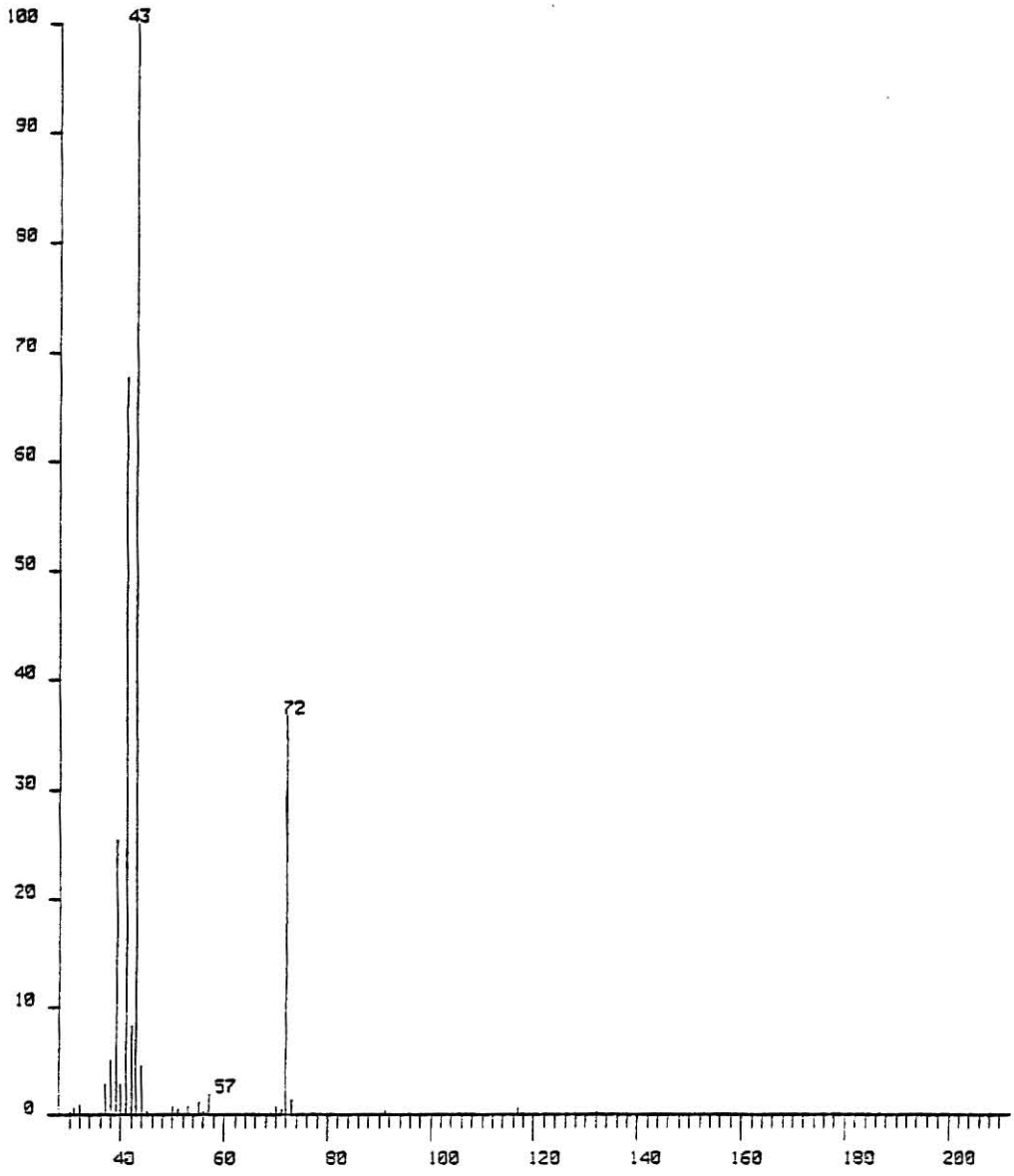


TABLE 7

Mass Spec. Analysis of Peak #11

Mass	Intensity/Base
43	100%
41	68%
72	37%
39	26%
57	2%

Proposed Structure

43 CH_3CO^+

72 $CH_3COC_2H_5^{\cdot +}$

41 $CHCO^+$

57 $CH_3COCH_2^+$

Chemical Name: METHYL ETHYL KETONE

Figure 16; Mass spectral fragmentation pattern of peak #12.

DS-55 MASS INTENSITY REPORT:
1757MZ.154 [TIC=4084, 100%=1597] EI

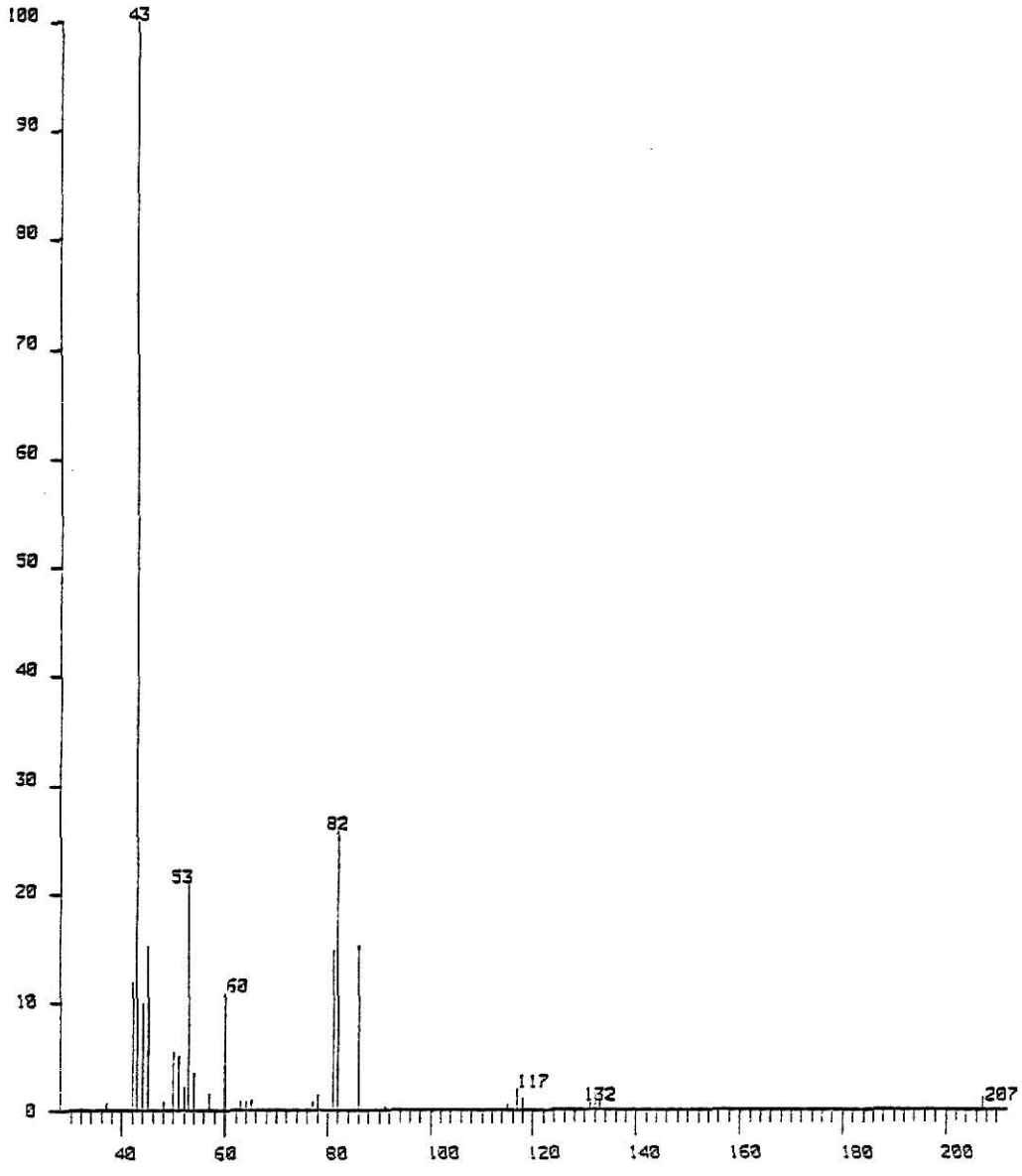


TABLE 8

Mass Spec. Analysis of Peak #12

Mass	Intensity/Base
43	100%
82	26%
53	22%
86	15%
45	15%

Proposed Structure

43 CH_3CO^+ and/or $C_3H_7^+$

86 $CH_3COCH(CH_3)_2^{+\cdot}$

53 $CCOCH^+$

Chemical Name: 3-METHYL-2-BUTANONE

TABLE 9

Olfactory response of RFB to the first half and second half volatile compounds of wheat separated by gas chromatograph.

	TIME REPLICATES (MIN)	NO. OF VISITS	TIME SPENT (MIN)	RELATIVE NO. OF VISITS	RELATIVE TIME SPENT
CONTROL TENAX	FIRST 10	7	0.09		
	SECOND 10	8	0.60		
	THIRD 10	22	2.16		
	30	37	2.85	1	1
FIRST HALF COMPOUNDS	FIRST 10	55	5.02		
	SECOND 10	41	4.01		
	THIRD 10	34	3.63		
	30	130	12.66	3.5	4.5
SECOND HALF COMPOUNDS	FIRST 10	26	5.51		
	SECOND 10	39	2.66		
	THIRD 10	22	7.44		
	30	87	15.61	2.4	5.4

* The relative value was obtained by taking each value from the specific compounds testing divided by the value of the control testing from the table 9 all through 17.

TABLE 10

Olfactory response of RFB to the first quarter and second quarter of the first half volatile compounds of wheat separated by gas chromatograph.

	TIME REPLICATES (MIN)	NO. OF VISITS	TIME SPENT (MIN)	RELATIVE NO. OF VISITS	RELATIVE TIME SPENT
CONTROL TENAX	FIRST 10	29	2.54		
	SECOND 10	11	1.05		
	THIRD 10	7	0.72		
	30	47	4.31	1	1
FIRST 1/4 OF THE FIRST 1/2	FIRST 10	28	3.38		
	SECOND 10	1	0.09		
	THIRD 10	11	8.31		
	30	40	11.78	0.9	2.7
SECOND 1/4 OF THE FIRST 1/2	FIRST 10	14	4.91		
	SECOND 10	24	4.22		
	THIRD 10	15	1.39		
	30	53	10.52	1.1	2.4

TABLE 11

Olfactory response of RFB to the first quarter and second quarter of the second half volatile compounds of wheat separated by gas chromatograph.

	TIME REPLICATES (MIN)	NO. OF VISITS	TIME SPENT (MIN)	RELATIVE NO. OF VISITS	RELATIVE TIME SPENT
CONTROL TENAX	FIRST 10	16	0.97		
	SECOND 10	25	2.09		
	THIRD 10	27	3.59		
	30	68	6.65	1	1
FIRST 1/4 OF THE SECOND 1/2	FIRST 10	40	4.63		
	SECOND 10	8	7.41		
	THIRD 10	16	8.50		
	30	64	20.53	0.9	3.1
SECOND 1/4 OF THE SECOND 1/2	FIRST 10	24	6.66		
	SECOND 10	27	5.25		
	THIRD 10	17	4.41		
	30	78	16.32	1.1	2.5

TABLE 12

Olfactory response of RFB to the compounds of peak #1 and peak #3

	TIME REPLICATES (MIN)	NO. OF VISITS	TIME SPENT (MIN)	RELATIVE NO. OF VISITS	RELATIVE TIME SPENT
CONTROL TENAX	FIRST 10	2	0.02		
	SECOND 10	10	0.31		
	THIRD 10	17	1.91		
	30	29	2.24	1	1
PEAK #1	FIRST 10	23	7.24		
	SECOND 10	30	3.25		
	THIRD 10	10	8.74		
	30	63	19.23	2.2	8.6
PEAK #3	FIRST 10	10	7.66		
	SECOND 10	16	7.34		
	THIRD 10	8	9.25		
	30	34	24.25	1.2	10.8

TABLE 13

Olfactory response of RFB to the compounds of peak #2 and peak #4

	TIME REPLICATES (MIN)	NO. OF VISITS	TIME SPENT (MIN)	RELATIVE NO. OF VISITS	RELATIVE TIME SPENT
CONTROL TENAX	FIRST 10	6	0.19		
	SECOND 10	0	0		
	THIRD 10	3	0.06		
	30	9	0.25	1	1
PEAK #2	FIRST 10	11	0.19		
	SECOND 10	0	0		
	THIRD 10	10	0.25		
	30	21	0.44	2.3	1.8
PEAK #4	FIRST 10	28	1.25		
	SECOND 10	7	0.24		
	THIRD 10	6	0.18		
	30	41	1.67	4.6	6.7

TABLE 14

Olfactory response of RFB to the compounds of peak #5 and peak #7

	TIME REPLICATES (MIN)	NO. OF VISITS	TIME SPENT (MIN)	RELATIVE NO. OF VISITS	RELATIVE TIME SPENT
CONTROL TENAX	FIRST 10	6	0.33		
	SECOND 10	3	0		
	THIRD 10	5	0.34		
	30	14	0.67	1	1
PEAK #5	FIRST 10	35	5.03		
	SECOND 10	34	6.63		
	THIRD 10	8	0.41		
	30	77	12.06	5.5	18.2
PEAK #7	FIRST 10	28	4.00		
	SECOND 10	22	0.97		
	THIRD 10	20	1.06		
	30	70	6.03	5.0	9.1

TABLE 15

Olfactory response of RFB to the compounds of peak #6 and peak #8

	TIME REPLICATES (MIN)	NO. OF VISITS	TIME SPENT (MIN)	RELATIVE NO. OF VISITS	RELATIVE TIME SPENT
CONTROL TENAX	FIRST 10	14	1.16		
	SECOND 10	18	0.88		
	THIRD 10	5	0.28		
	30	37	2.32	1	1
peak #6	FIRST 10	37	3.19		
	SECOND 10	40	6.84		
	THIRD 10	31	2.56		
	30	108	12.59	2.9	5.5
PEAK #8	FIRST 10	45	2.56		
	SECOND 10	32	1.56		
	THIRD 10	33	3.38		
	30	110	7.50	3.0	3.2

TABLE 16

Olfactory response of RFB to the compounds of peak #9 and peak #11

	TIME REPLICATES (MIN)	NO. OF VISITS	TIME SPENT (MIN)	RELATIVE NO. OF VISITS	RELATIVE TIME SPENT
CONTROL TENAX	FIRST 10	10	0.66		
	SECOND 10	8	0.13		
	THIRD 10	12	1.00		
	30	30	1.78	1	1
PEAK #9	FIRST 10	17	0.94		
	SECOND 10	4	0.25		
	THIRD 10	6	0.84		
	30	27	2.03	0.9	1.1
PEAK #11	FIRST 10	10	0.59		
	SECOND 10	21	4.13		
	THIRD 10	23	5.97		
	30	54	10.69	1.8	6.0

TABLE 17

Olfactory response of RFB to the compounds of peak #10 and peak #12

	TIME REPLICATES (MIN)	NO. OF VISITS	TIME SPENT (MIN)	RELATIVE NO. OF VISITS	RELATIVE TIME SPENT
CONTROL TENAX	FIRST 10	8	0.28		
	SECOND 10	41	2.41		
	THIRD 10	43	4.50		
	30	92	7.19	1	1
PEAK #10	FIRST 10	36	2.28		
	SECOND 10	54	4.34		
	THIRD 10	7	9.19		
	30	97	15.81	1.1	2.2
PEAK #12	FIRST 10	16	7.56		
	SECOND 10	40	2.38		
	THIRD 10	25	1.03		
	30	81	10.97	0.9	1.5

interference from human odors. As discussed before, peaks #1 through #3 came from the background, they might be H_2O, CO_2, etc.. Willis and Roth (20), (22) showed a positive reaction of T. castaneum and T. confusum to CO_2 and moisture. When the sample of wheat was added or drained, the solid sampler was in direct contact with air. Therefore, the components in the air were also trapped onto the TENAX-GC. This could explain the presence of H_2O and CO_2 in our sample. Another reason for the presence of water is that the water content in wheat is about 12%. When the volatile compounds were driven into the cold trap, water was also among the volatiles. This may be the explanation for the positive olfactory response of the insect to the first three peaks.

The control test was always carried out before two sets of specific materials were tested. A positive response was obtained in all of the control tests. There may be several explanations for this result: 1. All samples were collected, and all of the test materials were kept, in our laboratory. The vapors of the various chemicals in the laboratory might have absorbed onto TENAX-GC when we prepared the ball-shaped control materials. 2. Liquid nitrogen was used to freeze the test and the control material before the bioassay was done. It froze not only the volatiles from the wheat in the test material but also CO_2 and H_2O from the air into both the test and the control TENAX-GC.

Compared to the control test, every specific compound received a positive response. The compounds in peaks #3, #4, #5 and #7 elicited a stronger olfactory response than the others. Peak #3, background from air, was excluded in the comparison. The concentrations of peaks #4 and #5 as indicated on chromatograms were greater than the other compounds.

The higher concentrations of these compounds might explain the greater olfactory response. The relative amounts of all compounds in wheat are different. Each volatile compound from wheat sample was collected for the bioassay test, so the concentration differences must appear. In 1965 Kennedy (41) proposed that food selection of insects involves two basic types of chemical stimulants: (1) attractants, usually comprising fairly volatile compounds, to which insects orient by directed movement and (2) arrestants or aggregants, represented by scarcely volatile substances on which insects settle following contact gained by undirected movements. According to Kennedy's statement, we can assume that the compounds emanating from wheat are either attractants or aggregants to the red flour beetle.

CONCLUSION

According to the results we obtained from the bioassay, most of the volatile compounds were attractive to the insects. The concentration of each compound might play an important role in the various attractant or aggregant. The attractant or aggregant is due to either the combined effect or individual effect of the volatile compounds.

FUTURE WORK

Future bioassay tests should be performed, keeping the background contamination as low as possible. The RFB we used were 72-96 hours old. They are still young compared to their normal life span. Whether the insects of this age gave the best olfactory response to the food selection or not needs further investigation. The optimum concentration of volatile compounds for attracting the insect should also be determined.

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THE ISOLATION AND IDENTIFICATION OF VOLATILE COMPOUNDS IN WHEAT WHICH INDUCE AN AGGREGATION RESPONSE TO THE RED FLOUR BEETLE, TRIBOLIUM CASTANEUM (Herbst.)

by

VEN-SHING WANG

B. S., Providence College, Taiwan,
R. O. C., 1974

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1981

ABSTRACT

By using direct sample injection, a solid sampler was used to hold a wheat product (kernels and/or flour) in a heating block of a gas chromatograph and the volatile components were collected in a cold trap with TENAX-GC as the absorbent. Out of nine components separated, the four most active components were identified by using a G.C./M.S. as methanol, acetaldehyde, ethanol, and methyl ethyl ketone. The aggregation response was tested with red flour beetles, T. castaneum. While all nine of the compounds gave a positive response, four were definitely superior.