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Losses caused by granary weevil larva in wheat grain using digital analysis of X-ray images

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A b s t r a c t. The object of the study was spring wheat grain of the Torca variety infested with granary weevil (*Sitophilus granarius* L.). Weevil-infested kernels were X-rayed for detection of hidden endosperm damage which is connected with the reproductive cycle of the studied insect pest. Digital analysis of infested grain roentgenograms was used to determine the mass loss in wheat kernels. Infestation rate was tested in five periods: zero (control), 5, 10, 15 and 20 days from the moment of infestation. A parabolic dependence of the mass loss on infestation time was found. To determine the mass losses connected with insect growth, a new algorithm was developed, consisting of two steps. In the first step, the volume of a single loss was calculated using the grey scale. Grey values were taken from a calibration marker which was made of plexiglass. In the second step, the mass loss was obtained from the volume and density of endosperm.

K e y w o r d s: wheat, granary weevil, hidden/internal infestation, X-ray

INTRODUCTION

Globalization of the world trade makes movement of agricultural products easy across international borders. One of the disadvantages of this process is pest infestation which is adverse to the sanitary regime. Many countries introduce control measures of agricultural products to reduce the propagation of pests. Pest insects, which feed mainly on crops, cause 10 to 30% damage in both traded and elevator-stored grain every year (Karunakaran *et al*., 2004). Two types of pest infestation are distinguished: external and internal (hidden). The external infestation is connected with feeding of adult insects which are easy to remove from grain by using suitable sieves. Hidden infestation is linked with the reproductive cycle of pest insects which takes place inside the kernel. Larval stages not only eat kernels from the inside, but also leave there their metabolic products and moults. All these products must be removed from the grain as they deteriorate the grain quality. Some of these substances are regarded as allergens. For example, there is a 'storekeeper disease' which is triggered off by specific proteins produced by granary weevils (Herling *et al*., 1995). The internal infestation is very difficult to detect because of the lack of infestation signs on the kernel surface. The greatest damage to grain is linked with larva feeding. Hence, it is very important to develop a fast method of detection of internally-infested grain.

Many methods have been developed so far for the detection of pest-infested kernels, but none of them has been used in the monitoring of both storage and grain transport on a wide scale. It is connected with the high price of such apparatus and with the lack of technically well-trained staff. Furthermore, some of the methods are time-consuming, destroy kernels during the test, or do not detect hidden infestation. Among the methods used in research laboratories we may find: egg plug staining technique (Pearson and Brabec, 2007; Toews *et al*., 2006), flotation and cracking (Brader *et al*., 2002; Haff and Slaughter, 2004), acoustic method (Hagstrum *et al*., 1990; Neethirajan *et al*., 2007; Vick *et al*., 1988), immunoassay method ELISA (Enzyme-Linked Immunosorbent Assay) (Piasecka-Kwiatkowska *et al*., 2005), near-infrared hyperspectral imaging (Mahesh *et al*., 2008; Singh *et al*., 2009), soft X-ray roentgenography (Brader *et al*., 2002; Fornal *et al*., 2007; Grundas *et al*., 1999; Karunakaran *et al*., 2003, 2004; Schatzki and Fine, 1988).

X-ray examination has been the official method of the Association of Analytical Chemists (AOAC) for the inspection of internal insect infestation in grain or seed since 1961 (AOAC, 1980). X-ray imaging is a simple and fast method to detect both internal and external infestation. For example, testing of 1 kg grain sample lasts about 15 min

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(Neethirajan *et al*., 2007), whereas the Berlese funnel method needs about 5-6 h (Neethirajan *et al*., 2007). This technique does not destroy kernels and it also enables to observe larva motion inside the kernel. To monitor this motion, daily roentgenograms are made, or one by one X-ray images on which changes in the position of juvenile insects can be analysed (Karunakaran *et al*., 2002). Furthermore, the larval activity can be increased by warming cool grain, which makes the identification of live insect stages inside grain kernels more effective (Mankin *et al*., 1999).

The X-ray technique can be used for detection of any kind of insect pest in a variety of agricultural products (grain, fruits, vegetables). Consequently, improving X-ray technology for the purpose of grain monitoring has been an area of active research for many years. Two areas can be distinguished within this field of research. The first looks for improving X-ray equipment to generate images of higher resolution and lower noise, using CCD arrays and chips as X-ray detectors. The second area is the development of algorithms for automatic identification of features linked with infestation (Haff and Pearson, 2007).

The aim of the studies presented herein was to develop an algorithm for determining the mass loss connected with granary weevil (*Sitophilus granarius* L.) larva feeding in wheat (*Triticum aestivum* L.). This algorithm can be used to separate infested kernels from sound ones by using digital analysis of X-ray images. Furthermore, the developed procedure can help to reduce losses in grain caused by storage pests since food producers would not have to remove whole batches of grain but only the infested parts. The first step of the developed algorithm was calculation of the damage volume by using a plexiglas marker. The marker provided information about the grey scale, in other words the thickness of the X-rayed object. In the second step, the volume was converted into mass using average value of endosperm density. The level of the mass loss was determined for five infestation periods – zero (control – sound kernel), 5, 10, 15 and 20 days from the moment of infestation.

MATERIALS AND METHODS

The study was carried out on the Torca variety of spring wheat (*Triticum aestivum* L.). It belongs to an exclusive category (*E*), and thus it is often used to improve the quality of a lower class flour. Its kernels are red, vitreous and highly homogeneous. The grain is characterized by good milling value and contains an average amount of protein. The mass of one thousand kernels is 39.2 g (Descriptive List of Varieties, 2007). The Torca grain was obtained from the Strzelce Plant Breeding Station in Konczewice, Poland.

The introduction of adult insects in wheat samples took place at the Institute of Plant Protection in Poznań, Poland. Before the experiment, the grain of Torca variety was sifted to obtain homogeneous samples. The mean kernel diameter was larger than 2.4 mm. The studied grain was conditioned for seven days in an incubator. The air moisture and temperature in the incubator were kept on a stable level and equalled 75±5% and 26.5±0.5ºC, respectively. After grain conditioning, twenty pairs of adult granary weevils were introduced in a 400-kernel sample of the studied variety. The pest insects were not older than 24 h from their exit from kernels. The adult granary weevils fed on the grain for 5, 10, 15 and 20 days. Kernels from each infestation period were X-rayed and compared to the control sample (uninfested kernels).

The microfocus X-ray apparatus 'Electronica-25', made in Russia, was used for the acquisition of X-ray images. X-ray films Kodak XS1, size 13×18 cm, were the radiosensitive material. Magnification rate used in the studies was three-fold. The voltage of X-ray tube was fixed at 20 kV at the current of 60 μA and exposure time was 2 min.

Randomly selected 30 kernels were fixed on a thick paper with an adhesive. They were placed in transverse rows with the grooves and germs oriented downwards. For each sample, differing as to time of infestation, 90 kernels were X-rayed. Next, the X-ray films were scanned in a 8-bit scale by using an Epson Perfection V700 Photo scanner. The scanner resolution was set at 200 dpi.

Digital images of wheat kernels were characterized by resolution of 46 μm/pixel. The digital processing was made using the ImageJ program (available free on the website: http://rsweb.nih.gov/ij/), which was installed on a PC computer. The computer had an Intel Pentium III EB Coppermine 2 773 MHz processor, 512 MB SDRAM and an IBM-DTLA 305010 hard disc of 10 GB capacity.

Analysing roentgenograms of the wheat kernels infested by granary weevil, there were clearly visible areas which differed from the rest of the kernel in the hue of grey. Those areas were assigned to the internal and external infestation. Using the grey scale, the volume of both sound kernels and losses was obtained which was next converted to mass.

Grey values were obtained from a calibration marker which was made of plexiglass. The marker was placed on each X-ray film. It had five steps of thickness: 1.5, 2.5, 3.15, 3.9 and 5 mm. A scheme and an X-ray image of the calibration marker are depicted in Fig. 1.

The calibration marker represented the grey scale which was used to calculate the loss volume. Depth of pixels was received by assigning grey values to the steps of the calibration marker (0-255, zero means black and 255 means white).

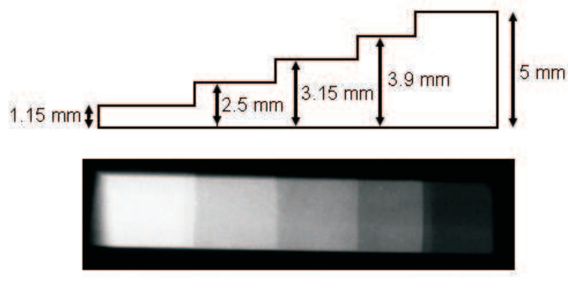

Fig. 1. Scheme and X-ray image of the calibration marker.

Grey values corresponding to marker steps were obtained by drawing a histogram of selected step in ImageJ. For the loss, the black (0) was assigned to the biggest step of the marker (5 mm), while the white (255) was linked with the least step (1.5 mm). The steps were assigned inversely for the control sample. Examples of X-ray images of sound and infested kernels are shown in Fig. 2. Grey value ranges connected with the calibration marker steps of a definite thickness are presented in Table 1 for both sound and infested kernels. The grey values listed in Table 1 were averaged through all calibration marker images. As seen from Fig. 2, if the loss image is darker the mass loss is greater. This results from the fact that a bigger number of X-rays reached the film since there was not an absorbent of definite thickness. The amount of absorbed/transmitted radiation is described by the Labert-Beer law.

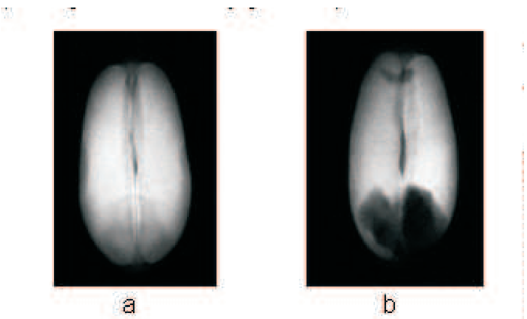

Fig. 2. Comparison of X-ray images of: a – sound, b – infested kernels.

Table 1. Grey value ranges and thickness of the calibration marker steps used for the Torca wheat variety

Thickness of calibration marker (mm)	Grey value ranges	
	Sound kernel	Infested kernel
1.15	0-20	205-255
2.50	21-80	146-204
3.15	81-146	87-145
3.90	147-214	31-86
5.00	215-255	0-30

The first step of volume calculation was determination of 'single pixel area' (mm^2). If the image resolution was 0.046 mm/pixel, the 'single pixel area' comes to 2.13 10^{-6} mm^2. The ImageJ program gives the size of a marked area in square pixels. If there the 'single pixel area' and the number of pixels comprised in the marked area are known, the area size is obtained in square millimetres. Next, using a histogram (distribution of grey values) of the marked area, the volume corresponding to this area was calculated. The obtained volumes were converted to mass (mg) using mean value of endosperm density given by Bochyński *et al.* (1991). The endosperm density is d_e = 1339 kg m^{-3}. Furthermore, the loss mass had to be divided by $\sqrt{3}$ to receive the real value of mass loss. This operation was made owing to the threefold magnification. All mass values presented in this article were calculated using the above algorithm.

RESULTS

The granary weevil larvae feed generally on grain endosperm since it is regarded as a depository of nutritious components. Figure 3 shows X-ray images of the larval stages of granary weevil which fed on the wheat grain for the five infestation periods. The ranges of the mass loss values corresponding to these stages are presented in Table 2.

The shape and size of the loss, which are connected with larval development of granary weevil, depend on infestation time as well as on laid eggs location *eg* back or side of the kernel. A 5-day infestation period gives defects in the form of an elongated tunnel or a round hole where the larva is clearly visible (Fig. 3b). The mass loss value changes from 0.1 to 1 mg during this period. The average mass loss is equal to 0.46 mg (standard deviation, SD = 0.2 mg). Damage of tunnel shape is also typical for the next infestation period. The mass loss is in the range of 1.01 to 1.7 mg. Mean value of the loss is 1.22 mg with SD amounting to 0.17 mg. Figure 3d depicts the larva after 15 days of its development. Mass loss in the shape of an ellipsoidal hole, connected with this period, varies between 1.71 and 2.8 mg. The average mass loss is equal to 2.1 mg (SD = 0.3 mg). After 20 days from the moment of infestation the granary weevil larva is clearly visible on the X-ray image, as seen in Fig. 3e. This damage is ellipsoidal in shape or consists of two parts: a round or ellipsoidal hole, in which the larva develops, and a tunnel. The

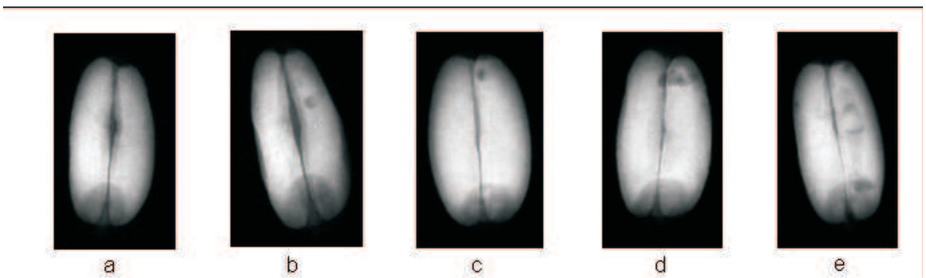

Fig. 3. X-ray images of mass losses connected with the reproductive cycle of granary weevil for: a – zero (control), b – 5, c – 10, d – 15, and e – 20 days from the moment of infestation. The pests fed on the Torca variety of wheat.

T a b l e 2. Mass losses connected with granary weevil larva feeding determined for the Torca variety of wheat

Infestation time (days)	Average mass loss (mg)	Ranges of mass loss (mg)		SD
		Min	Max	
5	0.46	0.1	1	0.2
10	1.22	1.01	1.7	0.17
15	2.10	1.71	2.8	0.3
20	3.86	above 2.8		0.35

tunnel was drilled by larva which looked for an appropriate place in the endosperm for further development. Mass loss is assigned to this infestation period if it is above 2.81 mg. The mean value of it is 3.86 mg with SD of 0.35 mg.

Kernels from the control group (Fig. 3a) constitute a mass model for the X-ray images of infested ones. The average mass of one sound kernel was calculated as 38.7 mg (SD = 5.4 mg). This value is similar to a single kernel mass obtained from the 1000-kernel mass which amounts to 39.2 mg (Descriptive List of Varieties, 2007).

During the study, we also wanted to determine the character of the mass loss as related to the infestation time. It corresponds to the feeding rate of granary weevil larva. The diagram presented in Fig. 4 indicates that the feeding rate probably has a parabolic character. Measuring points are fitted by a curve described by the equation $y=0.1x^2-0.03x+0.39$. Obviously, it is necessary to carry out further studies to determine the character of this rate precisely. In such future studies, roentgenograms will have to be taken more often. We will also have to take into account other wheat varieties and larger grain samples.

After analysing X-ray images of the studied kernels, it can be said that the losses occurred mainly in the kernel germ in a 5-day infested sample. They are connected with the feeding of adult granary weevils (Gołębiowska and Nawrot, 1978). In the endosperm of some kernels small defects can be already seen that probably correspond to oviposition or early stages of larval development. Furthermore, there is usually one loss in one kernel. Kernels from the 10-day infestation period are characterized by two kinds of losses, located both in the germ and in the endosperm. Defects caused by adult insects are still observed in the 15- and 20-day samples, but the number of larval losses is still increasing. The size of those second defects is also larger. Very surprising is the fact that multiple infestation was found on the X-ray images of 15- and 20-day samples since it is not observed in nature. Moreover, more than 90% of the studied sample is damaged and it could be considered as a source of toxins and impurities. It was found that there are no larval losses if more than 20% of the kernel is eaten by an adult insect.

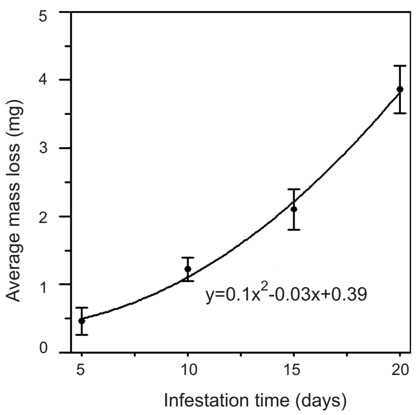

Fig. 4. Parabolic dependence of the mass loss linked with the granary weevil reproductive cycle and the infestation time. The insects fed on the Torca variety of wheat.

DISCUSSION

Values of the loss volume calculated by using the procedure developed by the authors seem to be false because of the different densities of endosperm (d_e = 1.34 g cm^{-3}) (Bochyński *et al*., 1991)) and plexiglass (d_p = 1.19 g cm^{-3}). This can be a problem if real loss of mass caused by internal infestation must be obtained. During this experiment, a calibration marker was made for kernels of different thickness. However, it was impossible to get a reliable dependence of grey scale and marker thickness because of the heterogeneity of kernel density. The advantage of the plexiglas calibration marker is that each marker step is characterized by the same density value.

On the other hand, this procedure gives more information about the infestation rate in comparison with the method which uses loss area only. For example, there were two defects that differed in the grey value ranges but had the same area. Comparison of X-ray images of those defects and of their histograms is shown in Fig. 5. Although the areas of both losses are equal to 83 square pixels (177 10^{-6} mm^2), their volumes amount to 0.58 mm^3 (0.45 mg) (Fig. 5a) and 0.42 mm^3 (0.32 mg) (Fig. 5b), respectively. These values are obtained by using the grey value ranges listed in Table 1. Both loss histograms differ in terms of the grey scale. The first range of grey values is from 85 to 177, while the second is from 159 to 241 (Fig. 5). These ranges indicate that the defect in Fig. 5a is darker than the other one. In other words, it is a bigger loss.

In the above mentioned examples, the areas of losses are the same, but one of the mass losses is larger by about 30%. Thus it can be said that volume converted to mass is an adequate feature to determine the infestation time or to localize the place of infestation on a wide scale using roentgenography. Furthermore, a single kernel mass calculated using this procedure is similar to the mass obtained from the 1000-kernel mass. The first one is equal to 38.7 mg while the second one amounts to 39.2 mg (Descriptive List of Varieties, 2007).

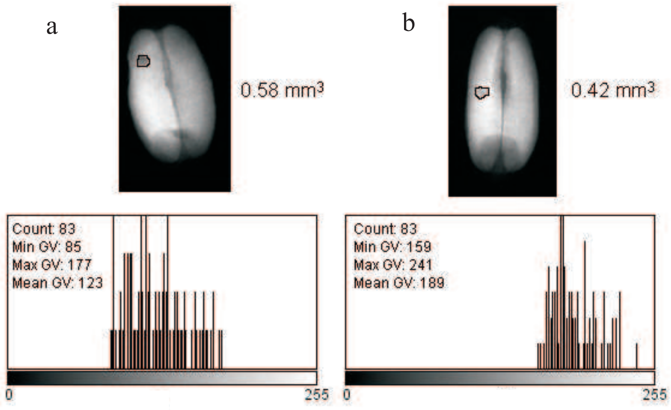

Fig. 5. Comparison of X-ray images and histograms of two losses which differ in grey values ranges but have the same area: a – 0.58 mm^3 (0.45 mg), b – 0.42 mm^3 (0.32 mg).

There are determined ranges of mass defects connected with the larval development of granary weevil for 5, 10, 15 and 20 days from the moment of infestation. However, Huff and Slaughter (2004) claim that it is impossible to determine a reproductive stage precisely, since statistical distribution of the stages depends on a number of factors *eg* insect population age, and food and space accessibility. In our case, it is necessary to carry out more detailed studies and impose a few conditions. First of all, adult granary weevils have to be separated from the grain after 5 days of infestation to avoid multiple infestation. Secondly, the X-ray images must be taken more often than every 5 days to define the precise size of larval defect and feeding rate. It could be possible to obtain an exact place of infestation if there were a database of mass loss values for different cereal species. Furthermore, there is a risk that frequent X-raying of infested kernels can affect the reproductive cycle of granary weevil by accelerating it. But according to the literature, soft X-rays have no effect on the larval development (Schatzky and Fine, 1988).

Multiple infestation is seen in the 15- and 20-day infested samples. It is not observed in natural conditions, because usually one kernel is settled by one larva. This fact was proved by Schatzky and Fine (1988) who found only two kernels double-infested by rice weevil (*Sitophilus oryzae*) in a 15000-kernel sample. They studied also wheat grain settled by the lesser grain borer (*Rhyzopertha dominica*). There were observed even triple-infested kernels, but this insect is smaller in comparison with weevil species (Schatzky and Fine, 1988). The multiple infestation in these studies is probably connected with the low number of kernels in the sample as well as with a high number of granary weevils. There was a 400-kernel sample settled by 20 pairs of adult insects, whereas a 500 g sample, containing about 15 000 kernels infested by about 300 insects, was used in other studies (Pearson and Brabec, 2007). For this reason, in our research, the adult weevils had to lay eggs in different places of the same kernel.

CONCLUSIONS

1. Determination of precise size of mass loss which is connected with the reproductive cycle of insect pests is possible.

2. The average values of mass loss for 5, 10, 15 and 20 days of infestation amount to 0.46, 1.22, 2.1 and 3.86 mg, respectively. The mass ranges obtained enable to determine precise time and place of infestation.

3. The suggested method can be used to develop an algorithm which will help to separate infested grain from the non-infested one in future studies. The creation of a model of granary weevil reproductive cycle in wheat can have a legislative significance, since the determination of exact time of infestation is very important for phytosanitary control.

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