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(6) LABORATORY EVALUATION OF THE USDA RAPID NONDESTRUCTIVE STORED PRODUCTS INSECT DETECTOR.

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Tests conducted in the laboratory determined that the insect detector (second prototype) could distinguish between a sample with three live insects per pound and an uninfested sample of stored food products. The detector could not differentiate between no infestation and one insect in 5-pound packaged commodities or a 1-pound sample of whole grain. The length of time necessary to test a sample and extrinsic interferences from various sources made the first and second prototypes of the hidden insect detector operationally unsatisfactory.

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INTRODUCTION

Insect infestations of stored products cause major economic problems throughout the world. Effective stored product insect control requires early, rapid and reliable detection of insect infestations. Various detection methods have been developed including X-ray,[1,2] pheromones to attract insects,[3,4] traps,[5,6] nuclear magnetic resonance,[7] electrical resistance,[8] microwaves,[9] sound,[10,11] and chemical indicators.[12] These methods were not satisfactory because they failed to detect low-level insect infestations in packaged commodities.

The present method for insect detection is outlined in Military Standard 904, Guidelines for Insect Infestation of Subsistence.[13] Briefly, the method begins with a detailed visual inspection of the package. The contents are then sieved onto a collection surface where the residue is examined for whole insects and insect parts. This procedure destroys the package and its contents and is time consuming.

Bruce and Street[14] described a system which had potential for detection of low-level insect infestations in stored products while maintaining package integrity. The system detected insect-produced carbon dioxide (CO_2) in the presence of atmospheric CO_2. First and second generation prototypes of this system were provided to the United States Army Medical Bioengineering Research and Development Laboratory (USAMBRDL), by the United States Department of Agriculture (USDA) Stored Products Insects Research Laboratory, Savannah, Georgia for laboratory evaluation under simulated operational conditions. The laboratory evaluation of the first prototype is given in Appendix A. This report concerns only the second prototype evaluation.

Purpose

The purpose was to evaluate the capability of the USDA detector system to detect insect infestations in packaged commodities without destroying package integrity. Parameters to be met were that detection occur in a relatively short period of time and that it be uninfluenced by CO_2 from sources outside the package.

Materials

The second prototype is based on a HORIBA Model PIR 2000 General Purpose Infrared Gas Analyzer. This instrument is capable of precision gas analysis based on nondispersive infrared ray absorption for continuously determining the concentration of a given component in a gaseous stream. For insect detection the gas analyzer was preset to detect CO_2.

Molecules of CO_2 absorb infrared radiation of a specific wavelength. The degree of absorption is proportional to molecule concentration at constant pressure. The infrared radiation emitted by the light source is modulated by a rotating chopper, then passed simultaneously through the sample and reference cells into the detector cell. If the sample cell contains more CO_2 than the reference cells, a decrease in the amount of radiation reaching the sample side of the detector cell results. This difference is registered as an electrical output which is amplified and directed to a meter/recording device.

The recording device, a Linear Model 141, is a multi-range potentiometric null balance servo recorder which provides an accurate, permanent graphic record of signal input. The DC input signal to the recorder is first filtered, then amplified by a preamplifier to a level which is less susceptible to noise and interference. This conditioned signal is then applied to a differential amplifier which continuously compares the conditioned signal to the feedback signal developed by the servo potentiometer. The difference between these two impulses is a positive or negative error signal which is amplified and used to drive the servo motor. The motor is coupled to the servo potentiometer in a direction which reduces the error signal to zero. Since the recorder pen is mechanically coupled with the servo motor and potentiometer, its position on the chart represents an accurate, and continuous graphic record of the input signal.

The insect detector weighs 42 lb and measures 7.5 x 14.5 x 22 in. The accessory equipment included a Drierite[R] rigid plastic cylinder (2.5 x 11 in), two different plastic shrink bags (15 x 21 in and 9 x 15 in), Tygon[R] tubing, Swagelok[R] quick connect fittings, A.C.[R] line filters, a 5-gallon glass jar, and a small wire cage (0.5 x 2 in). A plastic gutter screen (9.5 x 18 in), two wooden strips (0.75 x 9 in) and four No. 10 binder clips were additional accessories used only with the shrink bags.

The commodity test chamber was either the plastic cylinder, when a bulk sample of whole grain was examined, or the shrink bag, when a packaged commodity was tested for insect infestations. The inlet and outlet ports, positioned at opposite sides of the test chambers, were connected to the ends of two segments of Tygon[R] tubing and attached to the insect detector by Swagelok[R] quick connect fittings. The Tygon[R] tubing is fitted with filters to prevent extrinsic particles from entering the detector. The 5-gallon jar, which provides a stable source of air with minimum fluctuations of CO_2, had Tygon[R] tubing leading from the jar through the inlet port to a series of pumps which circulated the air through the insect detector. The wire cage facilitated introduction of a known number of insects into the test chamber and their retrieval after the test. The plastic gutter screen was used to wrap a packaged commodity to create a small air space between the package and shrink bag that allowed air to flow through the package. The wooden strips and binder clips were used to fasten the open end of the shrink bag.

Procedures

The insect detector was operated in the following manner:

Initial Setup

The switches were placed in the following positions:

Power - Off
Purge - Off
Mode - Either Ramp or Peak
Chart Recorder - Off
Recorder Input - 100 MV
Atten - CCW

The line cord of the instrument was plugged into a 115 V AC60 Hz power outlet.

One end of the air inlet hose was attached to the insect detector, and the other end was placed into a 5-gallon jar.

Sample Preparation

Two modes of sample testing were available and selected by means of the front panel Mode switch. The Peak mode first purged the sample and then stopped air flow for a period of time determined by the Test/Collect timer. During the quiescent period the system was sealed. At the end of the Collect interval, flow was restarted and any CO_2 collected in the sample was read out as a peak on the chart recorder.

The Ramp mode was an integrating mode in which air was continuously recirculated around a closed loop containing the sample and the CO_2 analyzer. In this mode any buildup of CO_2 was constantly displayed on the recorder chart as an increasing ramp function.

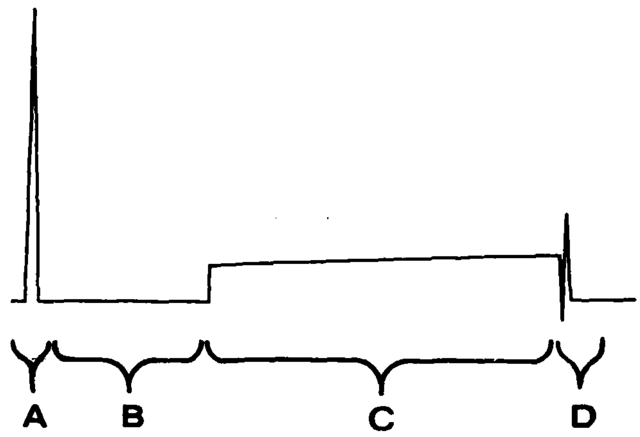
Three species of stored product insects were used as test specimens; the red flour beetle, *Tribolium castaneum*, the lesser grain borer, *Rhizopertha dominica*, and the rice weevil, *Sitophilus oryzae*. During the latter part of the investigation, only the red flour beetle was used.

Samples were evaluated by the insect detector using various levels of infestation, species of insects, testing times and modes, and types of commodities. The controls were obtained by placing the samples (packaged or bulk) into a freezer (-50°C) for at least 2 days to assure that no live insects were present. After removal from the freezer, the samples were allowed to reach room temperature and any condensate to evaporate before placing the contents into the commodity test chamber (shrink bag or plastic cylinder) and connecting the chamber to the insect detector. The infested samples were obtained by artificially introducing a known number of laboratory reared beetles into those samples which had previously been used as the controls. Both the Ramp and Peak modes were tested. Measurements were made of the peak and ramp heights which were recorded on the strip chart. These were examined to ascertain if there were detectable differences between the two modes of operation, and to determine if the peak and ramp heights consistently corresponded to a given number of artificially introduced insects in a sample.

RESULTS AND DISCUSSION

The Peak mode was generally more sensitive,[15] but required a fixed time cycle. Until the end of the cycle no information was available on the degree of infestation. The ramp mode, although not as sensitive, had the advantage of faster presentation of data. As soon as a positive slope on the ramp was established the test could be terminated. In the case of a heavily infested sample the test could be terminated in as little as 15 to 20 seconds. Field experience would determine whether either or both modes are desirable in a commercially produced system.

Typical Peak mode chart print-outs using the plastic chamber and the shrink bag are given in Figures 1 and 2, respectively. The plastic chamber was used for a 1-pound sample of whole grain wheat. The shrink bag was used for a 5-pound bag of flour or corn meal. The initial peak occurred each time either commodity chamber was opened to external air. Thus, when the insect detector was turned on to begin the test, this excess air was pumped through the CO_2 detector to flush the system. The CO_2, included in this bolus of air, caused the recorder pen to rise sharply before returning to baseline. This process usually took 20 seconds or less with whole grain. The short "purge" time is made possible because of the large amounts of interstitial air in a sample of whole kernel grain. With flour or corn meal, which had comparatively little interstitial air and was compacted when under vacuum, 10 to 15 minutes were needed for the system to purge and establish a steady baseline. This was essential before any sample could be tested for the presence of CO_2. A false reading was recorded if a test was conducted without adequate purging. After the initial "purge" peak, the baseline would level out during the rest of the purge cycle. The system then entered a collect phase where CO_2 was allowed to accumulate in the sample. At the end of the collect cycle, the system was flushed again. The CO_2



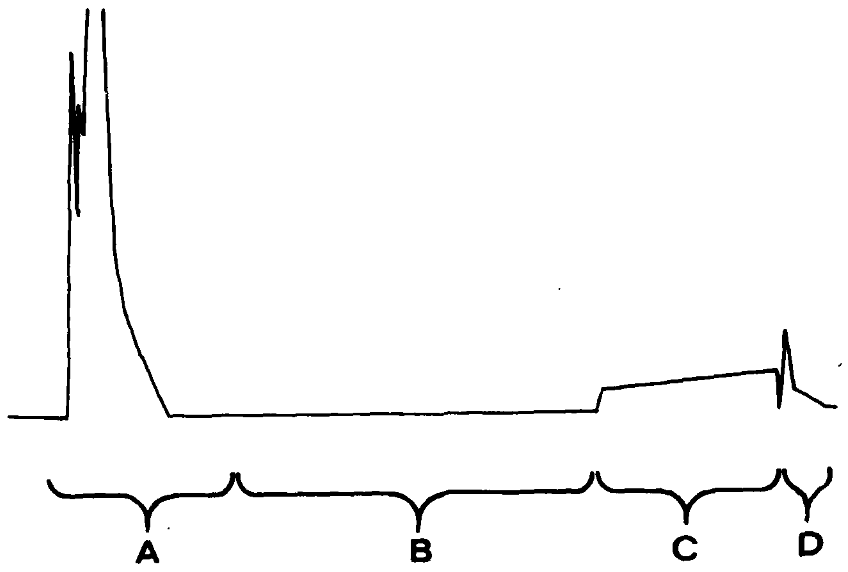
A - Initial peak - caused by opening the commodity test chamber to add or change a sample.

B - Purge cycle - flushing the system of any remaining gases from the previous run or those which entered while the test chamber was opened. Air was pumped through from an external air source.

C - Collect cycle - the detector was sealed off with no additional external air pumped through the system.

D - Peak - height indicates amount of internally produced CO_2.

Figure 1. Peak Mode Chart Print-Out of a 1-Pound Whole Grain Sample in the Rigid Plastic Cylinder.



A - Initial peak - caused by opening the commodity test chamber to add or change a sample.

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C - Collect cycle - the detector is sealed off with no additional external air pumped through the system.

D - Peak - height indicates amount of internally produced CO_2.

Figure 2. Peak Mode Chart Print-Out of a 5-Pound Packaged Commodity in the Shrink Bag.

given off from the sample in the commodity test chamber was recorded as a peak on a strip chart print-out. In this investigation the assumption was made than an insect infestation would produce the CO_2 and that the magnitude of the peak would be directly related to the number of insects present.

The insect detector could detect adult insect infestation in a 1-pound sample of whole grain wheat (Table 1) with some variations. Using the Ramp mode, the insect detector recorded a greater ramp height with commodities containing three rice weevils (12.00 mm) than with five rice weevils (8.32 mm). This seemingly indicated that three rice weevils produced more CO_2 than five rice weevils.

TABLE 1. AVERAGE PEAK AND RAMP HEIGHTS (mm) OF CO_2 PRODUCED BY THREE DIFFERENT SPECIES OF ADULT STORED PRODUCT INSECTS IN A 1-POUND SAMPLE OF WHOLE GRAIN WHEAT WITH A 5-MINUTE PURGE CYCLE AND 10-MINUTE COLLECT CYCLE

No.	Red Flour Beetles Mode		Rice Weevils Mode		Lesser Grain Borers Mode	
Beetles	Ramp	Peak[a]	Ramp	Peak[a]	Ramp	Peak
0	0.00	1.00	0.00	2.50	0.00	3.32
1	0.66	2.00	1.32	3.32	0.00	--
2	1.66	--	2.32	--	0.00	--
3	5.32	6.50	12.00	--	1.32	9.00
5	13.66	--	8.32	--	3.00	13.00
6	--	19.50	--	7.66	--	--

a. Five-minute purge, 5-minute collect.

Both modes involved a considerable expenditure of time. To decrease the time required to test a sample, the collect time was reduced from 10 to 5 minutes using the Peak mode. This reduced the sensitivity of the instrument, but it still could detect differences in red flour beetle or rice weevil infestation levels. The insect detector required a 10 minute collect time for samples containing the smaller lesser grain borer, which probably produced lesser quantities of CO_2 per individual.

The initial tests were conducted using whole grain wheat to learn how the detector reacted to different levels of insect infestation. However, the main interest of the Department of Defense and the major objective of this report was to evaluate the effectiveness of the insect detector in determining various levels of insect infestations in processed commodities. Additional tests were performed using the red flour beetle in 5-pound bags of corn meal and flour because fewer of these insects are allowed in processed food commodities. According to Military Standard 904,[13] a product in a military wholesale food facility can contain a maximum of six insects per pound of product unless the infestation involves *Tribolium* or *Trogoderma* insects, the limits of which are three and zero, respectively. However, any level of insect infestation would bar acceptance of a processed commodity by The Department of Defense.

Further tests were conducted using a beetle-infested 5-pound bag of corn meal at five different temperatures (20°-27°C) to simulate temperature fluctuations generally found in government warehouses. The results confirmed that red flour beetles respired more at 27°C than at 20°C (Table 2) as indicated by increased peak heights. These results agree with the observations by Hunter and Hartsell,[16] who found that increasing the temperature would increase CO_2 production in larvae of another stored product insect, *Plodia interpunctella*.

TABLE 2. PEAK HEIGHTS (mm) OF CO_2 PRODUCED BY ADULT RED FLOUR BEETLES IN A 5-POUND BAG OF CORN MEAL WITH A 15-MINUTE PURGE CYCLE AND 5-MINUTE COLLECT CYCLE

No. Beetles	Temperatures										
	20°C		21°C		24°C		25°C		27°C		Average 20°-27°C
0	1	0	4	1	1	0	3	2	0	2	1.4
1	1	0	3	1	0	1	3	2	3	1	1.5
14	4	4	4	4	4	4	8	6	8	3	5.2
15	2	4	3	4	6	4	7	5	5	2	4.3
30	6	11	9	8	10	8	9	4	8	9	8.2
45	9	6	12	8	14	11	10	6	17	16	10.9

With a certain degree of accuracy, repeatable results of peak heights corresponding to different levels of insect infestations were obtained by the insect detector, but there were variations. An uninfested sample could be distinguished from one with 15 red flour beetles (three insects per pound). The detector could not accurately differentiate between a sample having 14 beetles or 15 beetles nor between samples containing 1 beetle as opposed to 0 beetles. The results of tests conducted using a beetle infested 5-pound bag of flour at two different temperatures (24°-26°C) are given in Table 3. Fewer replications were conducted, but these results supported the findings of the tests conducted with samples of corn meal.

TABLE 3. PEAK HEIGHTS (mm) OF CO_2 PRODUCED BY ADULT RED FLOUR BEETLES IN A 5-POUND BAG OF FLOUR WITH A 15-MINUTE PURGE CYCLE AND 5-MINUTE COLLECT CYCLE

No. Beetles	Temperatures 24°C		26°C		Average 24°-26°C
0	2	1	0	2	1.3
1	2	3	0	4	2.3
14	5	4	6	6	5.3
15	5	4	3	4	4.0
30	5	5	7	8	6.3
45	--	--	17	12	7.3

Advantage

The insect detector was able to distinguish the difference between an uninfested sample and a sample which contained three *Tribolium* sp. (red flour beetles) per pound or six of any other genera of insects per pound of food commodity. These figures represent the maximum number of insects allowed in a stored food product in a military wholesale food facility.

Disadvantages

1. The insect detector could not detect low-level insect infestations in 5-pound packaged commodities or 1-pound samples of whole grain within a reasonable length of time (less than 5 minutes).

2. With whole grain samples a stable baseline could be established in 10 to 30 seconds while 10 to 15 minutes were required for baseline stabilization in packaged commodities.

3. The time required to establish a stable baseline obviously increased sample run time. A whole grain sample could be tested in 10 minutes or less but a packaged commodity would usually take at least 20 minutes. Additional time was required if more replications were desired.

4. There were variations in the recorded results.

5. If the machine was jarred only slightly, a false peak would be recorded. If the insect detector was placed on an unstable table or cabinet the baseline would be broad and wavy.

6. The sample chamber (shrink bag or plastic container) had to be placed on a cushioned surface away from the machine. This reduced the vibrations caused by the operation of the insect detector. The vibration reduced the respiration rate of the insects thereby reducing CO_2 production[15] which caused smaller peaks to be recorded.

7. The insect detector, capable of measuring CO_2, could not detect dead insects, insect parts, differences in species or their stage of development,[17,18] or other contaminants in packaged commodities. Also, lower temperatures decreases the respiration rates (CO_2 production) of insects.[16]

8. Any source of CO_2 near the machine caused it to register peaks on the recorder. If the operator stayed within 1 or 2 feet of the machine, peaks would be recorded from the CO_2 given off by the operator. The detector worked best when the operator set the machine to run a sample and left the vicinity. A solution to this problem would be to connect a closed source of air to the inlet port on the back of the insect detector.

9. Sometimes negative slopes were recorded when the Ramp mode was used.

10. The timer for the purge cycle was capable of running only a maximum of 5 minutes. Fifteen minutes were necessary to purge a stored product packaged commodity to obtain a stable baseline. Another timer could be installed to remedy this problem.

11. The shrink bag should be only slightly larger than the commodity to be examined. This would reduce some of the time it took for the bag to collapse around the commodity. Several different sized shrink bags would be necessary to accommodate different sized packages. Also, the best position of the inlet and outlet ports of the shrink bag varies for different commodities.

12. The inlet and outlet ports frequently collapsed against the bag and blocked the air flow. The operator then had to manually pull the bag away from the ports.

13. The shrink bag was made of very thin plastic which could be torn. Also, there may have been some leakage of air through this material.

SUMMARY AND RECOMMENDATIONS

The second prototype of the hidden insect detector showed much improvement over the first prototype. However, the second prototype still was unable to meet required performance criteria. The second prototype conceptually was technically usable and could detect three insects per pound of commodity, but other physical and mechanical problems made it operationally unacceptable. A system which had the same basic principle of operation, but was more refined to eliminate and minimize undesirable characteristics could have potential in detecting hidden insect infestations in military stored product warehouses. However, since live insects are not the only contaminants for which examinations must be conducted, it appears unlikely that such a device will ever replace manual inspection.

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APPENDIX A

COMMENTS CONCERNING THE FEATURES AND CHARACTERISTICS OF THE RAPID NON-DESTRUCTIVE STORED PRODUCTS INSECT DETECTOR SYSTEM (FIRST PROTOTYPE)

Advantages

1. Instrumentation and carbon dioxide detector appeared to have adequate sensitivity for detection of carbon dioxide concentrations and to changes in concentrations in bulk commodity sample handling systems.

2. Instrumentation demonstrated the capability to detect, display and record small changes (0.25 ppm) in carbon dioxide level with relatively short collect times when bulk product samples evaluated had relatively large interstitial spaces.

3. In the laboratory it was demonstrated that, with unpackaged bulk products with relatively large interstitial spaces, repeatable results could be rapidly obtained.

4. Printer provided an easily readable, rapid print-out of results.

Disadvantages

1. Results using this system with processed agricultural products having little interstitial space and capable of being compacting were unreliable and often not repeatable.

2. Purge time requirements (greater than 6 minutes) for processed products were excessive.

3. Leakage of vacuum from the shrink bag was inherent in this system. Degree of leakage varied considerably from run to run.

4. Initial design shrink bag provided with this system had valves which interfered with the sealing of the pack. This pack also ripped during initial testing.

5. System is too bulky. Weight and bulk could be reduced by rearranging components. Vacuum lines were longer than required.

6. Instrumentation required in excess of 30 hours for complete warm-up and stabilization of the baseline. To accommodate this, the system has to be on 24 hours per day.

7. The system is dependent on a 110/120 volt, 60-cycle electrical system, thus limiting its operating environment.

8. Internal insect infestation of the instrument could and did develop. However, this was noted only in the replaceable internal filter elements.

9. Instrumentation is sensitive to movement. Chart recordings reflected and plotted even slight movement of the system. If the instrument is moved during operation, additional stabilization time is required.

10. Changes in the CO_2 in the surrounding environment interfered with the accuracy of the readings.

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LABORATORY EVALUATION OF THE USDA RAPID NONDESTRUCTIVE STORED PRODUCTS INSECT DETECTOR

US ARMY MEDICAL BIOENGINEERING RESEARCH AND DEVELOPMENT LABORATORY
Fort Detrick, Frederick, MD 21701

Page 13, SUMMARY AND RECOMMENDATIONS

Line 9, delete last sentence in toto "However, since live insects . . . manual inspection."