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Detection of granary weevil *Sitophilus granarius* (L.) eggs and internal stages in wheat grain using soft X-ray and image analysis

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Abstract

In order to prevent grain mass and quality losses, rapid methods for early detection of insect infestation of cereal grain during trade and storage are urgently needed. Amongst many options, the soft X-ray method using roentgenograms is one of the most frequently applied. It has been shown that when some corrections for working parameters of the equipment used are made and some modification of the digital image analysis introduced, the soft X-ray method is suitable for accurate detection of granary weevil eggs laid in wheat kernels if at least 5 days after oviposition have elapsed.

Keywords: Wheat grain; Granary weevil; *Sitophilus granarius*; Eggs; X-ray detection; Digital image analysis; Algorithm

1. Introduction

Cereal grain losses during storage can reach 50% of the total harvest in some countries, a world-wide loss equivalent to thousands of millions of Euros per year (Dowell et al., 1999; Haff and Slaughter, 2004; Stermer, 1973). The major part of this loss is caused by insects and therefore the evaluation of simple, sensitive and reproducible instrumental methods for detection of living and dead insects in bulk grain as well as inside kernels is urgently needed in the grain industry.

The most popular methods for detection of insect-infested grain are based on visual detection of insect-damaged kernels, acid hydrolysis (insect fragment test), as well as milling and microscopical counting of insect fragments (Rosiński et al., 2004). The X-ray method, which is an official standard method in the USA, and the cracking and flotation method (Brader et al., 2002) are also widely exploited.

The changes in infested crushed kernels have been extensively investigated using electrical conductance in Perten SKCS equipment for grain hardness classification (Pearson et al., 2003), NIR spectroscopy (Ghaedian and Wehling, 1997; Maghirang et al., 2003; Ridgway and Chambers, 1996) as well as by the immunoassay method (sandwich ELISA) (Brader et al., 2002, Chen and Kitto, 1993; Kitto, 1991). The electrical conductance method is based on monitoring the conductance signals (135–140 points) for each single kernel during milling in a Single Kernel Characteristics System (SKCS) which is commonly used for wheat hardness determination (Pearson et al., 2003). This method is highly accurate for detecting older developmental stages of insects, the percentage of properly classified cases for small, medium, and large larvae and pupae being 24.5, 62.2, 87.5, and 88.6, respectively. The accuracy of this method depends also on insect species (rice weevil and lesser grain borer) and wheat type (soft or hard red winter wheat). Ghaedian and Wehling (1997) found

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NIR spectroscopy with a 5-factor principal component analysis (PCA) of the sound spectra to be a promising tool for accurate classification of infestation with late-instar granary weevil larvae at the spectral range of 1100–1900 nm; this range being the least sensitive to kernel moisture differences. More recently, Dowell et al. (1998, 1999), and Maghirang et al. (2003) developed an automated NIR system which afforded correct classification of infested kernels containing live insects at different stages of development (small, medium, and large larvae, and pupal stage) with an accuracy of 62%, 84%, 92%, and 94%, respectively. Finally, the immunoassay method, in which antibodies binding specific insect muscle protein myosin are used, seems to be the most promising method for the detection of infestation in grain consignments. This method is relatively quick, precise and sensitive for both grain and flour (Brader et al., 2002; Chen and Kitto, 1993; Kitto, 1991; Quinn et al., 1991).

The X-ray method is widely used as a test reference method. Thus, all modifications of the existing procedures making this method more accurate and easier to use are of great importance. The existing X-ray techniques enable the classification of at least four stages of insect development by measuring the area occupied by the insect, and an accurate classification is also possible based on visible insect morphology (Pearson et al., 2003). According to Brader et al. (2002), the time needed for a complete analysis is about 2.5 h, whereas the immunoassay method and the cracking and flotation method as a whole take less than 1 and 1.5 h, respectively. The possibility of significantly shortening the X-ray procedure has been recently suggested by Haff and Slaughter (2004) who proposed the use of real-time digital imaging instead of X-film for discrimination of infested kernels. Film observations gave, however, better accuracy (3% error rate) than the digital images (11.7%) for infestation by third-larval instars, while the error was less than 1% for both methods with a more advanced stage of larval development.

The separation of externally living insects for industry does not create a problem, e.g. simple sieving serves this purpose well. When wheat grain was infested by granary weevil the most important problem to solve was how to detect the hidden stages of larval development, particularly just after oviposition, inside the kernel.

The aim of the work presented here was to develop a procedure for use of soft X-rays to enable early and rapid identification of grain infested by granary weevil, *Sitophilus granarius* (L.), using an automated digital system of analysis and development of a special algorithm for simple data acquisition.

2. Materials and methods

2.1. Wheat samples

Grain of soft winter wheat varieties Begra and Korweta (*Triticum aestivum* L.) obtained from the Breeding Station Danko Choryń and durum spring wheat LGR 896/64a (*Triticum durum* Desf.) from the collection of the Institute of Genetic and Plant Breeding of the University of Agriculture in Lublin, Poland, were used in this study. Characteristics of the wheat grain used for granary weevil experiments are summarised in Table 1.

2.2. Sample preparation

Fifty wheat kernels were glued "crease up" to clear paper (0.10 mm thick × 7 cm × 6.5 cm) in an array of 6 rows and 8 columns +2 individual kernels. The contact paper was placed in a Petri dish of 12-cm diameter with a circular opening (2.5 cm with plastic screen of 120 mesh) in the cover.

Five-day-old adults of *S. granarius* (5 females and 5 males) bred in the Institute of Plant Protection, Department of Entomology, were placed in each Petri dish and incubated at 28 °C and 65% relative humidity (r.h.) for 3, 5, 7, 10, 20 or 30 days. After the allotted period, the insects were removed and an optical microscope Olympus Highlight 3100 (Olympus, Japan) was used for preparation of maps of egg laying in single kernels (plugs secreted over the egg were counted and marked on a graph—"expert score"). For each egg-laying period of the experiment and each wheat cultivar, five replicates of 50 kernels were

Table 1
Characteristics of wheat grain samples used

	Begra	Korweta	LGR
Moisture (%)	12.21±0.04	11.98±0.00	10.75±0.03
Bulk grain density (kg/m^3)	780.4±2.2	793.0±1.6	718.3±0.7
Thousand grain weight (TGW) (g)	51.84±0.57	44.31±0.24	34.44±0.18
Vitreosity (%)	73±4	81±1	93±3
Protein content $N_{og}\times 5.70$ (% d.m.)	13.5±0.04	12.60±0.05	15.60±0.10
Starch (% d.m.)	62.46±1.57	61.80±0.78	57.04±3.16
Reducing sugars (% d.m.)	0.60±0.01	0.51±0.01	1.21±0.02
Fibre (%)	2.30±0.09	2.35±0.08	2.90±0.06
Ash (%)	1.69±0.02	1.72±0.06	2.02±0.06