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Insect Detection Methods

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Abstract: *Cereals, oilseeds and legumes herein after collectively referred to as grains are critical to human survival. Cereal grains are the major grains produced and according to "Definition and Classification of Commodities" by Food and Agricultural Organization (FAO), are defined as grains cultivated for their dry-highly nutritious seed. During storage, grain undergoes various biological, physicochemical, interactions between environment and grain hence storage should be monitored and managed. Storage is an important link in the entire procurement and distribution system of food grains which are produced seasonally and consumed all year round. Storage of wheat is done to reduce the seasonal fluctuation and meet demand of future food, feed and seed purpose.*

Key words: *Cereals, Legumes, Oil seeds, Storage*

INTRODUCTION

Cereals, oilseeds and legumes herein after collectively referred to as grains are critical to human survival. Cereal grains are the major grains produced and according to "Definition and Classification of Commodities" by Food and Agricultural Organization (FAO), are defined as grains cultivated for their dry-highly nutritious seed (FAO 2014). Cereal grains (wheat, rice, maize, rye and barley) are staple food for majority of world population. Wheat (*Triticum* spp) is an important cereal crop cultivated worldwide, providing food for one-third of world's population (Curtis 2002; Cordain 1999). According to the production statistics the three major producers of wheat are China, India and United States, and major commercial producers for wheat trade are United States, Canada and Australia (FAS 2014).

WHEAT AND LOSSES IN WHEAT

Wheat is a seasonal crop consumed throughout the year, hence after harvest; storage for extended periods is required to meet the demand-supply in domestic and export markets. Harvesting of wheat is done at temperatures ranging from 10-35°C and moisture content ranging from 13-24% (wet basis (w.b.)) depending on the area of cultivation and harvest time (Jayas 1995). A process of reaping, threshing and winnowing is done to get the wheat kernels. The kernels are dried if needed, cleaned, cooled and stored for further processing. Storage of wheat is done on farm in indigenous storage structures according to the storage environment and area (Sharon et al 2014; Jayas 1995).

During storage, grain undergoes various biological, physicochemical, interactions between environment and grain (Jian and Jayas 2012); hence storage should be monitored and managed. Wheat has a very high importance in human diet and there is a need to provide wheat grains with high quality. The stored grain is to be protected from degradation until consumed; else it gets deteriorated or contaminated. This is termed as loss. India is the second largest producer of wheat, with a production of 92.5 million tonnes in 2013 (FAOSTAT 2013). Every year in India 10% of this production is lost during storage, transport and distribution, due to improper management. The grain gets deteriorated by various biotic and abiotic factors (Sharon et al. 2014; Rani et al. 2013). Canada ranks seventh (FAOSTAT 2013) in the production of wheat, however storage losses are less than 1% because most systems are well managed (Jayas 2012). Statistical estimates confirm a large

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increase in the output of cereal grains in 2014 in India and Canada (FAOSTAT 2013). Thus storage, management and protection of this huge production are needed from various pests, like insects, mites, microorganisms, birds and rodents. Insect infestation is to be controlled for maintaining the high quality of grain. This is achieved by the use of chemical fumigation quickly and easily. But now due to the concerns about health hazards and ozone layer depletion and ban of these chemicals in the Montreal protocol, there is a need of new research to be conducted for alternatives for chemical fumigation for disinfestation.

Storage is an important link in the entire procurement and distribution system of food grains which are produced seasonally and consumed all year round. Storage of wheat is done to reduce the seasonal fluctuation and meet demand of future food, feed and seed purpose. The production of wheat in the world increased due to green revolution and introduction of high yielding varieties. The world production of wheat in 2012-13 was 792 million metric tonnes (FAOSTAT 2013). Harvesting of wheat is done when wheat stalks turn brown or yellow from green, and the wheat heads tip towards the ground. A process of reaping (cutting the wheat stalk), threshing and winnowing is done to get the wheat kernel or seed. The wheat kernels are then dried (if needed), cleaned and stored for further processing. The safe storage guidelines of wheat are dependent on initial moisture content and seed temperature. The recommended guidelines are 10-14% mc and 5-20°C for storing more than 6 months (Nithya et al. 2011; Karunakaran et al. 2001; Brooker et al. 1992), and 14-18% mc at temperatures 20-45°C for 6 months to 2 weeks (AAFC 2010).

Storage of wheat is done on farm in heaps, indigenous structures or in bulk storage structures, bags, bins or silos depending on the area where wheat is cultivated. Stored wheat is a living entity which continuously undergoes respiration and biological changes, being desirable and undesirable. The undesirable changes are undesired color, contamination, sprouting, hot spots formation etc., which cause pests infestation causing huge losses. The losses are quantitative and qualitative occurring due to physical (abiotic), biological (biotic), chemical (breakdown of produce and pesticides) and engineering (structural and mechanical, aspects) factors (Jayas 1995). The grain losses during storage are directly related to the financial losses. Scientific storage is essential to reduce the food grain losses. Annually, the total economic losses in the world due to stored-product pests and microorganisms in grains and oilseeds can be in the millions of dollars (White 1995; White 1992). Increase in global awareness about the consumption of clean grains and its products, has increased the demand for food grains with the highest quality and insect presence should be avoided. Countries like Canada have legally defined zero tolerance for stored-grain insects (Canada Grain Act, 1975). In this scenario, serious attempts are needed to control insects in stored grains at early stage of infestation for pest management in wheat.

LOSSES DUE TO INFESTATION IN WHEAT

The loss of wheat grains due to insect pests is estimated to be 10-20% of produce yearly (White *et al.* 2011; Oerke 2006), depending on the storage system adopted and environmental conditions. The losses caused by insect pests are loss of quality and quantity. The post-harvest losses of grain are caused by the unscientific storage, insects, rodents, micro-organisms etc. Insect pests like *Cryptolestes ferrugineus* (Stephens), *Tribolium castaneum* (Herbst), *Tribolium confusum* (Jacquelin du Val), *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.), *Sitophilus granarius* (L.) and *Trogoderma granarium* (Evert) are mostly found in cereal grains during storage. Among these insects, one of the major insects infesting wheat is the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) a predominant insect in grain storage and handling systems all over the world (White et al. 2011; Alagusundaram et al. 1995). It is a cosmopolitan and a secondary pest infesting broken kernels. It attacks already broken wheat kernels and feeds the germ end of the kernel. It develops internally under the seed coat (Jian *et al.* 2006). The female adults lay eggs on the wheat kernel which hatch into larvae in 3 d, having four instars; which develops to pupae and emerges to adults in 25 d, at 30 ± 1°C and 70 ± 5% relative humidity in wheat of 14.5% moisture content (w.b.). The adults and the larval stages of this beetle are mobile (move), the pupae and eggs are immobile life stages. The larval stage feeds on the wheat germ. Rusty grain beetle infestation damages the grain internally (Bell 1994) causing loss in germination of the seed. The insect causes heat production thus leading to the formation of hot spots. If the infestation of this beetle is not managed, it leads to huge losses.

These losses are prevented by proper storage conditions, monitoring grain for presence of insects (detecting) and controlling their growth. This is done by using proper detection and control techniques.

Detecting the presence of insect pests is done using various methods (Neethirajan *et al.* 2007) and then the corrective measures are to be taken before the infestation can reduce grain quality. The various control or disinfestation techniques used for controlling insect infestation are physical (Banks and Fields 1995), biological (White *et al.* 2011) and chemical disinfestation methods. Producers have a limited number of options to control insect infestation in stored grains. The most common are fumigation with phosphine, carbon dioxide or methyl bromide (MB), treating grain with aerosols (methoprene) or contact insecticides (diatomaceous earth, malathion and synergized pyrethrin) (Arthur 2012) or new safe chemicals (spinosads or carbon dioxide). Alternatives to these methods are needed because of concern for worker safety with phosphine, concern over chemical residues (Popp *et al.* 2013), insect species becoming resistant to phosphine (Nayak *et al.* 2012), insect resistance to contact insecticides (Opit *et al.* 2012), strict regulations for standardization of new safe chemicals and regulations on methyl bromide phase out (MBTOC 2013; Fields and White 2002). The new technologies for alternative to fumigation should not cause harm to the environment and also provide control to the insect infestation. Use of temperatures (extreme, moderate and low) is one of the alternatives to these treatments (Fields *et al.* 2012; Beckett *et al.* 2007; Qaisarani and Banks 2000; Banks and Fields 1995; Fields 1992).

Insect infestation in grain is caused by mixing of already infested product, material or equipment with clean produce. Damage caused due to insect pests is by the direct consumption of kernels and germ which causes germination reduction, reduction in seed viability, musty odour formation, rotting of the surface, moisture migration in storage, heat production (hot spots) and contamination of grain by insect parts (White 1995). Conditions of high moisture content, optimum temperature for insect growth, excessive broken kernels and dockage provide the favourable conditions for the infestation.

Most insects thrive between temperature ranges between 16 and 45°C. Insects are found in both raw and processed food, and have adapted to feed on a wide array of stored commodities: cereal grains, legumes, tobacco, dried fruits and nuts, wood and even cloth fabric or carpet (White *et al.* 2011). Stored-product insects are found at different locations of the post-harvest pathway: in warehouses, elevators, flour mills, processing plants and stores (Arthur *et al.* 2014; Flinn *et al.* 2010; Flinn *et al.* 2007). Many stored-product insects crawl or fly long distances (Perez-Mendoza *et al.* 2004), which enables them to spread to new unexploited habitats. Insect infestation also leads to health risks such as pathogenic microbial infection (Hill 1990) and allergic reactions. All these lead to reduced market value and economic loss of stored grain (Hagstrum and Subramanyam 2006). Insect pests like *Cryptolestes ferrugineus* (Stephens), *Tribolium castaneum* (Herbst), *Tribolium confusum* (Jacquelin du Val), *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.), *Sitophilus granarius* (L.) and *Trogoderma granarium* (Evert) are mostly found in cereal grains during storage. Of which the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) is a predominant pest in stored wheat.

INSECT IDENTIFICATION METHODS

Sampling methods for insect identification

Insect identification is necessary for designing a control mechanism in stored grain. Monitoring of grain and its inspection is very important for storage, be it short or long term. Inspection is done by sampling procedures. Insect infestation estimation is done by collecting the sample of stored grain and checking for the presence of insect pests and damage indications (Neethiranjan et al, 2007). Sampling is done using devices of grain probe, trier, or spear (Mohan et al. 2012; Jian et al. 2010; Jian et al. 2008), which could be mechanical or manual. The sampled grain is tested for quality analysis (moisture content) and sieved to check for insect presence (live insects and insect parts).

Alonso-Amelot *et al.* (2011) compared the seven methods used for identifying the insects in rural areas and these were visual inspection, weight loss checking, standard volume to weight ratio, grain count and weight, percentage of damaged grains on weight basis, one-thousand grain mass and mass of grain of 1000 kernels including dust. Damage caused by rusty grain beetle could be identified by the presence of distinct burrowing hole in the germ area made by the emerging adult (Sinha and Watters 1985). Dissection of the wheat kernels is also done under microscope to check the presence of insect infestation; which is very time consuming and commercially not used.

Berlese funnel method (Fig. 1.1) is the common detection method used for insect infestation (Minkevich et al. 2002; Smith 1977), where 1 kg of wheat grain is sampled from the storage unit and is then sifted for adults using sieve. The grain is then

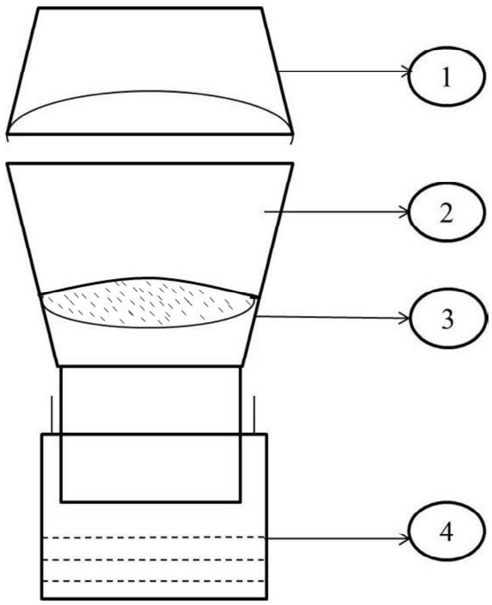

Figure 1.1: The "Berlese funnel" method used to detect insect presence (1: Light Bulb; 2: Metal funnel; 3: Metal screen; 4: Glass jar with 50 ml of 70% alcohol or water)

placed inside a funnel with a metal screen attached to the bottom. The funnel with grain sample is placed under incandescent light or under a heating source (or light source) for a minimum of 6 h. It works on the principle that insects generally move away from heat. This detection technique is mostly followed in grain elevators in Canada. Rusty grain beetle larvae are found to be more than 90% in the terminals, as they stay intact inside grain and are not damaged during transit (White *et al.* 2011).

The method of cracking and flotation method which is an immunoassay technique taking 1 h and 1.5 h, respectively is used for detection of insect presence (Brader *et al.* 2002). Use of aggregation or sex pheromones for monitoring insects is another widely used technique. Stored-product insects can be detected with a variety of traps (Mohan 2012; Vick *et al.* 1990), some using food attractants or synthetic insect pheromones. A great number of insect species are attracted towards light of different wavelengths, thus use of visual lures is done for stored pest management. Visual lures to attract insects use fluorescent bulbs or ultraviolet wavelength emitting bulbs. As different species respond uniquely to specific portions of the visible and invisible spectrum, these visual lures did not gain much popularity in grain storage insect control (Neethirajan *et al.* 2007).

Mohan (2012) reported the use of low cost traps, use of UV-traps, TNAU automatic insect removal grain bin traps (Indian Patent 1773/CHE/2008) (Mohan 2008), pitfall traps, probe traps, indicator devices for low volume storage facilities in warehouses which will ensure safe removal of insects. The pitfall traps are redesigned using less expensive material and are used to detect presence of adults. Epsky and Shuman (2001) designed a technology developed by OPI Systems® in Calgary, Canada which can identify insect falling through the probe trap with infra-red beam; this identifies the insect based on the size of the insect

Carbon dioxide detection in storage structure can be monitored to detect the presence of insect infestation and it is also reported to be toxic to the insects causing mortality (Rameshbabu *et al.* 1991). Other techniques like electric conductance, acoustics, odours detection are also used in insect detection, but all these techniques are very sensitive and cost intensive. Adult insects are often easily trapped or detected by above mentioned techniques but the detection of immature insect stages is limited (Laopongsit and Srzednicki 2010). The above mentioned methods are time consuming, sometimes destructive and have to have huge sample collections to detect infestation.

Instrumental methods for detecting insect infestation

Instrumental methods for detecting the stored product pests using the computer vision techniques are simple, sensitive and accurate giving reproducible results. These methods have potential for use in the grain industry. The methods mostly researched are microcomputer controlled video image analysis (Zayas and Flinn 1998) which can detect free living insects but not internal infestation.

Hyerspectral imaging has a huge potential to detect insect infestation and can detect internal infestation (Singh *et al.* 2010; Singh *et al.* 2009). Thermal imaging is used for detection of termites, *C. ferrugineus* presence (Manickavasagan et al. 2008) and it is also detection of fungal infestations (Chelladurai *et al.* 2010) in wheat. Gowda and Alagusundaram (2013) and Vadivambal and Jayas (2010c) reviewed the use of thermal imaging for improving the quality of food grains during storage and concluded that sensing temperature changes can be an effective method to monitor grain quality and insect infestation.

Near infrared reflectance (NIR) spectroscopy using wavelength from 780-2500 nm is used to detect rice weevils presence and its life stages and to detect internal infestation in wheat (Paliwal *et al.* 2004). NIR spectroscopy can also be used to determine if the insect present is dead or alive (Elizabeth *et al.* 2002). The thermal imaging system is also useful for the detection *C. maculatus* in mung bean (Kaliramesh *et al.* 2013).

Soft X-ray imaging system for insect detection

Soft X-ray imaging has become a well-established method for the inspection of certain agricultural products and to identify defects and contaminants. Soft X-rays are electromagnetic radiations which have low energy, a wavelength in the range of 0.01 to 10 nm (10^{-10} to 10^{-9} m) (Fig. 2.2) and frequencies in the range 30 petahertz to 30 exahertz. The X-ray imaging can be used to clearly identify internal insect infestation in grains at all stages of development, as well as the extent of internal loss of grain kernels due to insect feeding (Karunakaran *et al.* 2004b). This technique can be used for identification of insect pest in a variety of agricultural products (grain, fruits, and vegetables) (Nawrocka *et al.* 2012). Nawrocka *et al.* (2012) used soft X-ray imaging to identify the mass losses of wheat kernels infested by the granary weevil. The mass loss was calculated using the soft X-ray images in gray scale taken from 20 to 66 d after infestation and assigning morphological features obtained from the X-ray images into six categories (sound, small larva, medium larva, large larva, pupae and emerged) based on the mass loss, to give an indication of the time and stage of infestation. Haff and Pearson (2007) developed an automatic recognition algorithm for detection of wheat kernels infested with larvae of granary weevil, *Sitophilus granarius* (L.) and olives infested with the olive fly, *Bactrocera oleae* (L.). The detection accuracy was reported to be 90%.

Alfatini *et al.* (2013) describes the potential to use a combination of X-rays and digital image processing technique to identify and describe the stage and degree of infestation. Karunakaran *et al.* (2002) studied the feasibility of soft X-ray method to detect the infestations caused by various cereal grain pests on wheat kernel. Karunakaran *et al.* (2003) reported 98% accuracy in detection of larvae of *Sitophilus oryzae* (L.). The automated line-scan X-ray system was developed, which proved to be more accurate and rapid than Berlese funnel in inspecting grain infestation. Karunakaran et al. (2004a) determined the potentiality of soft X-ray imaging system in identification of the infestation caused by *Rhyzopertha dominica* (F.) and *Cryptolestes ferrugineus* (Karunakaran *et al.* (2004b)). Detection of wheat kernels with red flour beetle were also reported (Karunakaran *et al.* 2004c). Parametric, non-parametric statistical classifiers and a back propagation neural network (BPNN) were used to differentiate between infested and uninfested wheat kernels. BPNN was reported to have higher accuracy of 97% for classifying infested and uninfested kernels.

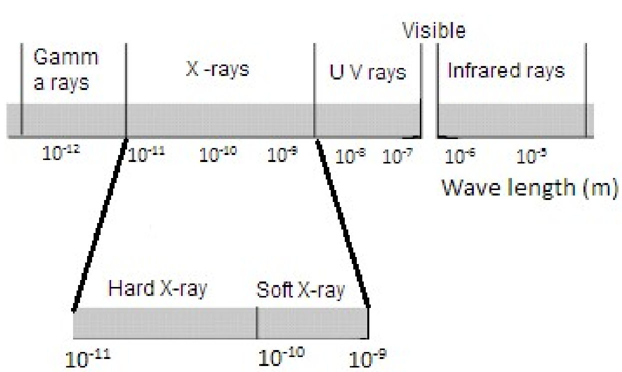

Figure 2.2: The electromagnetic spectrum

Summary

Other than detection of insect infestation, soft X-ray technique can also be very effective in identifying the mechanical damage in grains that are used for seed purposes (Neethirajan *et al.* 2007). Of all the available techniques, X-ray imaging is a non-destructive and rapid technique (Neethirajan *et al.* 2007).

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