THE ODOR COMPONENTS OF THE DEFENSE MECHANISM OF THE "GREEN WORM", AMYNTHAS HUPEIENSIS AND WHEAT INSECT REPELLENT IN BAY LEAVES, (LAURUS NOBILIS, L.)

by

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PART I

THE ODOR COMPONENTS OF THE DEFENSE MECHANISM OF THE "GREEN WORM", AMYNTHAS HUPEIENSIS

INTRODUCTION

Anglers have used several methods to attract fish such as by appealing to vision, hearing and lateral line (vibration) senses[3]. However, a fish's ability to recall odor is probably better than its ability to recall what's seen, heard or felt[3]. Scientists have calculated how sensitive some fish are to molecule concentrations in water. For example, sharks are able to detect food odor at a concentration of 0.0001 parts per million[4]. Salmons have been shown to be repelled by mammal skin in a concentration of 1 to 80,000,000,000[4]. The knowledge of chemical senses in fishes has produced several scent products such as "Gator Bait", "Dr. Juice Elexir", "Formula" and "Crawfish". These scent baits are based on amino acids.

In northeast Kansas, there is a species of earthworm called "green worm" known as the catfish bait[1][2]. It is a popular bait and reported to be very effective for catching catfish. This worm gives off a sickening sweet long lasting odor when it is handled roughly, particularly so if stuck with a fish hook. The odor produced is believed to be part of the defense mechanism used by these worms. Preliminary tests with some of the "odor" on a piece of paper towel placed in a water tank containing several different species of fish indicated that it was quite effective as a bait for fish that are primarily odor feeders such as channel cat, drum and bullheads. The purpose of this work was to isolate and identify those compounds responsible for producing the odor and for attracting

the fish.

This "green worm" was tentatively identified[5][6] as Amynthas hupeiensis, which is considered as an "exotic" in that it is not native to this area but is believed to be an import from China many decades ago. It looks like an ordinary "fishing worm" but it is dark olive drab green color and is about 8-10 cm long. It has the perculiarity of curling into a ball when it is first handled.

This worm is found along the banks of the Kansas river as far as Waubausee Co., on the Wakarusa, Marais des Cygnes rivers and Dragoon creek in Osage and Shawnee counties; along most bigger streams in Brown, Doniphan, Atchinson, Jackson, Jefferson and Leavenworth counties; and on the banks of the best place of all, the Delaware river in Brown, Jefferson and Atchinson counties.

The work pattern followed is;

1. Develope a method to extract the "odor" from the worm.
2. Separate the extracted sample into various components with the use of gas chromatograph and a suitable column.
3. Identify each compound using GC/MS.

EXPERIMENTAL

A. Sampling of volatile odor from the worm.

Several methods have been tried to collect the odor sample as free of other compounds from the worm as possible. It was observed earlier in the investigation that a single drop of chloroform, hexane or ethanol placed in the water containing the worm would immediately induce the production of the odor. Then, by trapping the headspace gas, it was expected that the odor could be easily separated from the other compounds associated with the worm. However, this method proved unsatisfactory as the relatively large amount of irritant compound overloaded the chromatogram and overlapped the odor components, making a good separation impossible.

It was then discovered that just leaving the worm in a round bottom flask for a few minutes would also cause it to produce the odor without the addition of any irritants. However, the amount of odor produced was too small to be evaluated. Therefore, pre-concentration of the sample is required.

Pre-concentration of the sample has been used by several members of our research group[10][11]. The recycled air system used by Verma was tried. This system uses an air pump which recycles the air from the sample to the TENAX-GC trap without the addition of external air. Using this method, a reasonable amount of sample was obtained, but there was an increased amount of contaminants from either the tubing or the pump

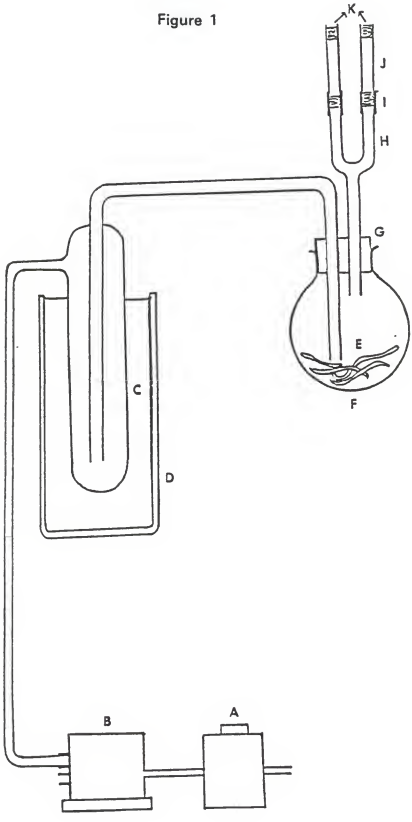
itself, trapped with the sample causing difficulties in making a good separation.

In the improved system discussed below, those contaminants were eliminated by a cold trap and by replacing the plastic tubing by glass tubing. (Refer to Figure 1). The apparatus used for collecting the odor consists of a Neptune Dyna Pump (B), controlled by a Variac (A). The flow rate was adjusted to approximately 200 mL/min. The incoming air was first pumped through a cold trap (C) cooled with liquid nitrogen (D). This was to ensure that the air was free of vapor and other organic contaminants present in atmospheric air. The air was then passed into a 100 mL round bottom flask (F), containing the worms (E). The vapors from the worms were absorbed in the two vials (J), containing TENAX-GC, held in placed by glass wool plugs (K). These vials were made of glass tubing, 7 mm x 3 cm. The TENAX-GC used here was first conditioned by placing it in a column and heating to 250°C for 12 hours with He carrier gas flowing at 50 mL/min. TENAX-GC was used due to it established suitability as a medium for the trapping and transferring of volatile organics to a gas chromatograph[13].

Four live worms (8-12 cm long) were washed with distilled water, wiped dry with a paper towel and placed in the 100 mL round bottom flask (F), previously well cleaned and flushed with nitrogen. The rubber stopper (G) was then fitted into place and the Y-tube (H) attached. The pump was turned on and air allowed to pass through the system for 5 minutes to flush it out. The two TENAX-GC containing tubes (J) were then

Figure 1 : Trapping system

Figure 1



attached to the Y-tube with small sections of rubber tubing. As the worms began to die, they gave off the vapor desired which was then flushed out of the flask and adsorbed on the TENAX-GC. This trapping process was continued for 4 hours, then the pump was turned off and the tubes containing the TENAX-GC were removed and saved for further analysis. A control experiment was run using the same apparatus for the same length of time and the same conditions but without the worms. This control sample is used as the baseline for the analysis.

B. GC separation and GC/MS identification of compounds.

1. Equipment

To introduce the sample collected in TENAX-GC into the gas chromatograph, a solid sampler previously used by V.S. Wang[12] was used. This solid sampler consists of a plunger and a sample compartment. A small portion of the TENAX-GC from the trap tubing was placed into the sample compartment and was introduce into the gas chromatograph by pushing in the plunger.

The compounds evaporated from the TENAX-GC were concentrated at the beginning of the GC column using a U-shaped trap, made of 3 mm x 15 cm stainless steel tubing. This trap was immersed in liquid nitrogen. One end of the trap was connected to the injection port and the other was capped off for later connection to the separating column. TENAX-GC, a porous polymer of 2,6-diphenyl p-phenylene oxide is used as the packing material due to its established suitability in terms of relative innertness and sufficient thermal stability[14][15]. It has been used previously in our laboratory[10][11][12].

Separation of the volatile compounds was accomplished using a Tracor model 560 gas chromatograph equipped with flame ionization detector (FID). The column used was made of 1.8 m by 3 mm stainless steel tubing, packed with TENAX-GC (60/80 mesh).

Mass spectra of the separated compounds were obtained using a Finnigan model 4000 GC/MS.

2. Sample

The worms, (Amnythas hupeiensis) were obtained from the Joseph Underwood farm at Ottawa, Kansas. They were found along the banks of the Marais des Cygnes river. They could readily be obtained at a depth of 20-30 cm on the wettest, shadiest, portion of the top of the bank during the months of May and June and again in September if it was wet. Very few could be obtained at other times and then only with considerable effort.

3. Chemicals

All chemicals were obtained commercially, were reagent grade, and used after distillation.

a) Benzaldehyde	:	Mallincrodt Chemical Works.
b) Phenethyl alcohol	:	Lot No. 5312EL, Aldrich Chemical Company
c) Phenylacetaldehyde	:	Lot No. 0927TK, Aldrich Chemical Company.
d) 3-Tolualdehyde	:	Lot No. 2421PK, Aldrich Chemical Company
e) 2-Tolualdehyde	:	Lot No. 2306TJ, Aldrich Chemical Company

f)	4-tolualdehyde	:	Lot No. 0922CK, Aldrich Chemical Company
g)	3-Ethyltoluene	:	Lot No. 0521BL, Aldrich Chemical Company
h)	2-Ethyltoluene	:	Lot No. 1526DK, Aldrich Chemical Company
i)	4-Ethyltoulene	:	Lot No. 0313JK, Aldrich Chemical Company
j)	TENAX-GC	:	Lot No. 780830/33 Applied Science Lab. Inc.

3. Procedure

TENAX-GC was removed from the glass tubing, and was mixed thoroughly. Then, a small portion of the TENAX-GC was placed into the sample compartment of the solid sampler. The sample was introduced into the gas chromatograph by pushing in the plunger. The injection port was heated to 230°C. The sample was heated for 10 minutes. Helium with a flow rate of 50 mL/min was used as the carrier gas to drive the volatile compounds into the cold trap. This procedure was repeated twice in order to get a sufficient amount of test compounds to be analyzed.

The trap was then removed from the liquid nitrogen. The capped end was then connected to the column in the GC. The conditions under which all of the chromatograms were obtained were as follows:

Column	:	1.8 by 3 mm stainless steel with TENAX-GC packing.
Carrier gas	:	Helium
Flowrate	:	30 mL/min
Injection port temp.	:	200°C
Detector	:	FID

Detector temp.	:	250°C
Initial temp.	:	120°C
Initial hold	:	3 minutes
Final temp.	:	200°C
Final hold	:	3 minutes
Program rate	:	8°/min
Attenuation	:	8

A background chromatogram was obtained using the control sample. Then, appropriate corrections were made on the sample chromatogram.

For the GC/MS identification, the sample was collected as describe previously. The volatile compounds were trapped in the U-tube and connected to the separating column, for the GC/MS analysis.

The identified compounds were confirmed by the following methods;

1. Comparison of the mass spectra of each compound with the spectra in the library of the data system of the GC/MS.
2. Comparison of the gas chromatographic retention time of the compound in the sample with that of the pure compound.
3. Spiking each compounds in the sample with the pure compounds.
4. Comparison of the mass spectra of the sample with that of the known pure compound.

RESULTS AND DISCUSSION

A. Identification of the volatile compounds from the worm.

For collecting the odor from the worm, the trapping system discussed earlier was used. Using this system, several improvements were obtained:

1. The worms produced an increased amount of odor.
2. Contaminants from the system and the worm were markedly reduced.
3. Solvent effects were eliminated.

For the separating column, several column packings were tried, such as;

10% Carbowax 20M on Chromosorb P (80/100 mesh, Johns Mansville Sales Corporation.)

Porapak P (50/80 mesh, Waters Associates, Inc. Batch No. 1834.)

Porapak R (80/100 mesh, Waters Associates, Inc. Batch No. 1159.)

Super Q (80/100 mesh, Alltech Associates, Inc. Applied Science Labs. Lot No. 6035.)

However, these materials were not sufficiently stable at high temperature and produced undesirable peaks during subsequent chromatography. Since TENAX-GC was used as the trapping material, this packing was tried, and found to be successful.

Chromatograms of the background (Fig. 2) and that of the sample (Fig. 3) were obtained. When compared to the background, 3 peaks were obtained from the sample. The peaks preceding peak #1 were due CO_2, N_2 and O_2 that were trapped

Figure 2 : Chromatogram of background.

Figure 3 : Chromatogram of volatile compounds from the sample.

Figure 2

Chromatogram of background

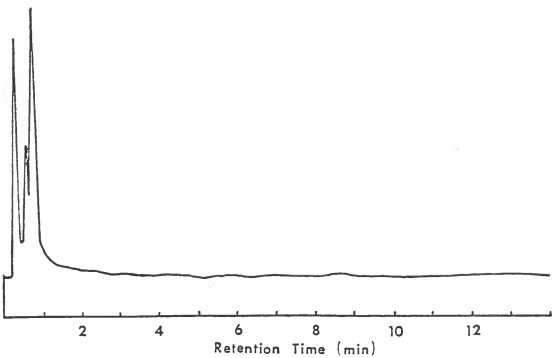
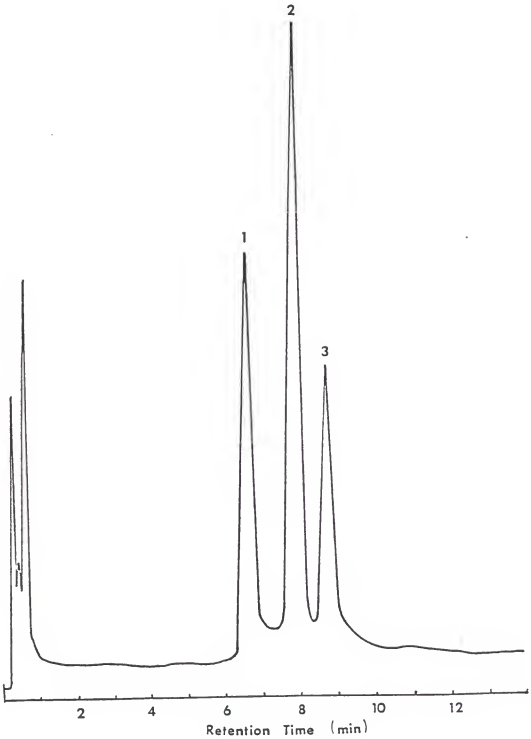


Figure 3

Chromatogram of volatile compounds from the sample.



during the cold trap process. From the chromatogram of the background, all room air contaminants were removed and therefore no contaminants were trapped together with the sample.

a. Identification of peak # 1.

(Refer to Fig. 4). The mass spectral data shows a large parent peak, m/e 105, and also a large peak corresponding to the $(p-H)^+$ ion. These characterize an aromatic aldehyde. The peak due to the loss by α- cleavage of the aldehyde group, $(p-CHO)^+$, is also a charateristic of an aldehyde. The $(p-1)^+$ ion, C_6H_5CO, eliminates CO to form phenyl ion, m/e 77, which in turn eliminates $HC\equiv CH$, forming $C_4H_3^+$ ion, (m/e 51). The proposed compound for peak # 1 is benzaldehyde (Table 1). The addition of pure benzaldehyde to the sample shows an increase in the area of the peak under investigation (Fig. 6, Fig. 7). In addition, the mass spectra of pure benzaldehyde recorded (Fig. 5), shows a matching spectra.

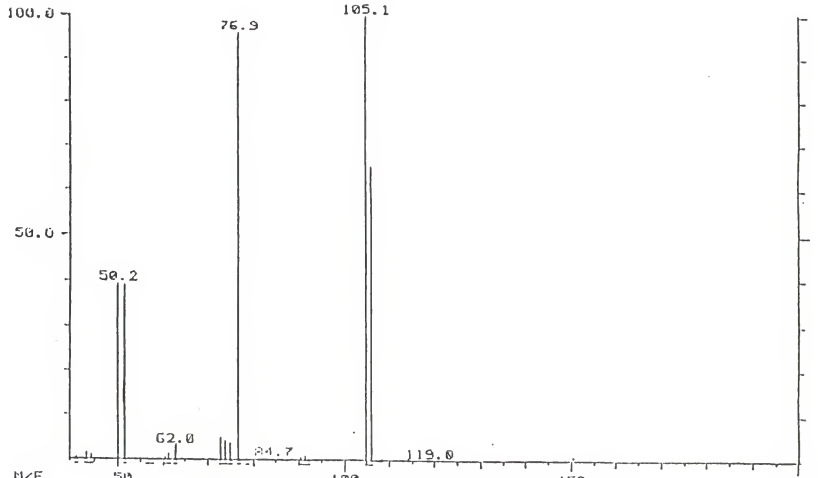
b. Identification of peak # 2.

(Refer to Fig. 8). The mass spectral data for peak #2 shows a reasonably large parent peak, m/e 120. The peak corresponding to the $(p-1)^+$ ion is not observed and the $(p-29)^+$ ion peak is the base peak. n-propyl benzene is a good possibility since an aromatic ring stabilizes the molecular ion. Also, a prominant peak at m/e 91, possibly $C_6H_5CH_2^+$ is indicative of an alkyl substituted benzene ring. However, this possibility is not likely since the retention time for n-propyl benzene does not match that of the sample (Table 2).

Figure 4 : Mass spectral fragmentation pattern of peak #1.

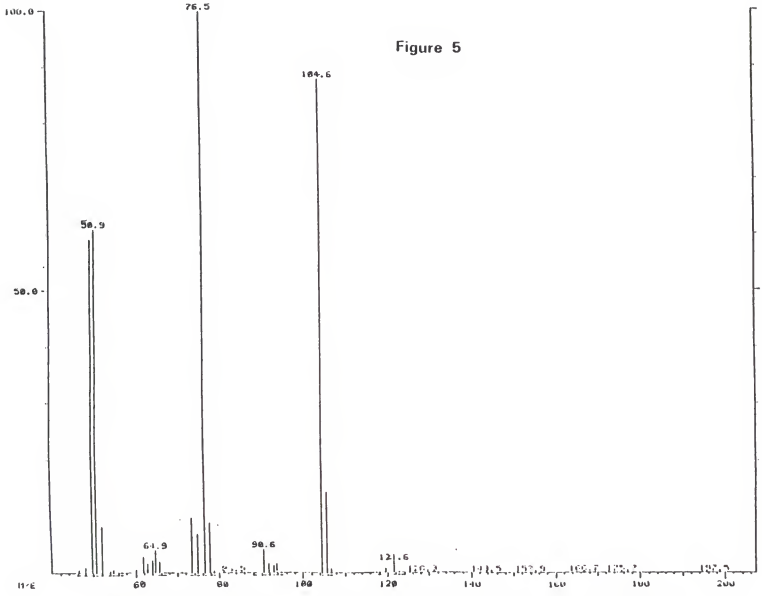
Figure 5 : Mass spectral fragmentation pattern of pure benzaldehyde.

Figure 4



Mass spectral fragmentation pattern of peak #1

Figure 5



Mass spectral fragmentation pattern of pure benzaldehyde

TABLE 1

Mass Spec. analysis of peak # 1

Possible fragmentation patterns

Mass	Intensity	Fragments
106	65%	
105	100%	
77	96%	
51	39%	$C_4H_3^+$

Proposed structure

Benzaldehyde

Figure 6 : Chromatogram of sample.

Figure 7 : Chromatogram of sample spiked with benzaldehyde.

Figure 6

Chromatogram of sample

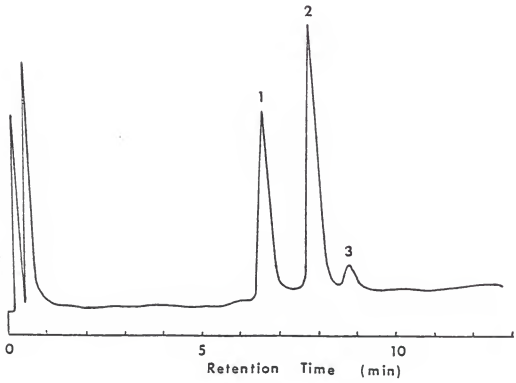
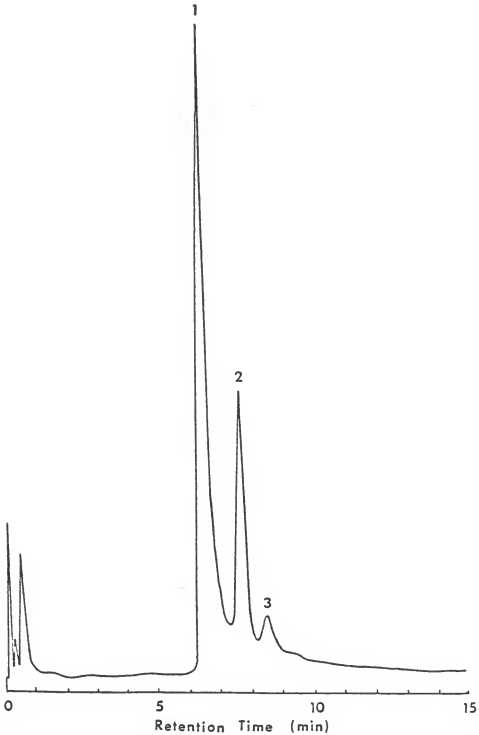


Figure 7

Chromatogram of sample spiked with benzaldehyde



An aromatic aldehyde is another possibility. This includes phenylacetaldehyde and the three isomers of tolualdehyde. The retention time for these four compounds were obtained (Table 2). It was found that phenylacetaldehyde, 3-tolualdehyde and 2-tolualdehyde have the same retention time as the compound in the sample. However when the mass spectra of those compounds were obtained, 3- and 2-tolualdehyde produced a large peak at m/e 119, $(p-1)^+$ ion (Fig. 10, Fig. 11). Since the mass spectra of the compound in the sample did not show any peak at m/e 119, the possibilities of those two compounds are eliminated. The mass spectra of pure phenylacetaldehyde (Fig. 9), matches well with that of the unknown. Phenylacetaldehyde has a base peak at m/e 91, due to the benzyl ion from the loss of the CHO group. Figures 12 and 13 show the addition of pure phenylacetaldehyde to the sample, which causes an increase in the peak area of peak #2. In addition, the odor of this compound matches that given off by the worms and the tolualdehydes do not.

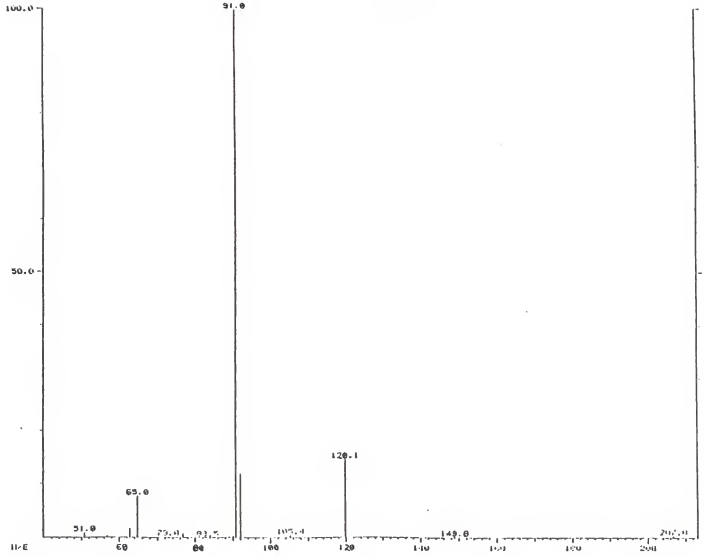
c. Identification of peak #3.

(Refer to Fig. 14). The mass at 122 is taken as the parent peak. The base peak at m/e 91 is indicative of a benzyl group, which is supported by the masses at 77 and 65, indicating a benzene ring. The mass at 92 is due to the loss of a CH_2O ion. A possible compound is phenethyl alcohol. Methyl benzyl ether is another possibility due to the large peak at m/e 91, a possible loss of OCH_3^+ ion. However this possibility is not likely as the mass spectra of methyl benzyl ether has a

Figure 8 : Mass spectral fragmentation pattern of peak #2.

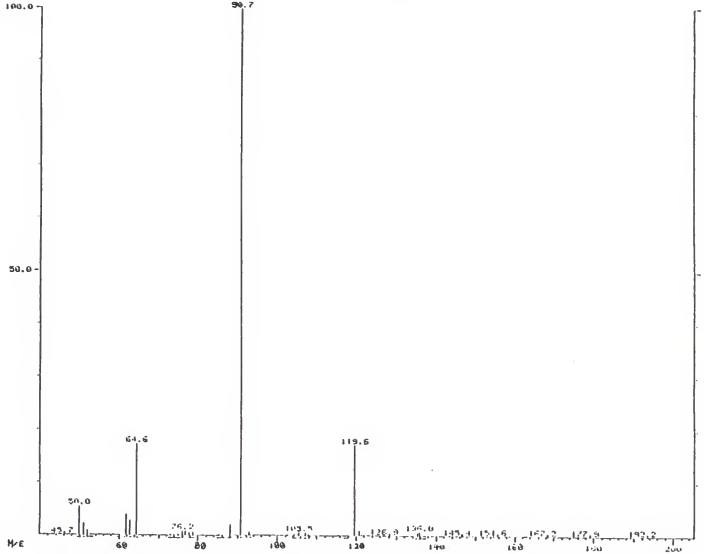
Figure 9 : Mass spectral fragmentation pattern of pure phenylacetaldehyde.

Figure 8



Mass spectral fragmentation pattern of peak #2

Figure 9

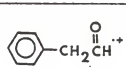


Mass spectral fragmentation pattern of pure phenylacetaldehyde

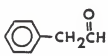
TABLE 2

Mass Spec. Analysis of peak # 2

Possible fragmentation patterns

Mass	Intensity	Fragments
120	15%	
91	100%	
92	12%	
65	8%	C_5H_5

Proposed Structure

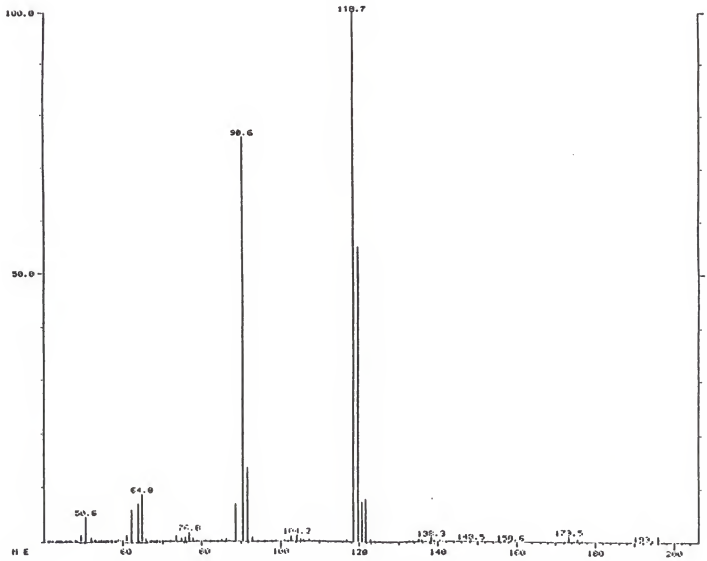


Phenyl acetaldehyde

Figure 10 : Mass spectral fragmentation pattern of 3-tolualdehyde.

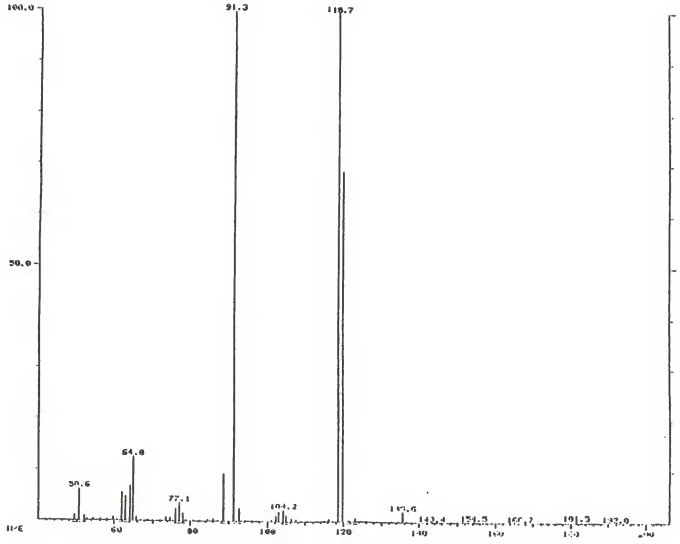
Figure 11 : Mass spectral fragmentation pattern of 2-tolualdehyde.

Figure 10



Mass spectral fragmentation pattern of 3-tolualdehyde

Figure 11



Mass spectral fragmentation pattern of 2-tolualdehyde

Figure 12 : Chromatogram of sample.

Figure 13 : Chromatogram of sample spiked with phenylacetaldehyde.

Figure 12

Chromatogram of sample

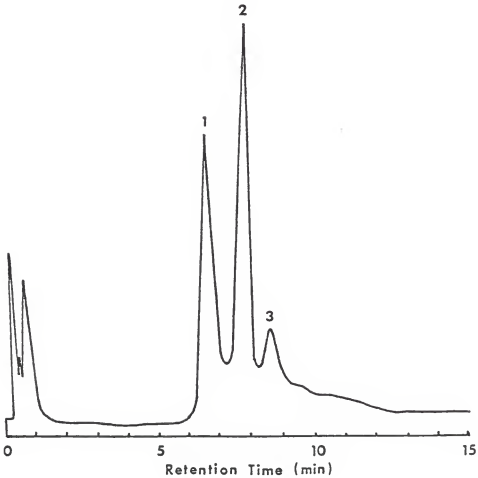


Figure 13

Chromatogram of sample spiked with phenylacetaldehyde

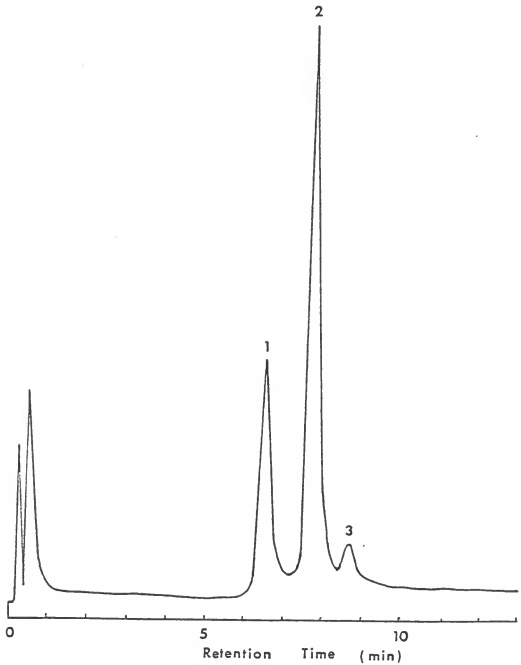


TABLE 3

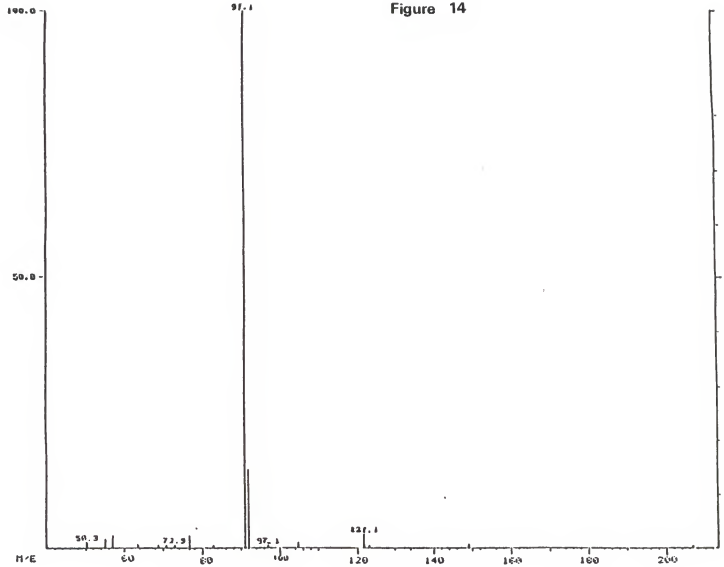
Retention times of related suspected compounds for peak # 2

Compound	Retention time
Unknown 2	8 min 00 sec
n-propyl benzene	6 min 45 sec
2-tolualdehyde	8 min 00 sec
3-tolualdehyde	8 min 00 sec
4-tolualdehyde	8 min 10 sec
2-ethyl toluene	6 min 15 sec
3-ethyl toluene	6 min 00 sec
4-ethyl toluene	6 min 00 sec

Figure 14 : Mass spectral fragmentation pattern of peak #3.

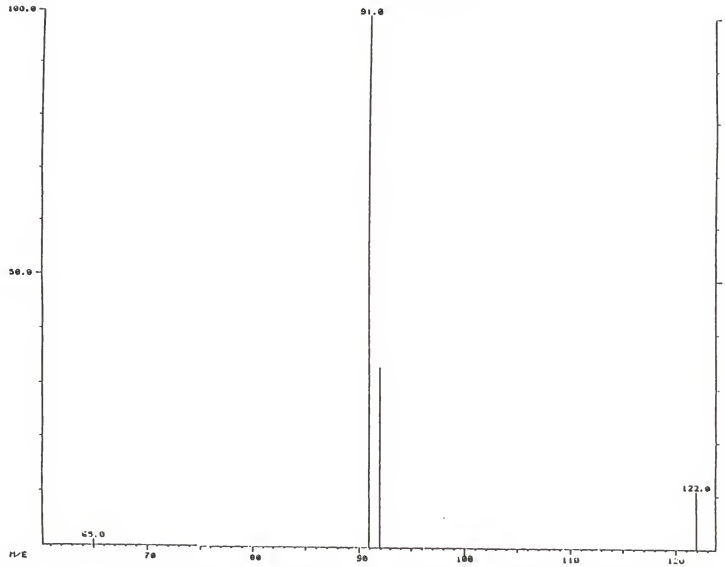
Figure 15 : Mass spectral fragmentation pattern of pure phenethyl alcohol.

Figure 14



Mass spectral fragmentation pattern of peak #3.

Figure 15

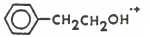


Mass spectral fragmentation pattern of pure phenethyl alcohol

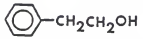
TABLE 4

Mass Spec. analysis of peak #3

Possible fragmentation patterns

Mass	Intensity	Fragments
122	3%	
91	100%	
92	15%	
77	2%	

Proposed structure



Phenethyl alcohol

Figure 16 : Chromatogram of sample.

Figure 17 : Chromatogram of sample spiked with phenethyl alcohol.

Figure. 16

Chromatogram of sample

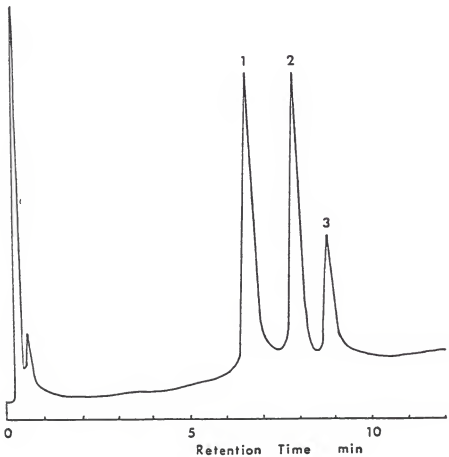
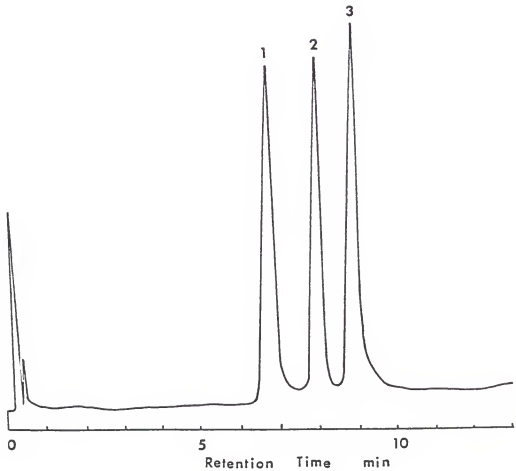


Figure 17

Chromatogram of sample spiked with phenethyl alcohol



large parent ion peak[20]. It also has a large peak at m/e 121, $(p-1)^+$ ion[20]. Figures 16 and 17 shows an increase in the area of peak #3 when pure phenethyl alcohol is added to the sample. In comparison with the mass spectra of the pure phenethyl alcohol, the mass spectra of the unknown in the sample matches well with that of the pure compound (Fig. 15).

B. Quantitative analysis of the sample.

Since the detector usually response somewhat differently for different compounds, an internal normalization technique was chosen for the quantitative analysis of the sample[27]. Standards of each compound were prepared and 1 μL of each standard was individually injected into the gas chromatograph. This procedure was repeated and the average area of each compound was measured using an Apple II computer. From the data obtained, a response factor, F, for each compound was calculated (Table 5). Then, using these response factors, the weight percent of each compound in the sample was obtained (Table 6).

CONCLUSION

From the GC/MS analysis, the compounds making up the odor produced by the worms are benzaldehyde, phenylacetaldehyde and phenethyl alcohol. The weight ratio of benzaldehyde : phenylacetaldehyde : phenethyl alcohol is 1.61 : 3.30 : 1.00.

Tables 5 and 6 : Quantitative analysis of the sample.

TABLE 5

Compound (Standard)	Weights taken ($x10^{-6}$)	Weight (%)	Average Peak Area	Area (%)	Area / Wt %	Response factor, F
Benzaldehyde	1.043	33.80	3.14	33.69	0.9967	1.0000
Phenyl acetaldehyde	1.023	33.30	2.48	26.61	0.7990	0.8016
Phenethyl alcohol	1.017	32.90	3.70	39.70	1.2067	1.2107
Total	3.083	100.00	9.32	100.00		

TABLE 6

Compound (in sample)	Area	Area %	Wt %	Fraction
Benzaldehyde	3.31	29.4	27.22	1.61
Phenyl acetaldehyde	5.44	48.4	55.84	3.30
Phenethyl alcohol	2.49	22.2	16.94	1.00
Total	11.24	100.0	100.00	

PART II

WHEAT INSECT REPELLENT IN BAY LEAVES (*LAURUS NOBILIS*, L.)

INTRODUCTION

Insects are known to often cause extensive damage to stored grain products[1][2]. With the growing food shortages around the world, this damage has to be minimized.

Researchers have put a lot of effort into devising methods to control insects in stored grain products, which includes the use of inert gases, radiation, pathogens, growth regulators, insecticides and others[3]. Pesticides are known to control insects effectively.

Ethylene dibromide, EDB, had been used for years to effectively treat stored products. However, in February 1984 the Environmental Protection Agency (EPA) announced a ban on the use of EDB as a pesticide for grain. This action was taken when research by the National Cancer Institute and others determined that EDB caused cancer, birth defects, and genetic and reproductive disorders in test animals[4].

The use of pesticides is not the best solution due to several other reasons such as the development of resistance among insects and also the persistance of the residues of certain pesticides which may pollute the environment[8].

Due to the several disadvantages of pesticides, other ways of controlling insects are becoming more important. For example, the use of insect repellents offers a hope of protection of stored grain from insects. Repellents have the advantges of being more specific and may have low mammalian toxicity[5]. Today, several effective, persistant, and

economical repellents have been discovered[5]. In addition, some active naturally occuring materials have been extracted from plants and chemically identified. For example, three plant materials that are common in Pakistan, rhizomes of Curcuma longa (L.) (tumeric), leaves of Azadirachta indica A. Juss. (neem) and leaves of Trigonella foenum-graecum L. (fenugreek) have been sucessfully used in common practice to protect cereal grains against stored-product insects[6]. Seven other plant species were tested and evaluated for their repellancy ability[5].

Bay leaves, also known as laurel leaves (Laurus nobilis L.), were added to wheat and the wheat supposedly remained insect free for several months. However, this was not verified under controlled conditions. M. Verma[7] worked with bay leaves and has separated and identified 19 volatile compounds. The purpose of this project was to test several of those compounds individually for their repellent effects, using the red flour beetle (Tribolium casteneum. (Herbst.). Hard red winter wheat flour was chosen as the test material.

EXPERIMENTAL

1. Equipment

The equipment used for detecting the repellency response of the insects to compounds present in bay leaves was the same as that described by Laudani and Swank[8] and modified by Berndt[9]. (Refer to Figure 1). It was a choice test apparatus consisting of a circular wooden platform (A), 50 cm in diameter, with a metal rim (B). The metal rim was 30 cm high. There were 12 holes (C) of 9 cm diameter cut equidistantly spaced around the periphery of the platform. The holes were cut to fit a 5 cm high plastic container (G) , which could be inserted and removed easily. A modification was made on the lid of this apparatus. Three large holes were cut out and replaced by wire screen. This was done to prevent the test compound from saturating the apparatus. A metal tube (D) was passed through the middle of the lid and an inverted metal pan (E) was affixed to the end of the tube. The tube served as a means of introducing the test insects, and the pan served to restrain the insects until they were released. The apparatus was pivoted so that it can rotate.

This apparatus was placed in a room with a constant room temperature of 74±1°C and a constant humidity (67±3% R.H.), which were checked daily.

Fig. 1a : Cross-sectional view of the apparatus

Fig. 1b : Top view of the apparatus (without the lid), showing the position of the containers.

Figure 1a

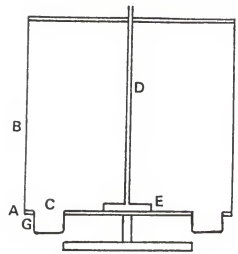
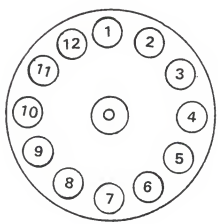


Figure 1b



2. Test Insects

Adult red flour beetles, *Tribolium casteneum,* (Herbst.), about 3 weeks old were used as the test insects. They were provided by the USDA Grain Marketing Research Laboratory, Manhattan, Kansas.

3. Wheat flour

Hard red winter wheat flour used was obtained from the Grain Science Department, Kansas State University.

4. Chemicals

All chemicals were reagent grade and used after distillation.

1,8-Cineole	: Lot No. 1523PH, Aldrich Chemical Company
Phenyl hydrazine	: Matherson Coleman & Bell, Norwood, Ohio.
l-Linalool	: Pfaltz & Bauer, Stamford, Conn.
Benzaldehyde	: Mallincrodt Chemical Works.
Geraniol	: Pfaltz & Bauer, Stamford, Conn.
Propionic acid	: Fisher Scientific Company.
Isobutyric acid	: Lot No. 721324, Fisher Scientific Company
α- pinene	: Lot No. 8118MJ, Aldrich Chemical Company.
Acetone	: Lot No. 720622, Fisher Scientific Company
Diethyl ether	: Lot No. 723335, Fisher Scientific Company
Phellandrene	: Pfaltz & Bauer, Stamford, Conn.
2-Methylbutyraldehyde	: Lot No. 321AL, Aldrich Chemical Company.
Bay leaves	: Lot No. 5629, Helix Spices.

4. Bioassay procedure

The effectiveness of bay leaves as a repellency was first tested using dried bay leaves. Two grams of bay leaves were crushed and mixed with 900 gm of wheat flour. Then 6 of the plastic containers, (#2,#4,#6,#8,#10,#12) were filled with the treated flour while the others were filled with untreated flour to serve as a control. These containers were then placed into the appropriate position in the apparatus. (refer to Figure 1b.)

Approximately 500 adult red flour beetles were then poured down the tube. After several minutes the restraining metal pan was pulled up, the insects were free to move inside the apparatus and enter the containers of their choice.

After 24 hours, the 12 containers were removed from the apparatus and the insects in each container were counted and recorded.

The compounds present in bay leaves were tested individually. Concentrations of 100 ,50 , 25 , 10 and 5 ppm were prepared using 0.0900 ,0.0450 , 0.0225 , 0.0090 and 0.0045 gram of tested compound for 900 grams of wheat flour. Dilutions were made using distilled deionized water. For the untreated flour the same amount of distilled deionized water was added. Then the concentration which gave the best response was repeated.

RESULTS AND DISCUSSION

Attractancy-repellency effects were measured by the following formula[10]:

$$A = \frac{N_c - N_b}{N_t}$$

where: A = attractancy (+) or repellency (-); N_c = number of insects in the test container; N_b = number of insects in the control container; N_t = total number of insects in both containers.

Refering to Table 1, bay leaves had a negative A value indicating that they acted as a repellent to red flour beetles. There were 19 compounds isolated from bay leaves[6], and 15 of these compounds were tested individually. The clorinated compounds that were found in bay leaves[6] were not tested. This includes Chloroform, Ethylene trichloride and Methylene chloride. These were believed to be fumigant residues and not natural components of the bay leaves. The results obtained are shown in Table 1.

From the results of the repellency test (Table 1), 3 compounds; piperidine, benzaldehyde and geraniol were found to be quite effective repellents. Benzaldehyde has the higest repellency factor.

Table 1 : Results of repellency test.

TABLE 1

Compound tested	No. of red flour beetles in treated wheat	: in untreated wheat	Repellency factor (A)	Average A
Bay leaves	a. 174	297	-0.26	-0.21
	b. 214	300	-0.17	
Benzaldehyde	a. 121	354	-0.48	
(50 ppm)	b. 129	403	-0.52	-0.42
	c. 126	238	-0.31	
α-pinene	a. 284	160	0.28	
(50 ppm)	b. 237	173	0.16	0.22
	c. 250	158	0.23	
Acetone	a. 243	186	0.11	
(50 ppm)	b. 273	217	0.04	0.10
	c. 190	215	-0.06	
2-Methyl butyraldehyde	a. 274	264	0.02	
(10 ppm)	b. 277	255	0.04	0.08
	c. 348	248	0.17	
Diethyl ether	a. 257	216	0.09	
(50 ppm)	b. 235	223	0.03	0.06
	c. 185	159	0.08	

TABLE 1 (cont.)

Compound tested		No of red flour beetles in treated wheat	: in untreated wheat	Repelency factor (A)	Average A
α-phellandrene (50 ppm)	a.	251	285	-0.06	
	b.	213	248	-0.08	-0.07
	c.	231	266	-0.07	
l-Linalool (50 ppm)	a.	261	193	0.15	
	b.	237	227	0.02	0.05
	c.	220	224	0.01	
Geraniol (50 ppm)	a.	171	301	-0.28	
	b.	208	350	-0.25	-0.22
	c.	151	191	-0.12	
Propionic acid (50 ppm)	a.	219	234	-0.03	
	b.	201	207	-0.01	-0.06
	c.	164	209	-0.12	
Isobutyric acid (25 ppm)	a.	169	158	0.03	
	b.	259	224	0.07	0.09
	c.	223	162	0.16	
Ethanol (50 ppm)	a.	179	219	-0.10	
	b.	244	230	0.03	-0.05
	c.	179	206	-0.07	

TABLE 1 (cont.)

Compound tested	No. of red flour beetles in treated wheat	: in untreated wheat	Repellency factor (A)	Average A
o-xylene	a. 198	186	0.03	
(50 ppm)	b. 183	201	-0.05	-0.03
	c. 178	210	-0.08	
Piperidine	a. 177	385	-0.31	
(50 ppm)	b. 129	315	-0.29	-0.32
	c. 159	307	-0.34	
1,8-Cineole	a. 277	234	0.08	
(10 ppm)	b. 247	231	0.03	0.07
	c. 239	199	0.09	
Phenyl hydrazine	a. 206	205	0.00	
(25 ppm)	b. 217	236	0.04	0.02
	c. 283	286	0.00	

CONCLUSION

From the results listed in Table 1, bay leaves do contain a mixture of compounds which act as a repellent for red flour beetles. Although not all of the compounds present in bay leaves were tested, 3 compounds; Piperidine, Benzaldehyde and Geraniol were found to be quite effective repellents.

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THE ODOR COMPONENTS OF THE DEFENSE MECHANISM OF THE "GREEN WORM", *AMYNTHAS HUPEIENSIS*

AND

WHEAT INSECT REPELLENT IN BAY LEAVES, (*LAURUS NOBILIS*, L.)

by

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

PART I - THE ODOR COMPONENTS OF THE DEFENSE MECHANISM OF THE "GREEN WORM", AMYTHAS HUPEIENSIS

The annelid, Amnythas hupeiensis, secrets a strong odor when its surface is roughened or ruptured. This odor is believed to be part of the defense mechanism used by these worms.

The odor was pre-concentrated on TENAX-GC and introduced into a gas chromatograph using a solid sampler. The volatile compounds were collected in a cold trap with TENAX-GC as the absorbent, before transferring them into a gas chromatographic column. The compounds seperated were identified by using a G.C/M.S, as benzaldehyde, phenyl-acetaldehyde and phenethyl alcohol.

PART II - WHEAT INSECT REPELLENT IN BAY LEAVES, (LAURUS NOBILIS, L.)

Bay leaves were found to act as a repellent for red flour beetles, Tribolium Castaneum (Herbst.). The repellency response of fifteen compounds were tested and three compounds, Piperidine, Benzaldehyde and Geraniol were found to be quite effective.