Detecting insect infestation in stored wheat grain using solid phase microextraction (SPME)

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Abstract

Rapid detection is necessary for monitoring early infestation by insects of stored wheat grain and indicating the need for fumigation. This study investigated a potential new method, solid phase microextraction (SPME), for detecting insect infestations in stored wheat. It is a technique for isolation of volatile compounds in the headspace. The results show that volatile pheromones (VP) produced by common stored grain insects can be used to indicate an infestation. The amounts of pheromone released varied between species. Lesser grain borer (LGB), *Rhyzopertha dominica* Fabricius produced the highest amount of VP among the species investigated. It was followed by red flour beetle (RFB), *Tribolium castaneum* Herbst, rusty grain beetle (RGB), *Cryptolestes ferrugineus* Stephens and flat grain beetle (FGB), *Cryptolestes pusillus* Schonherr, respectively. This resulted in different sensitivities when SPME was used for detection. A single specimen of RFB and LGB could be successfully detected in 1 kg of wheat whereas groups of 15 and 20 of RGB and FGB male beetles were required to be detected in the same quantity of grain. Extraction time and temperature affected the sensitivity of the SPME method. Longer exposure times and higher temperatures allowed more analytes to be collected. Consequently, extraction for 4 h at 50°C appeared to be the best condition for extracting pheromones produced by the beetles in this study. The method was further adjusted to suit industrial conditions. Detection of a single LGB can still be successfully achieved even though the time and the temperature were reduced whereas for the remaining species, the minimum number of insects required had to be increased by about 40-60% in order to detect VP in headspace. Lastly, SPME also demonstrated potential to be used in the larger scale where a total of 7 male insects can be detected in 20 kg of grain.

Keywords: Solid Phase Microextraction (SPME), wheat grain, pheromones, lesser grain borer (LGB), sensitivity

1. Introduction

Insect infestations cause quantitative losses to stored grains as the kernels are fed upon and damaged by insects. Also, the appearance and sensory properties can be altered through physical damage and contamination by feces, webbing and body parts of insects (Bulla et al., 1978). This leads to the loss in economic value of grain. Therefore, application of insect detection technologies is of interest to the grain industry in order to reduce losses and improve grain and grain product quality.

There are many insect detection techniques available today. The commercial techniques include manual sampling, traps and probes, which are the most basic tools used on farms, while manual inspection, sieving, flotation and Berlese-funnels are more advanced techniques used in grain handling facilities (Neethirajan et al., 2007). Adult insects are often easily trapped or detected by these techniques but detection of the immatures is limited to some extent. Hence, detection of immatures requires the application of other techniques to grain

which demonstrate higher sensitivity and accuracy. X-ray imaging and near infrared reflectance (NIR) spectroscopy have been extensively studied since they operate in a rapid and non-destructive manner. Also, they can detect young insects and hidden insect infestation which cannot be detected by visual inspection. However, the cost of these technologies is relatively high and they require trained labour for operation (Milner et al., 1950; Neethirajan et al., 2007). Due to high costs of detection technologies, some researchers are now investigating odour detection techniques to be applied on infested grain since odour detection has high sensitivity and accuracy with moderate costs.

Odour is a useful tool in monitoring grain quality. It has become part of standard methods for grading grain in United States (Ram et al., 1999). However, the operations done by humans are often affected by fatigue after inhaling the target analytes for repeated trials, which leads to limited samples for analysis and possible inaccurate results. Therefore, volatile detection/isolation is a practical technique that may substitute humans and also eliminate the associated problems. Studies related to volatile/odour often involve discrimination, detection and extraction of particular volatile components (VC)/odours. Headspace techniques are the most appropriate for detection of the VC that are vaporised from the sample under ambient conditions. VCs are collected from the headspace (Steinhart et al., 2000; Augusto et al., 2003), condensed and identified using gas chromatographic techniques. This study focuses on headspace techniques because those could be operated under low temperature and the samples are not destroyed.

Among the VC of particular interest in detecting the presence of insects in stored grain are the pheromones. Pheromones are defined by Wilson (1963) as 'those substances secreted by animal to influence the behaviour of another animal of the same species'. In insect population, pheromone is used for communication. As it is odorous, it allows insects to pass their messages a long distance. The molecular weight (MW) and structure of most pheromones are often low and simple (Regnier and Law, 1968; Thomas, 1997). Because of these properties information can easily spread over the large insect population.

Solid phase micro extraction (SPME) was selected to detect grain infestation by common stored grain pests in this project due to its high sensitivity, is easy to operate, mobile and has moderate costs which appeared to meet the industry goal and outweighs the drawbacks (Pillonel et al., 2002). Moreover, it has not yet been applied to monitor insect infestation consequently there is a gap that this study attempts to cover. Hence, the aim of this study was to investigate the potential of SPME for detection of pheromones as an indication of insect infestation in stored wheat grain.

2. Materials and Methods

2.1. Materials

Wheat samples, variety Baxter, were supplied by Grain Growers Association Ltd. To preserve the grain, it was stored in a freezer at -20°C and allowed to thaw overnight at room temperature before use in experiments. Insect cultures were obtained from CSIRO Entomology and Evolutionary and Ecological Functional Genomics, CSIRO Ecosystem Sciences, Canberra. Four species were investigated in this study: *R. dominica* (LGB), *T. castaneum* (RFB), *C. ferrugineus* (RGB) and *C. pusillus* (FGB). Insect cultures were maintained in an environmental chamber with controlled temperature and relative humidity. LGB were reared on 1 kg of wheat grain at 30ºC, 70% r.h. while RFB, RGB and FGB were raised under the same conditions on 1 kg of flour and crushed wheat with 3% brewer's yeast respectively. The same generation of each species was obtained using five hundred adult

insects on appropriate feed and stored for three weeks. The insects were then sieved out and the new generations of insects that emerged (approximately one week old) were used throughout all the experiments. Male beetles were used for determination of experimental sensitivity, consequently each species was sexed before the examination. Males LGB were determined by selecting only the beetles that produced the aggregation pheromone using SPME method as there were no other reliable methods (Edde, 2012). For the other species, the gender was distinguished according to methods proposed by Ho (1969) for RFB, Reid (1942) for RGB and Department of Agriculture and Food Western Australia (2011) for FGB respectively. Synthesised pheromone standards of LGB and RFB were supplied by Trécé Inc (Adair, OK, USA), and 1-pentadecene was purchased from Sigma Aldrich.

2.2. Methods

The methodology used for extraction and identification of pheromones is described in detail in Laopongsit et al. (2014). This refers to the development of the SPME and SPME-GC/MS method, fibre selection, determination of indicator compounds, determination of sensitivity and development of streamlined method of determination of volatile components.

3. Results and Discussion

3.1. Development of SPME method

With regard to SPME fibre selection, most of wheat volatile components were collected by PDMS-CARB-DVB while PA gave the lowest recoveries. PDMS-DVB also appeared to extract many volatile components as PDMS-CARB-DVB but there was a difference in the abundance of each volatiles collected where the peak that eluted later was significantly higher than the earlier components. The initial work has shown that the optimum condition for extraction of VC from 2.5 g of non-infested wheat was as follows: PDMS-CAR-DVB fibre, 50°C and 4 h extraction time. Therefore VCs from infested wheat were extracted under the same conditions.

VC identified as being produced by live LGB were considered to be potential indicators of infestation. Of these compounds, ten were found in infested grain. Seven of them were poorly identified and showed as minor peaks on the chromatogram (not shown here). Consequently, they were not of interest. In contrast, the remaining three were the major components reported by Seitz and Saucer (1996) as the volatile pheromones (VP) of LGB. They are Dom 1, (s)-1-methylbutyl-2-methyl-2(E)-pentenoate, Dom 2, (s)-1-methylbutyl (E)-2, 4-dimethyl-2-pentenoate and Dom 3, 1-methylbutyl (E)-2-methyl-2(E)-hexenoate, respectively (see Fig. 1).

A total of 37 compounds (3 were unidentified) were found to be produced in grain sample infested with RFB. After subtracting VC from non-infested grain, there were still 2 VC remaining as possible indicators after extraction of VC from insect culture alone (20 beetles). The two compounds were 4,8-dimethyltridecanal or Triboloure (Tbl) and 1-pentadecene (Fig. 2). Two metabolites reported to be uniquely secreted by RFB out of which Tbl is the aggregation pheromone of this *Tribolium* species (Suzuki et al., 1975a; Suzuki, 1980). Again, both were confirmed by comparing the mass spectra of synthesised Tbl provided by TRÉCÉ and 1-pentadecene purchased from Sigma Aldrich.

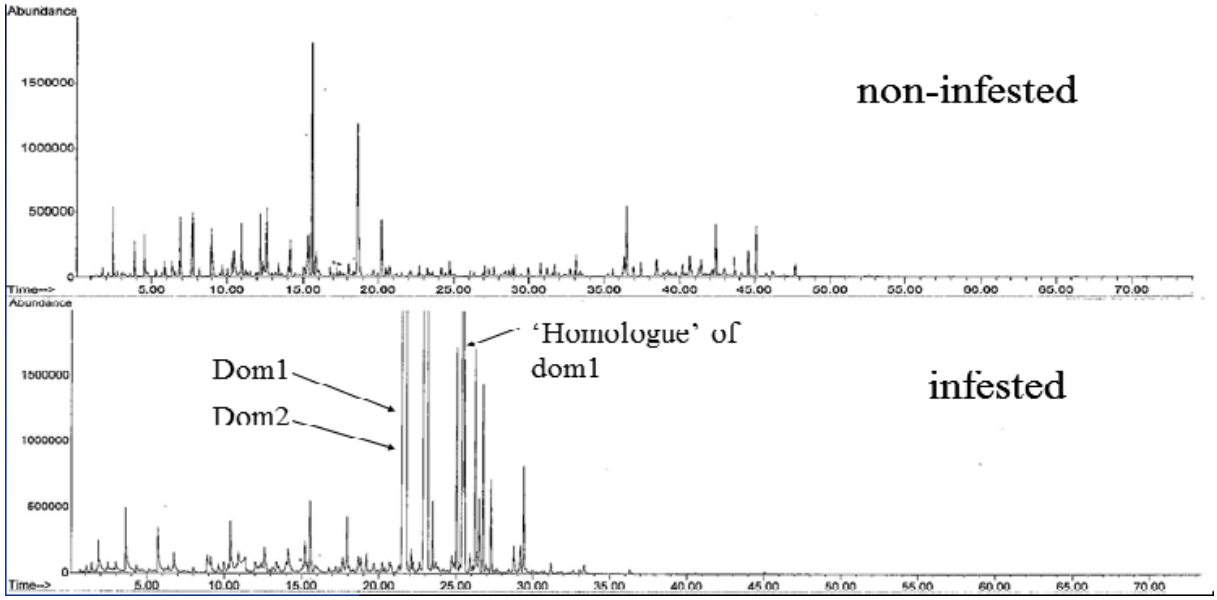

Figure 1 Pheromones detected in wheat sample infested with LGB.

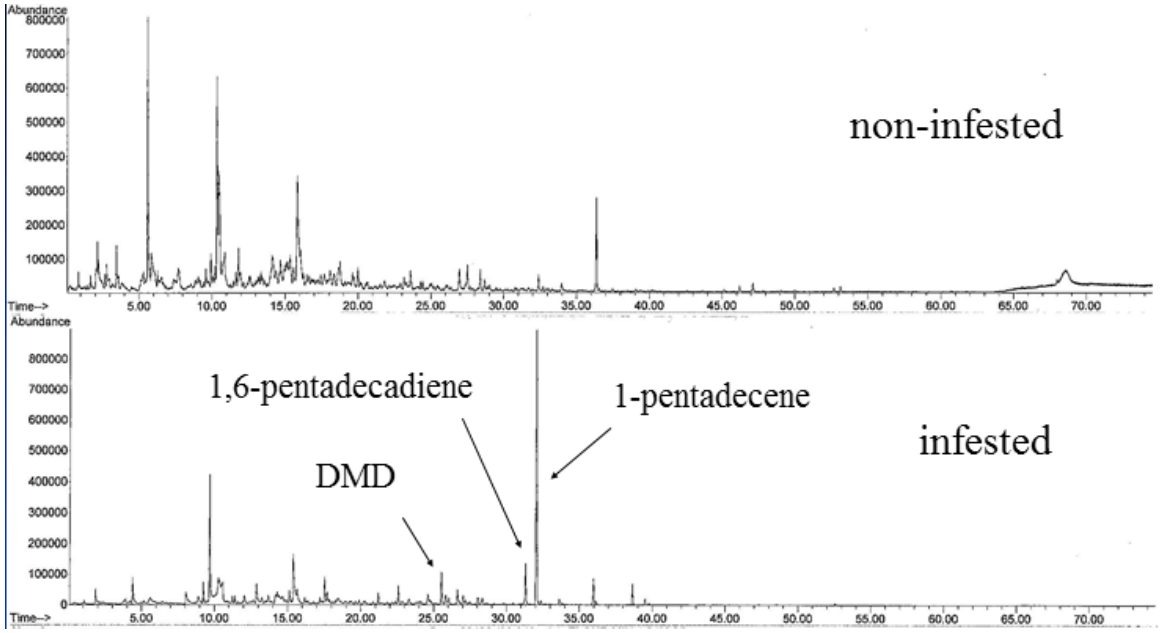

Figure 2 Pheromones detected in wheat sample infested with RFB.

As for the determination of the detection limit, the following method was found to produce the optimum results:

- Jars with infested wheat grain:
  - 10 jars of 1 kg of cereal (wheat for LGB & flour for RFB) infested with 1 insect

- 2 jars with 5 LGB and RFB
- 2 jars with 10 LGB and RFB

- One jar with non-infeste wheat (control)
- Incubate at 30°C 60% r.h. for 25 days
- Extract with SPME under optimum condition

As a result, it could be shown that the sensibility of the method was as follows:

- LGB

  A single LGB could be detected in 1 kg of wheat
  Amount obtained varied

- RFB

  A single RFB could be detected in 1 kg of flour
  Amounts obtained also varied

- RGB and FGB

  15, 20 of RGB and FGB male beetles were detected in 1 kg of wheat

4. Conclusions

VP produced by insects may be used as indicators of infestation in grain. The SPME can successfully isolate those VP from the grain matrix and insects. Dom 1, Dom 2 and Dom 3 were isolated and identified to be the aggregation pheromone of LGB and they were the unique compounds that are released only by this species. For RFB, Tbl, aggregation pheromone was found along with 1-pentadecene. These are the most common volatile metabolites that are found in all Tribolium species. The aggregation pheromone of each insect species can be used to indicate infestation for particular species.

This study has highlighted the potential of SPME to detect insect infestation in grain. SPME appearS to be easy to operate but is sensitive to time and temperature of extraction. With its application in detection of insect infestation, SPME still requires further development to improve its in order to be able to detect insects that produce relatively low amount of pheromones.

As shorter and less complex extraction was more acceptable for the industrial practice, the extraction method was adjusted to suit this purpose. However, this has reduced the sensitivity of detection in most of the insects species investigated. However, the detection of VP released by LGB appeared to be the most promising as they produced a high concentration of VP that it could be detected even at lower temperature. Most importantly, LGB is a primary pest which attacks the intact grain before other species like RFB, RGB and FGB. Consequently, detecting LGB in grain does not only indicate grain damage but also assists in preventing the emergence of infestation by a secondary pest.

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