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# The effect of some biochemical and technological properties of wheat grain on granary weevil (*Sitophilus granarius* L.) (Coleoptera: Curculionidae) development

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## Abstract

The granary weevil (*Sitophilus granarius* L.) is the most harmful pest of stored grain and other stored products in temperate climates. Because the granary weevil is a primary insect pest that can feed on intact kernels, it causes the most damage to cereal grain in storage or shipment; however, it also can develop on acorns, sunflower seeds, and noodles, although rarely. Chemical, technological, and nutritional properties of food can affect insect development rates. Therefore, the objectives of these studies were to examine granary weevil development and population dynamics in chemically and technologically defined grain of three wheat varieties. Selected biochemical and technological properties of two winter wheat varieties, Begra and Korweta (*Triticum aestivum*), and a durum wheat, LGR896/64a (*Triticum durum*), from two crop years were studied. The following grain properties were examined: moisture, total protein, reducing sugar, starch, fibre, ash content, 1,000 kernel mass, vitreosity, weight by volume, kernel hardness and diameter, as well as grain amylolitic activity and inhibitory action against *S. granarius* $\alpha$-amylase. A precise knowledge of differences in these grain parameters as they relate to insect development rate can be useful in determining biological factors responsible for insect resistance. Recently, physical and biological methods are becoming more widely recognized as alternatives or supplements to hazardous chemical pesticides used for the control of stored-food pests. All studied wheat cultivars showed statistically significant differences among determined parameters. LGR896/64a showed the lowest inhibitory activity against granary weevil $\alpha$-amylase and the highest amylolitic activity, reducing sugar, and total protein contents, as compared with Begra and Korweta varieties; however, these parameters did not influence insect development rate. Kernel diameter and hardness, vitreosity, 1,000 kernel mass, weight by volume, and thickness and hardness of the seed coat had the greatest influence on resistance of LGR896/64a wheat grain against *S. granarius*.

*Key words: Sitophilus granarius* L., wheat varieties, biochemical, physical and technological grain properties.

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## Introduction

The granary weevil (*Sitophilus granarius* L.) is the most harmful pest of stored grain and other stored products in temperate climates. It can develop on wheat, rye, barley, oats, corn, and rice, and it is rarely found in on acorns, sunflower seeds and noodles (Andersen 1934). Adults cause damage by destroying kernels, mainly germs, producing debris, and raising grain temperature and water contents, facilitating the invasion of secondary insect pests, mites, bacteria, and fungi. Larvae develop inside the kernel and consume about 64 % its content (Campbell and Sinha,1976). Under natural, fluctuating conditions of temperature and relative humidity of warehouses, the pest can have three to five generations per year. These abiotic parameters have the greatest influence on granary weevil rate of development and population increase (Schwartz and Burkholder, 1991). Chemical, biochemical, nutritional, physical, and technological properties of cereal grain have fundamental influence on development rate of pests. The amount of protein, enzymes, inhibitors, starch, lipids, and fibre, as well as some physical and technological factors including water content, hardness, vitreosity, weight by volume, and 1,000 kernels mass, are characteristic features of each variety.

Knowledge of all of the above mentioned parameters of grain can help to understand the influence of differences in given food value in relation to the development rate of feeding insects. Ecological factors increasing/delaying pest development or being growth regulators may be used as an element of integrated pest management (IPM). Chemical control of stored-product pests is very hazardous since protected products are used for human food or domestic animal feed. Increased concern by consumers over insecticide residues in processed cereal products, the occurrence of insecticide-resistant insect strains, and the precautions necessary to work with traditional chemical insecticides call for new approaches to control stored-product insect pests (Fields, 2006). It seems that biological methods integrated with physical methods should be a safe alternative for protection of stored grain in warehouses (Warchalewski et al., 2000).

The insect resistance mechanisms of cereal grains are complex and depend on physico-chemical and biochemical properties of the grain and on the subsequent biochemical and physical adaptation of postharvest insects to these properties. Stored seeds may have high resistance to insect pests because of the lack of vital nutrients or the presence of compounds that adversely affect insect development (Warchalewski et al., 2002). Enzyme inhibitors from plants are promising candidates for new biocontrol agents (Oppert at al., 2004). The enzyme inhibitors impede digestion through their action on insect gut digestive $\alpha$-amylases and proteases, which play a key role in the digestion of plant starch and proteins (Franco et al., 2002). However the highly active $\alpha$-amylase inhibitors in insects appear to have limited influence on their development parameters (Warchalewski at al., 2002). On the other hand, recently obtained positive results of biochemical screenings of inhibitors in a search for increasing resistance against insects' $\alpha$-amylases by application of direct mutagenesis in existing plant inhibitors will offer side benefits of transgenic technology (Franco et al., 2002).

Literature on this subject is rather rich and deals with all cereals. Suitability of different Polish wheat varieties and hybrids for granary weevil development were tested earlier (Nawrot, 1981). Many of authors stated some real differences in egg-laying, rate of development, and adult survival of primary pests on triticale (Dobie and Kilminster, 1978), wheat (Toews et al., 2000), and maize (Dobie,1974). Dobie (1974) proposed a special formula for quantifying susceptibility of grain and pulses varieties.

The objectives of these studies were to examine granary weevil development and population dynamics in biochemically, physically, and technologically defined grain of three wheat varieties.

## Materials and methods

Wheat grain of three Polish varieties - Begra, Korweta (*Triticum aestivum*), and LGR 896/64a (*Triticum durum*) - grown in two consecutive years on two different experimental fields - Begra and Korweta in Choryñ and LGR 896/64a in Lublin - were used in this study.

### Analytical methods

- Moisture of wheat grain was determined using moisture-air oven methods drying at 135 °C, according to Standard Method AACC-Method 44-19/1982.
- Wheat grain total protein content (N x 5.7) was determined according to modified Kjeldahl method-ICC Standard No. 105/2:194 with the use of the Foss Tecator Apparatus from Sweden.
- Vitreosity of grain was determined according to the Polish Standard Method PN-70/R-74008.
- 1,000 kernel mass, expressed in grams was determined according to the Polish Standard Methods PN-68/R-74017.
- Weight by volume was carried out according to Polish Standard Method PN-73/R-74007.
- Starch content was determined by polarymetric method, according to Standard Method AACC-Method 1975.
- Ash was estimated by burning a sample at 900 $^{o}$C and measuring the residue.
- Fiber content was determined according Scharrera-Kürschner method as published by Gawêcki and Jeszka (1986).
- Physical measurements were determined with the use of the Single Kernel Characterization System - SKCS 4100 (Perten Instruments North America 1995). The SKCS measures kernel weight (WT), moisture content (MC), diameter (DM), and hardness index (HI) at a rate of two kernels per second and reports the average and standard deviation of 300-kernels taken randomly from a 25-gram sample. To measure kernel moisture content and hardness index, electrical conductance and compression force are monitored and stored by the SKCS while a kernel is being crushed between a wheel and crescent (Martin et al. 1993).
- To obtain the biologically active proteins from cereal grains, three steps of water extraction in three independent runs was used (Warchalewski et al., 1997). Before extraction, cereal grains were ground into whole flour using a laboratory mill.

Determinations made on all extracts were:

- Protein contents according to the Lowry et al. methods (1951) at $\lambda$ = 750 nm.
- Reducing sugar contents according to methods with DNS at $\lambda$ = 530 nm (Hostettler and Denel, 1951).
- Amylolitic activity according to Bernfeld method (1955) modified by Warchalewski and Tkachuk (1978).
- Inhibitory activities against *Sitophilus granarius* L. - imago $\alpha$-amylases, according to modified Bernfeld method by Warchalewski (1978).

### Biological tests

Five-day-old adults of the granary weevil were used in experiments. They were collected from laboratory cultures maintained at 26 °C and 65 % r.h., and sexed by examining the rostrum and abdominal shape (Halstead, 1963). Fifty kernels were placed in plastic vials 55 mm long by 25 mm in diameter, closed with a cotton cloth, and infested with 10 beetles (5 males and 5 females). After 3, 5, 7, 20 and 30 days of feeding, the beetles were removed and the amount of dust produced was weighed. Kernels were returned to vials. Starting 36 days and continuing until 64 days after the initiation of the experiment, vials were checked daily for emerged adult progeny to determine numbers of progeny produced and development time. There were five replicates for each treatment. Biological experiments were conducted at 26 °C and 70 % r.h. in darkness.

### Statistical analysis

Tukey's, Fisher's, and Student's tests were used to evaluate significant differences in the quantitative results.

## Results and discussion

In Table 1 are shown selected biochemical, physical, and technological properties of studied wheat grain which can have influence on *Sitophilus granarius* L. development parameters. In the first year crop, all determined properties were significantly different among the studied wheat with the exception of starch content, while in the second year crop, starch, fiber, ash, 1,000-kernel mass, and weight by volume were significantly different.

LGR 896/64a, which was grown in different a location in relation to the *Triticum aestivum* varieties in the two consecutive years of harvest, showed significantly higher values of protein and reducing sugar contents as well as twice the amylolitic activity. The enzymology of the insect digestive tract in relation to the initial stages of large food polymers (starch and protein) reflects the biochemical adaptation of these postharvest insects to their preferred foods. According to Baker (1986), $\alpha$-amylase is the main digestive enzyme of the granary weevil; therefore significantly high amylolitic activity of wheat could be considered as a resistance factor in response to the insect digestive enzyme. On the other hand, inhibitory activity against *Sitophilus granarius* $\alpha$-amylase shown in Table 1 was seven times higher in Begra and Korweta wheat varieties than in durum wheat. Although, the highly active insect $\alpha$-amylase inhibitors appear to have limited influence on the developmental parameters of *S. granarius*, some reduction of insect population might be expected (Warchalewski et al., 2002).

**Table 1.** Selected biochemical, physical and technological properties of wheat grain harvested in the two consecutive years.

| Wheat variety | | BEGRA | | KORWETA | | LGR 896/64a | |
|---|---|---|---|---|---|---|---|
| Year crop | | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| Protein content | [% d.wt.] | 13.5 a | 14.41 d | 12.60 b | 14.36 d | 15.60 c | 14.66 e |
| Reducing sugar | [% d.wt.] | 0.60 a | 0.38 d | 0.51 b | 0.38 d | 1.21 c | 0.56 e |
| Starch | [% d.wt.] | 62.46 a | 67.39 d | 61.80 a | 61.76 e | 57.04 a | 59.68 f |
| Fiber | [%] | 2.30 a | 2.35 d | 2.35 b | 2.25 e | 2.90 c | 2.48 f |
| Ash | [%] | 1.69 a | 1.71 d | 1.72 b | 1.51 e | 2.02 c | 1.68 f |
| 1,000 kernel mass | [g] | 51.84 a | 47.58 d | 44.31 b | 58.04 e | 34.44 c | 39.44 f |
| Weight by volume | [kg/hl] | 78.04 a | 81.90 d | 79.30 b | 81.10 e | 71.83 c | 76.70 f |
| Vitreosity | [%] | 73.0 a | 94.0 d | 81.0 b | 94.0 d | 93.0 c | 95.0 d |
| MC | [%] | 11.78 | 12.83 | 12.24 | 12.73 | 10.96 | 11.58 |
| HI | [-] | 46.71 | 64.23 | 67.86 | 71.14 | 81.34 | 84.98 |
| WT | [mg] | 52.86 | 47.48 | 45.72 | 57.20 | 37.53 | 39.71 |
| DM | [mm] | 3.30 | 3.02 | 3.05 | 3.44 | 2.74 | 2.78 |
| Amylolitic activity | [UAA/100 g d.wt.] | 13082 | 17025 | 13620 | 12043 | 26559 | 23978 |
| | [UAA/ mg protein] | 5.7 | 7.0 | 5.7 | 5.6 | 9.2 | 8.4 |
| Inhibitory activity against | [UAA /100 g d.wt.] | 217563 | 234767 | 201971 | 210932 | 28495 | 47168 |
| *S. granarius* $\alpha$-amylase | [UAA/mg protein] | 95.6 | 97.1 | 84.8 | 98.8 | 9.9 | 16.5 |

Value followed by different letter within a harvest year are significantly different ($p < 0,05$).

In the second year of harvest, due to different agro-environmental conditions, Begra and Korweta varieties showed higher values of protein content than in the first year. Protein content tends to be higher in vitreous than in mealy wheat and vitreosity is often associated with hardness. As can be seen in Table 1, in the first year, varieties Begra and Korweta showed lower vitreosity compared to durum wheat. In addition the highest values of vitreosity in all studied wheat grain samples were correlated with the highest values of hardness index determined by SKCS. It is well known that hardness or softness of wheat grain is genetically controlled, therefore growing conditions have limited influence on grain hardness.

Insect development parameters are shown in Tables 2 and 3. The amounts of dust producing by parental beetles is indicative of feeding intensity. Amounts of dust in Begra and Korweta varieties were higher than in LGR 896/64a wheat grain, presumably due to the higher grain hardness of durum wheat (Table 1). Similar significant differences were found in progeny numbers. Very low, but significant, differences also were observed in development periods. The reason for these patterns might be very low levels of $\alpha$ -amylase inhibitors in wheat grain of LGR 896/64a. Another interesting phenomenon observed was prolongation of the development period of progeny due to age of parental adults. Progeny of older parental adults took longer to develop.

**Table 2.** Developmental parameters of the granary weevil (*Sitophilus granarius* L) in studied wheat grain varieties.

| Wheat variety | Time of feeding (days) | Dust production (mg) | | Progeny number | | Development time (days) | |
|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| LGR 896/64a | 3 | 3.40 | 3.47 | 18.66 | 16.00 | 40.05 | 43.10 |
| | 5 | 4.86 | 5.60 | 27.33 | 31.83 | 40.89 | 41.65 |
| | 7 | 6.80 | 7.00 | 26.16 | 34.00 | 42.65 | 42,59 |
| | 10 | 10.06 | 9.30 | 32.50 | 39.33 | 44.60 | 42.96 |
| | 20 | 20.80 | 20.65 | 35.30 | 48.00 | 48.69 | 46.59 |
| | 30 | 39.6 | 35.48 | 39.00 | 50.83 | 51.90 | 48.05 |
| Korweta | 3 | 2.98 | 4.03 | 22.83 | 21.33 | 39.73 | 42.75 |
| | 5 | 5.00 | 6.05 | 34.00 | 31.66 | 40.11 | 42.33 |
| | 7 | 6.68 | 8.35 | 38.33 | 44.50 | 41.86 | 42.49 |
| | 10 | 9.74 | 12.32 | 42.33 | 46.17 | 43.07 | 43.42 |
| | 20 | 20.72 | 26.08 | 51.50 | 52.16 | 46.82 | 48.05 |
| | 30 | 56.70 | 57.00 | 52.16 | 62.00 | 51.44 | 52.71 |
| Begra | 3 | 3.24 | 4.35 | 23.00 | 25.66 | 39.10 | 40.80 |
| | 5 | 4.96 | 7.88 | 29.60 | 35.83 | 40.36 | 41.20 |
| | 7 | 7.96 | 8.17 | 36.50 | 37.83 | 41.60 | 41.60 |
| | 10 | 10.34 | 11.55 | 37.80 | 44.50 | 43.97 | 41.95 |
| | 20 | 21.64 | 24.18 | 50.50 | 49.67 | 47.30 | 46.28 |
| | 30 | 54.62 | 57.25 | 57.80 | 52.83 | 52.00 | 50.63 |

**Table 3.** Statistical differences in average developmental parameters of studied wheat grain varieties.

| Variety | Dust (mg) | | Progeny number | | Development time (days) | |
|---|---|---|---|---|---|---|
| | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| LGR 896/64a | 14.25 B | 13.58 B | 30.92 B | 36.66 B | 44.79 A | 44.16 B |
| Korweta | 16.97 A | 18.97 A | 40.19 A | 42.97 A | 43.83 B | 45.23 A |
| Begra | 17.12 A | 18.90 A | 39.22 A | 41.05 A | 44.05 B | 43.74 B |
| $LSD_{0.05}$ | 1.62 | 1.49 | 2.33 | 2.65 | 0.65 | 0.65 |

The insect resistance mechanisms of cereal grains are complex and depend on physico-chemical and biochemical properties of the grain and on the subsequent biochemical and physical adaptation of postharvest insects to these parameters (Warchalewski et al., 2002). Grain, in contrast to green plant tissues, does not posses any insect defensive compounds such as alkaloids, saponins, non-protein amino acids, terpenoids, or phenols. Its chemical composition and food quality for insects does not radically change during the storage period. Simply speaking, grain feeding insects have stable food without specific defensive compounds changing in amounts during storage and insect development. When searching for possibilities for wheat genetic modification, one should take into account biochemical factors such as protein inhibitors that are non-toxic for humans or livestock and physical barriers like hardness and fiber which do not influence grain processing.

From our earlier studies some grain properties such as grain hardness, non-protein nitrogen, and intrinsic wheat proteinaceous inhibitors of $\alpha$-amylase have a negative effect on some growth parameters (Nawrot et al., 1985, Warchalewski et al., 2002).

In spite of the favorable biological activities, such as the highest amylolitic and the lowest antiamylolitic activities, of durum wheat LGR 896/64a, this wheat was highly resistant to granary weevil infestation probably due to higher protein, vitreosity, and higher fiber content as well as grain hardness. Also, lower 1,000-kernel mass, weight by volume, and kernel diameter, which are indicative of smaller size and kernel shape of durum wheat LGR 896/64a, could discourage granary weevils from laying eggs inside the kernels. Grain susceptibility index (Dobie, 1974), which includes number of progeny produced and development time, is a very good measure to compare food quality of different grain varieties. In this experiment, the index for LGR 896/64a was 7.63, while in the case of Korweta and Begra it was 8.36 and 8.38, respectively. It confirms higher susceptibility of the two *Triticum aestivum* varieties. According to Dobie (1974), the most important property of grain in pest infestation is its hardness.

Determination of the factors which can delay even a few days of larval development in one generation could be a crucial point in Integrated Pest Management (IPM) or computer advisory systems used to manage grain quality parameters and storage conditions (Fleurat-Lessard, 2002).

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