Early detection of insect infestation in stored grain based on head space analysis of volatile compounds
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Abstract
Insect infestation is a common problem for stored grain. Insects can cause quantitative losses as kernels are consumed by insects. Also, the appearance and organoleptic properties can be altered through physical damage and contamination by faeces, webbing and body parts of insects, respectively. Therefore, several detection techniques have been developed and applied to stored grains. Some methods demonstrate very high sensitivity but are relatively costly, and thus, are not affordable to the industry; whereas cheaper methods lack detection sensitivity. Consequently, volatile isolation techniques could be an alternative approach to monitor insects in stored grain. Odour detection is a useful tool for monitoring grain quality. It has become part of standard method for grading grain in the United States. Although identification of volatile compounds produced by fungi species has been extensively studied in most types of grains, this has been less done for insect infestation. This paper reviews the literature related to detection of insect infestation in stored wheat by using volatile isolation techniques.

Keywords: Grain infestation, Insect detection methods, Volatile isolation techniques, Solid phase microextraction

1. Introduction
Insect infestation is a common problem for stored grain. It can cause quantitative loss to grain as kernels are consumed by insects. Also, the appearance and sensory properties can be altered through physical damage and contamination by faeces, webbing and body parts of insects (Bulla et al., 1978). This leads to the loss in economic value of the grain. Therefore, application of insect-detection technologies have raised a lot of interest in the grain industry in order to reduce loss and improve grain and grain-product quality.

There are many insect detection techniques available. Commercial methods include manual sampling, traps and probes which are the most basic tools used on farm. Manual inspection, sieving, floatation 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, unlike immature insects, where detection is still limited to some extent. Hence, this requires the application of other techniques to grain which demonstrate higher sensitivity and accuracy. X-ray imaging and nuclear infrared reflectance (NIR) spectroscopy have been extensively studied since they are rapid and non-destructive methods (Milner et al., 1950; Karunakaran et al., 2003; Karunakaran et al., 2004; Rajendran and Steve, 2005; Fornal et al., 2007; Neethirajan et al., 2007; Haff and Toyofuku, 2008). Also, they can detect young insects and hidden insect infestation which cannot be seen by visual inspection. However, the cost of these technologies is relatively high and they require trained labour to operate (Milner et al., 1950; Neethirajan et al., 2007). Due to the high costs of detection technologies some researchers are now investigating odour detection techniques to be applied on infested grains 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). Along with odour evaluation by the human nose, technologies and techniques have been developed to help extraction for detection of particular odours. They could be classified into two categories: distillation and headspace techniques. The conventional method is distillation where volatiles could be extracted from sample matrices through distillation process. This practice is time-consuming and the sample could be easily decomposed; whereas, the headspace method
often involves shorter time without interrupting sample matrices at all. Therefore, it is suitable for fast screening test and the operations which have high output volume.

Many studies have focused on detection of fungal spoilage and contamination in grain by using volatiles (Borjesson et al., 1989; Magan, 1993; Magan and Evans, 2000; Olsson et al., 2002; Paolesse et al., 2006). However, reference to application to insect infestation is very limited in literature. Therefore, the aim of this paper is to review the potential of using volatiles to detect insect infestation in stored grain on the basis of published studies done so far. Headspace analysis will be focused upon here as it is more applicable to grain industry.

2. Insect pheromones

Insect pheromones are a substance which insects secret to influence the behaviour of other insects (Wilson, 1963). As it is odorous, it allows insects to pass their messages over long distances. Consequently, in the grain industry, this has been applied to stored grain with the aim to prevent insect infestation. Synthesized pheromone or chemical attractant was enclosed in a rubber or plastic case. Then, the insects will be lured and trapped inside the case. Pheromone traps are usually placed far away from stored grain so they can be safely protected from insect pests (Rajendran and Steve, 2005).

In terms of insect infestation detection, pheromones are potentially useful because they are important for insect communication. Hence, if volatile isolation detection techniques are able to detect and identify the chemical compounds that are emitted by insects as pheromones in grain, the results could be used to detect infested grain.

3. Volatile extraction/detection technique based on headspace analysis

3.1. Dynamic headspace extraction (DH)

DHS (or purge and trap) can be operated by flushing a stream of air or inert gas to purge volatiles in the headspace, then an adsorption tube is used to trap all the organic compounds carried by the gas. Many sorbent materials are available today. Tenax resin is probably the most widely used since a wide range of organic volatiles can be adsorbed, particularly aromatic compounds. Besides, it is made of porous polymers which are similar to the materials packed in GC columns. Thus, this allows desorption of the analytes at high temperatures close to those of the GC (Wampler, 1997). Consequently, the volatiles trapped from DHS can be directly introduced into the GC or GC-MS (Wampler, 1997; Rouseff and Cadwallader, 2001).

DHS-GC is a conventional volatile extraction method. Consequently, it has been used to analyze grain odour more widely than other volatile extraction techniques. The initial works of DHS-GC in grain studies involved identification of particular components in grain volatile mixtures and detection of off-odours in grains which were caused by microorganisms (Seitz et al., 1999; Sayaslan et al., 2000; Seitz and Ram, 2000). In terms of insect-damage detection, although it is a conventional method, application of DHS to infested grain is still limited in the literature. This may be because it is more time consuming and more complex than SPME and electronic nose. However, it also been used along with these techniques in some studies (Seitz and Saucer, 1996; Seitz and Ram, 2004).

3.2. Headspace solid phase microextraction (SPME)

SPME is a modern technique that is rapid, inexpensive and good for heat sensitive materials (Richter and Schellenberg, 2007). Headspace analysis of SPME involves insertion of a coated silica fibre above the sample, allowing the adsorption of the volatile compounds for a certain time. Concentrated volatiles can be readily obtained without interference from food matrices and other non-volatile compounds from the headspace (Richter and Schellenberg, 2007). Subsequently, the SPME needle is removed and inserted into the GC. Once the fibre has been placed in the GC inlet, heating causes the volatile compounds, adsorbed by the fibre, to be released into the GC column. Finally, volatiles are separated and characterised by GC or GC-MS (Martos and Pawliszyn, 1998; Reineccius, 2002; Turner, 2006).

Like DH, SPME has been widely applied to detect fungal volatiles in grains more than insect infestation (Jelen and Grabarkiewicz-Szczesna, 2005; Paolesse et al., 2006). However, very limited studies have used SPME to detect insect infestation in grain. Seitz and Ram’s (2004) study is probably the only study that generated results that can be used to monitor insect damage in stored wheat (Seitz and Ram, 2004; Fernandes et al., 2010). Some compounds from wheat infested by lesser grain borers were identified by
SPME coupled with GC-MS in their experiment. However, it has not yet been proven to be a reliable method of monitoring insect infestation, because no validation studies have been conducted. There are two more studies with a similar aim, namely to use SPME-GC-MS to identify metabolites and pheromones which are the unique characteristic of particular insect pests (Arnaud et al., 2002). Therefore, it could be concluded that the current position of the SPME application in stored-grain research are still growing; some studies have started using SPME to detect and identify pheromones and their metabolites which may have potential to be used to monitor insect infestation in grains. If this technique is successful, SPME is likely to be applied to grain industry because it is relatively inexpensive and simple to perform.

3.3. Electronic nose

Electronic nose (E-nose) is an excellent approach in odour analysis. It has been widely used in food and flavour industries for over 20 years (Rajendran and Steve, 2005). Persaud and Dodd (1982) were the first to introduce the E-nose with the aim of mimicking the discrimination of the human olfactory system. It is comprised of an array of chemical sensors which are used to detect odour above the sample with different selectivity (Persaud and Dodd, 1982; Marti et al., 2005; Rock et al., 2008).

Studies of E-nose application to grain with the aim of detecting insect infestation are slowly expanding. It was first reported by Stetter et al., (1993), where wheat samples were discriminated as good, sour and insect infested. In this study, 83% of wheat samples were successfully classified by the gas sensor, chemical parameter spectrometry (CPS). In a paper by Hu (2006), E-nose technology was used to detect insect damage in rice. Degrees of insect damage (after 10, 15 and 24 h) could be detected and differentiated. The longer the infestation time, the better E-nose was able to identify infested grain. In the following year, Zhang and Wang (2007) published a paper focusing on similar experiments. They showed that fifteen percent of insect damage could be determined by E-nose. Moreover, volatile profile of different ages of grain was also classified. Future trends in insect damage detection by E-nose seem to improve the detection more and more. With a better pattern analysis software and chemical sensors, E-nose has potential to not only discriminate but also to identify the components of volatile mixtures produced during insect infestation.

4. Conclusions

This paper has highlighted the relevant literature related to detection of insect infestation in stored grain. Current techniques applied to stored grain are still too low in sensitivity to detect insect infestation and the high sensitivity tools such as x-ray and NIR are too costly. Volatile isolation techniques may demonstrate moderate sensitivity which will compromise with the cost. Therefore, it appears to be an attractive technique to be used as an alternative approach to detect insects in stored grain. Identification of volatile compounds that indicate insect infestation would be the goal for the development of this technique. Headspace techniques appear to be the most suitable technique for volatile extractions because they are non-destructive methods. DH, SPME and electronic nose are the main headspace methods on which grain researchers are currently focusing. Eventual validation of laboratory findings in field trials will provide information on the potential for adoption of these techniques at an industrial scale.

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