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Phenolic and lipophilic compounds of wheat grain as factors affecting susceptibility to infestation by granary weevil (*Sitophilus granarius* L.)

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Summary

The impact of certain groups of polyphenolic (phenolic acids and alkylresorcinols) and lipophilic compounds (total lipids, fatty acids, sterols, tocols and carotenoids) on susceptibility of bread wheat (*Triticum aestivum L.*) kernels to *Sitophilus granarius* infestation was studied. In the experiments, six cultivars of spring wheat with comparable protein content, endosperm hardness and overall technological quality were used. Twenty grams of grain were infested by 10 pairs of beetles and stored for one week or eight weeks at 28±2 °C and relative humidity of 60%. The intensity of growth and feeding of *S. granarius* varied significantly in the used cultivars. The antixenosis effect of the studied grain chemicals, observed after one week of infestation, was the lowest for Łagwa cv., which was characterized by the highest total lipid and sterol contents. Other cultivars showed a similar antixenosis effect. For antibiosis effect, the most attractive for *S. granarius* infestation was Ostka Smolicka cv., which was characterized by the lowest content of total phenolic acids. In contrast, the highest antibiosis effect was found for Arabella and Izera cvs. with the lowest values of sterol content and average values of other determined phytochemicals.

Keywords: alkylresorcinols, infestation by pests, lipophilic compounds, phenolic acids, wheat grain

Introduction

Post-harvest losses of cereal grain during storage may be as high as 83% of mass, especially in tropical and subtropical areas (Pingale, 1964). In temperate zones, the losses to the stored grains and products range from 5% to 15% (Rajendran and Sriranjini, 2008). The main reason for these losses is an attack of insect pests (Fornal et al., 2007). According to FAO estimates, grain losses from pests are about 96 million tonnes annually (Nietupski et al., 2006). Chemical pesticides can reduce these losses, but their use is detrimental to the environment and to the health of the warehouse staff. Additionally, there is a possibility of residual toxicity in the final products used in the feeding of people and animals (Singh and Kaur, 2018). An additional problem with the use of chemical control is the increment of the pest resistance to almost all groups of pesticides (Georghiou, 1990; Singh, 2017). Although the use of plant natural repellents (powders, essential oils and extracts) as an alternative to synthetic compounds is possible (Singh, 2017), the basic strategy is still limited to creating the least favourable conditions (temperature, humidity) for their development (Niewiada et al., 2005).

The most dangerous pest of stored grain in temperate climates is the granary weevil (*Sitophilus granarius* L.) (Campbell and Sinha, 1976). It is considered as a cosmopolitan species (Padin et al., 2002). Grain weight losses caused by *S. granarius* range from 2% to 5% in temperate countries (Ignatowicz, 1999; Rajendran and Sriranjini, 2008). Adult beetles eat from 0.5 to 1 mg per day (Nawrot, 2001) and produce from 0.1 to 0.3 mg of dust (Piasecka-Kwiatkowska, 1999). Larvae of *S. granarius* develop inside the grains and destroy more than half of the grain volume (Hansen and Steenberg, 2007). In the interior of the infested kernels, there are moults or dead individuals. However, the number/mass of tolerable insect fragments is not regulated by European regulations, milling products made from grains heavily infested by *S. granarius* are harmful to humans and animals (Perez-Mendoza et al., 2005) and may cause atopic bronchial asthma in employees of cereal-mills (Skjold et al., 2007). Additionally, the infestation by *S. granarius* results in an increase in kernel moisture content and temperature, which contributes to an increase in enzyme activity (grain sprouting), facilitates the invasion of secondary insect pests, bacteria, mites and fungi (Nawrot et al., 2006; Grineva et al., 2014) and favours the infection of the grain with mycotoxins produced by *Aspergillus* and *Fusarium* species (Ragunathan et al., 1974).

Susceptibility to postharvest infestation by granary weevils is highly variable between wheat cultivars. For example, the most and the least susceptible cultivars can differ approx. 8-fold in insect fertility and approx. 3-fold in insect growth index and grain weight loss after three months of storage (Mebarkia et al., 2010). In a similar study conducted by Khan et al. (2014) on the resistance against rice weevil (*S. oryzae*), the extreme wheat genotypes differed by at least 2-3-fold in the number of damaged grains, percent weight loss and adult population during an experiment conducted over five months. In turn, Fourar-Belaifa et al. (2011) found a 5-fold difference in the number of *S. oryzae* after a 160-day storage of three wheat cultivar grains. Similarly, Nawrot et al. (2006) determined significant differences of the number of offspring and the mass of produced dust between two bread wheats (cvs. Begra and Korweta) and durum wheat (cv. LGR896/64a). The phenomenon of various wheat cultivars susceptibility to pest damage is related to many chemical compounds, such as the content of total protein or gluten, total lipids and cuticular lipids (Nawrot, 1983; Niewiada et al., 2005; Mebarkia et al., 2010; Nawrot et al., 2010) and physical kernel features, such as endosperm hardness or vitreosity and thickness of seed coat (Nawrot et al., 2006; Fourar-Belaifa et al., 2011), but it is still not fully explained. These relationships are much better understood for maize. According to López-Castillo et al. (2018), maize kernel resistance against maize weevil (*Sitophilus zeamais)* and large grain borer (*Prostephanus truncates)* is manifested by antibiosis and antixenosis mechanisms. The cited authors concluded that kernel-pest interactions are determined by biophysical factors (pericarp thickness/toughness, kernel hardness and endosperm vitreosity) and biochemical factors (hydroxycinnamic acids, hydroxyproline-rich glycoproteins, extensins, zeins, arabinoxylans, peroxidises and phenolic acid amides) under the control of genetic factors. The result of these interactions is kernel modification, which leads to limited

accessibility or toxicity to invading pests (López-Castillo et al., 2018).

The objective of the study was to determine if the susceptibility of grain of bread wheat cultivars to *S. granarius* infestation is influenced by certain groups of grain polyphenolic and lipophilic compounds, since these secondary metabolites are highly varied between various wheat grain samples. To avoid the impact of grain protein content and endosperm hardness, only grain samples of similar values of these features were used. Based on a one week and eight-week infestation experiment, the antixenosis or antibiosis effect of the determined chemical compounds was determined.

Material and methods

Biological material

Wheat kernel - *S. granarius* interaction was determined for six spring cultivars of bread wheat: Arabella, Izera, KWS Torridon, Łagwa, Ostka Smolicka and Tybalt obtained from the Department of Experimental Evaluation of Varieties in Radostow, Pomeranian voivodeship, Poland, from the harvest in 2014 (Fig. 1). The used grain samples were similar in moisture content (13.7 - 13.9%), protein content (13.9 - 14.2%), endosperm hardness (14.4 - 14.8 N) and grain overall quality (results of preliminary analyses) and represented the same technological class (quality A – bread wheat) according to the guidelines and criteria of the Research Centre for Cultivar Testing in Poland (COBORU, 2018).

Granary weevils originated from the mass breeding conducted in the Department of Entomology, Phytopathology and Molecular Diagnostics of the University of Warmia and Mazury in Olsztyn on wheat kernels of the Korweta cv. (*Triticum aestivum*).

Experimental setup for infestation

The experiment was performed in 10 replicates for each cultivar, using 80 mm vinyl vials and 35 mm height with a single-centimetre ventilation opening. Each replicate containing 20 g of whole kernels was infested with 20 five-day adults, 10 males and 10 females, sexed by examining the rostrum and abdominal shape, as reported by Halstead (1963) and Dinuta et al. (2008). Infested kernels were placed for seven days in a SANYO MLR-350H air-conditioned chamber for incubation in 28 ± 2 °C and 60% relative humidity. After this time, the adults were removed and the percentage of damaged kernels (DK), the grain weight loss (GWL), the number of eggs laid (NE) and the mortality of the parents (MP) were determined. The kernels were further incubated for insect development during eight weeks. After that time, the selected parameters were recorded: the number of the adult progeny (AP), the total grain weight loss (TGWL), the weight of the dust/flour production (FP). The Dobie susceptibility index (DI) was calculated according to the formula given by Dobie and Kilminster (1978). Based on the DI, used wheat cultivars were assigned as moderately resistant/susceptible to pest infestation with values in the 7-8 range (Dobie, 1974).

Determination of chemical composition

In this part of experiment intact grain samples, manually cleaned from broken and damaged kernels, were utilized. Before subsequent extraction of chemicals, grains were milled to obtain particles smaller than 400 μm.

Phenolic acids

Chromatography analysis was performed by the RP-HPLC technique according to the method described by Bojarska et al. (2011). Total phenolic acids were extracted by triplicate with diethyl ether from alkaline hydrolysate of milled grains, while free phenolic acids from untreated samples were extracted according to Konopka et al. (2012). An Agilent Technologies (Santa Clara, USA) 1200 series system fitted out with a photodiode detector and with an Agilent Technologies Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 μm) at the temperature of 30 °C was used. The mobile phase consisted of water : acetonitrile : formic acid (88:10:2, v/v/v). The isocratic flow rate was equal to 0.8 mL/min. Detection was performed at the wavelength of 260 and 320 nm. Identification and quantification of phenolic acids was performed by comparison of the retention times and absorption spectra of the sample with reference standards (Sigma-Aldrich, St. Louis, MO, USA). The content of phenolic acids was determined from calibration curves of reference phenolic acids (Sigma-Aldrich, St. Louis, MO, USA) and expressed as μg per g of a sample dry mass (DM). The repeatability for phenolic acid determination (expressed as coefficient of variation) was at least 2.1 and 3.5% (for free and total form, respectively). Limit of quantification was at least 0.05 μg/g of sample DM, while linearity of calibration curves was confirmed in range of 1-150 μg/mL. Free phenolic acids content is presented as a sum of identified compounds.

Alkylresorcinols

Extraction of alkylresorcinols was done with the use of acetone (10 mL/g of a sample) according to the method described by Sampietro et al. (2009) with small modifications. Firstly, samples were sonicated for 15 minutes in an ultrasonic bath (InterSonic, Olsztyn, Poland) and subsequently put aside in a dark place at room temperature for 48 hours. After that time, the 15-minute ultrasonic treatment was repeated. Then, the extract was centrifuged in an Eppendorf centrifuge (Eppendorf, type 5810 R, Hamburg, Germany)

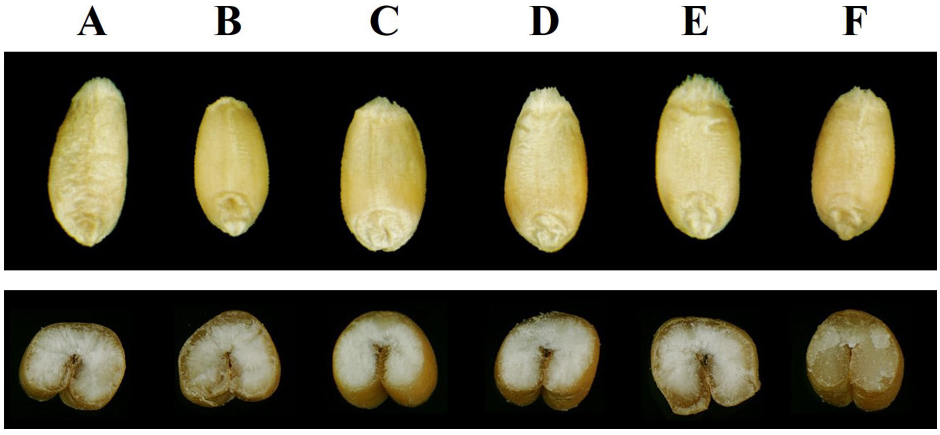

Fig. 1: Images of kernels of the studied spring wheat cultivars: Ostka Smolnicka (A), Arabella (B), Izera (C), Tybalt (D), KWS Torridon (E), Łagwa (F).

at 1,000 rpm for 10 minutes, at 22 °C. The supernatants were collected in clean flasks and evaporated to dryness on a vacuum evaporator (Büchi, type R-210; Büchi Labortechnik). The residue was dissolved in 1 mL of methanol and centrifuged on a centrifuge (Eppendorf, type 5417 R) at 1,600 rpm for 10 minutes. Colour reaction was performed by adding 2 mL of 0.05% Fast Blue RR reagent (4-benzoylamino-2,5-dimethoxybenzenediazonium chloride hemi (zinc chloride) salt) (Sigma-Aldrich, St. Louis, MO, USA) diluted with methanol (1:5) and 10 mL of 10% K_2CO_3 solution to each extract. Absorbance measurements were carried out after 20 min at 480 nm using a UNICAM UV/Vis UV2 spectrophotometer (ATI Unicam, Cambridge, United Kingdom). The content of alkylresorcinols was calculated using a standard curve prepared for olivetol and expressed in μg/g of grain DM. The repeatability for olivetol determination (expressed as coefficient of variation) was 2.5%. Limit of quantification was 0.05 μg/g of sample DM, while linearity of calibration curve was confirmed in range of 1-15 μg/mL. The composition of alkylresorcinols was determined with the use of the GC-MS QP2010 PLUS, manufactured by Shimadzu (Kyoto, Japan). For this purpose extract re-dissolved in methanol and centrifuged was transferred into vials, and evaporated under a nitrogen stream. To the residue pyridine and N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) (Sigma-Aldrich, St. Louis, MO, USA) were added, and the mixture was heated at 60 °C for 60 min. After complete derivatization heptane was added, and the mixture was analysed using the GC-MS QP2010 PLUS manufactured by Shimadzu (Kyoto, Japan). Alkylresorcinols were separated on a ZB-5ms capillary column (30 m × 0.25 mm × 0.25 μm) (Phenomenex, Torrance, USA), and with helium as a carrier gas with a 0.9 mL/min flow rate. The temperatures were as follows: injector – 230 °C, column – 70 °C increased to 230 °C at 15 °C/min, and to 310 °C at 3 °C/min, and maintained for 10 min, GC-MS interface – 240 °C, ion source – 220 °C. Electron energy was set to 70 eV. The total ion current (TIC) mode was used for quantification (100-600 m/z range). Alkylresorcinols were identified using retention times and mass spectra of peaks.

Lipids

The content of lipids was determined by Soxhlet method with hexane according to Polish Standard PN-EN ISO 659:2010P.

Fatty acids

The fatty acids methylation was carried out by heating the vials at 70 °C for 2 hours according to the method described by Zadernowski and Sosulski (1978). Obtained methyl esters were analysed by gas chromatography with a GC-MS QP2010 PLUS (Shimadzu, Japan) system according to the parameters described by Czaplicki et al. (2016). Separation of fatty acids methyl esters was performed on a BPX70 (25 m × 0.22 mm × 0.25 μm) capillary column (SGE Analytical Science, Victoria, Australia) with helium as a carrier gas at a flow rate of 0.9 mL/min. The column temperature was programmed as follows: a subsequent increase from 150 °C to 180 °C at the rate of 10 °C/min, to 185 °C at the rate of 1.5 °C/min, to 250 °C at the rate of 30 °C/min, and then 10 min hold. The interface temperature of GC-MS was set at 240 °C. The temperature of the ion source was 240 °C and the electron energy 70 eV. The total ion current (TIC) mode was used in 50-500 m/z range. The results were expressed as percentages of total fatty acids.

Sterols

The content of sterols was determined according to the GC/MS method described by Roszkowska et al. (2015), with modifications. Extraction of sterols from finely milled grain was carried out using acetone (10 mL/g of a sample). The collected extracts were evaporated to dryness at temperatures below 50 °C in a vacuum evaporator (Büchi, type R-210; Büchi Labortechnik, Flawil, Switzerland). The residue was dissolved in ethanol, and 5α-cholestane (Sigma-Aldrich, St. Louis, MO, USA) solution was added as an internal standard. The mixture was saponified by adding 0.5 mL 10 M KOH solution in ethanol at a temperature of 70 ºC for 30 min. The mixtures were transferred to separatory funnels containing deionized water, and non-saponifiable components were extracted twice with diethyl-ether. The collected ether layers were washed twice with 0.5 M KOH and four times with deionized water. The ether fractions were filtered through a filter with anhydrous sodium sulphate and evaporated in a vacuum evaporator at 45 °C. The dry extract was re-dissolved in hexane, centrifuged on a centrifuge (Eppendorf, Hamburg, Germany), transferred into vials, and evaporated under a nitrogen stream. To the residue pyridine and N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) (Sigma-Aldrich, St. Louis, MO, USA) were added and the conditions of derivatization, separation and identification were identical to those in alkylresorcinol analysis. Sterols were identified using retention times and mass spectra of peaks, and their content was determined based on the concentration of the internal standard, and was expressed as μg of 5α-cholestane per g of grain DM. The repeatability for 5α-cholestane determination (expressed as coefficient of variation) was 2.5%. Limit of quantification was 0.05 μg/g of sample DM.

Tocols

The content of tocols was determined according to the method described by Konopka et al. (2012). Each sample of finely milled grain was extracted using n-hexane (10 mL/0.5 g of sample). The collected extracts were evaporated to dryness at temperatures below 50 °C in a vacuum evaporator (Büchi, type R-210; Büchi Labortechnik) and then rediluted in n-hexane and subsequently centrifuged (10 min, 25,000 g) in a 5417R-type Eppendorf centrifuge (Eppendorf AG, Hamburg, Germany). Chromatography analysis was performed by the RP-HPLC technique according to method described by Phenomenex (2015). The analysis was carried out using an Agilent Technologies 1200 RP-HPLC apparatus, equipped with a fluorescence detector from the same manufacturer. Separation was performed on a Phenomenex Kinetex F5 column (100 mm × 2.1 mm × 2.6 μm). A methanol (A)-water (B) gradient was used for elution of the tocols. The gradient was programmed: 0-1 min 85% of A, until 15 min increased to 100% of A and persisted until 17 min. Then it decreased to 85% of A and was stable until 23 min. The flow rate was equal to 1.2 mL/min. The fluorescence detector was set at 296 nm of excitation and 330 nm of emission. Tocols were quantified using standards of α-, β-, γ-, and δ-tocopherols (Sigma-Aldrich, St. Louis, MO, USA). Their content was calculated using external calibration curves, and was expressed as μg/g of grain DM. The repeatability for α-, β-, γ-, and δ-tocopherol determination (expressed as coefficient of variation) was 2.5%. Limit of quantification was respectively 0.45, 0.4, 0.4 and 0.2 μg/g of sample DM, while linearity of calibration curve was confirmed in range of 0.02-16 μg/mL.

Carotenoids

The content of carotenoids was determined according to the method described by Czaplicki et al. (2016). To each 10 g of fine milled grain sample, placed in brown glass flask, were added 20 mL mixture of hexane, acetone, absolute ethanol and toluene (10:7:6:7, v/v/v/v), 5 mL of 40% KOH in methanol, 1 mL of 0.1% BHT in ethanol and β-apo-8'-carotenal (Sigma-Aldrich, St. Louis, MO, USA) as an internal standard. The solutions were vigorously shaken in Multi Rotator RS-60 (Biosan, Riga, Latvia) in the dark at room temperature for

16 h saponification. After saponification 10% Na_2SO_4 was added to each sample and extraction of carotenoids were performed fourfold with hexane. Collected extracts were evaporated to dryness using vacuum evaporator (BÜCHİ R-200 Type). Finally, the dry extracts were re-dissolved in methanol and dichloromethane mixture (45:55, v/v) and subsequently centrifuged (10 min, 25,000 g) in a 5417R-type Eppendorf centrifuge. The chromatographic analysis was carried out using a 1200 series Agilent Technologies chromatograph equipped with a diode array detector (DAD). Separations were performed at 30 °C on YMC-C30 column (250 mm × 4.6 mm × 5 µm; Komatsu, Japan) and YMC-C30 pre-column (10 mm × 4.6 mm × 3 µm). A methanol (A) vs. methyl tert-butyl ether (MTBE) (B) gradient was used for elution. The gradient was programmed: 0-5 min 95% of A, until 25 min decreased to 72% of A and kept on decreasing until 33 min to 5% of A. Then it increased to 95% of A and was stable until 60 min. Absorbance was measured at 450 nm. Carotenoids were identified based on the retention times of standards and their spectral properties, and their content was determined based on the concentration of the internal standard, and was expressed as µg of β-apo-8'-carotenal equivalent per g of grain DM. The repeatability for β-apo-8'-carotenal determination (expressed as coefficient of variation) was 2.5%. Limit of quantification was 0.05 µg/g of sample DM, while linearity of calibration curve was confirmed in range of 1-150 µg/mL.

Statistical analysis

All chemical analyses were done in triplicate and analysed using Statistica 12.5 PL software (StatSoft, Kraków, Poland) at $p \leq 0.05$ significance level. The differences of grain samples composition were determined using ANOVA with Duncan's test. The relationships between biological susceptibility to pest infestation and chemical composition of used grain were examined using principle component analysis (PCA).

Results and discussion

Wheat cultivar differentiation based on susceptibility to granary weevil infestation

In the first stage of the study, selected indices related to the intensity of growth and feeding of *S. granarius* for six spring wheat cultivars were determined (Tab. 1). Host plant attractiveness for insects to laying eggs and feeding (antixenosis mechanism) was assayed 7 days after grain infestation. It was found that the percentage of damaged kernels ranged from 67.7% (Izera cv.) to 82.6% (Łagwa cv.), while the percentage of grain weight loss was from 1.71% to 2.80%. At the same time, the number of folded eggs varied from 291 (Tybalt and Izera cvs.) to 349 (Łagwa cv.). The mortality of the beetles was relatively low and ranged from 0 (Łagwa cv.) to 4.38% (KWS Toridon cv.). Considering the results of all these infestation indices, Łagwa cv. was the most attractive for *S. granarius* to laying eggs and feeding (the low visible antixenosis mechanism). After 8 weeks of infestation, the antibiosis mechanism was determined, which refers to adverse biologically consequences on the life cycle of pests as a result of feeding activity on the resistant host plant. Symptoms of the antibiosis mechanism can include: larval mortality, increased mortality of pupae, failure of the adult out of the pupae, low adult fertility, short insect life cycle and other forms of abnormality (Sulistyo and Inayati, 2016). For the used wheat cultivars, the contrast antibiosis effect was stated for Arabella and Izera cvs. (the highest effect) and Ostka Smolicka cv. (the lowest effect) with the values of an adult progeny 160 and 266, respectively. It was also confirmed by a final percentage grain weight loss close to 28% and 45.8%, respectively.

Wheat cultivar differentiation based on the profile of phenolic and lipophilic compounds in grain

Phenolic compounds act as natural plant pesticides and protect plants against external aggression and predators (Drewnowski and Gomez-Carneros, 2000). In cereal grain, the main representatives of these compounds are phenolic acids and alkylresorcinols (Konopka et al., 2017). Most of the phenolic acids are deposited in pericarp cell walls and cells of the aleurone layer (Hansen et al., 2002; Lopez-Castillo et al., 2018), while alkylresorcinols (AR) as a phenolic lipids (Ross et al., 2003) are mainly (99% of them) deposited in an intermediate layer of the caryopsis, including the hyaline layer, testa and inner pericarp surrounding the cereal kernel (Landberg et al., 2008). Phenolic compounds content in wheat grain is highly affected by genetic factors (species/cultivar) and by environmental conditions (mainly biotic stress).

The phenolic compound contents and compositions of the used wheat cultivars are presented in Tab. 2. Grain contained from 332 µg/g (Ostka Smolicka cv.) to 482 µg/g (KWS Torridon cv.) of total phenolic acids (TPA). Among them, ferulic acid prevailed with a share from 79.6% (Ostka Smolicka cv.) to 86.0% (Tybalt cv.). The second in quantity was sinapic acid and its share varied from 8.9% (KWS Torridon cv.) to 15.6% (Ostka Smolicka cv.). Other identified phenolic acids (p-coumaric, protocatechuic, vanillic, p-OH benzoic and caffeic) were less frequent. Only up to 1.8% of these acids existed in an unbound state. The highest content of unbound phenolic acids was determined in grain of the Ostka Smolicka cv. (5.89 µg/g). In

Tab. 1: Parameters of development of *S. granarius* on grain of the studied spring wheat cultivars after 1 week and 8 weeks of storage.

	Parameter	Ostka Smolicka	Arabella	Izera	Tybalt	KWS Torridon	Łagwa
Parameters at 1 week	DK [%]	69.0±2.5^{b}	71.2±2.5^{b}	67.7±4.9^{b}	69.1±8.0^{b}	69.9±6.5^{b}	82.6±2.4^{a}
	NE [pieces]	322±12^{ab}	330±14^{a}	291±19^{b}	291±32^{b}	299±27^{b}	349±13^{a}
	GWL [%]	2.80±0.33^{a}	1.71±0.47^{b}	2.31±0.68^{ab}	1.71±0.39^{b}	1.85±0.54^{b}	2.11±0.22^{ab}
	MR [%]	3.75±3.54^{ab}	0.63±1.77^{ab}	1.25±2.31^{ab}	3.75±3.54^{ab}	4.38±3.20^{a}	0.00±0.00^{b}
Parameters at 8 weeks	AP [pieces]	266±31^{a}	160±16^{c}	160±18^{c}	189±16^{b}	186±11^{b}	189±16^{b}
	TGWL [%]	45.8±7.5^{a}	28.7±3.8^{b}	28.2±3.9^{b}	36.2±7.4^{b}	34.7±9.2^{b}	36.2±7.4^{b}
	DI	7.97±0.18^{a}	7.25±0.14^{c}	7.25±0.16^{c}	7.48±0.12^{b}	7.46±0.08^{b}	7.48±0.12^{b}
	Resistant category	MR/S	MR	MR	MR	MR	MR

Abbreviations: DK – percentage of damaged kernels, NE – number of eggs, GWL – percentage grain weight loss, MP – parent mortality, AP – adult progeny, TGWL – percentage grain weight loss, DI – Dobie Index, S – susceptible cultivar based on DI, MR – moderately resistant cultivar based on DI
The results are presented as mean values ± standard deviation. Different letters in the same raw indicate significant differences (P≤0.05).

Tab. 2: Quantification of phenolic and lipophilic compounds in grain of the studied spring wheat cultivars.

Parameter	Ostka Smolicka	Arabella	Izera	Tybalt	KWS Torridon	Łagwa
Total phenolic acids (mg/g of DM)	332±16^{a}	441±13^{c}	342±10^{a}	472±4^{d}	482±17^{d}	404±2^{b}
Ferulic	265±14^{a}	373±10^{d}	284±8^{b}	406±3^{e}	414±11^{e}	341±0^{c}
Sinapic	51.8±1.4^{b}	49.2±2.4^{ab}	43.4±2.2^{ab}	43.3±6.5^{ab}	42.7±4.5^{ab}	42.3±1.7^{a}
p-coumaric	5.92±0.22^{a}	5.82±0.21^{a}	5.36±0.23^{a}	7.69±0.09^{b}	8.56±0.62^{c}	8.23±0.10^{bc}
Protocatechuic	2.13±0.01^{a}	5.09±0.32^{c}	3.26±0.02^{b}	5.81±0.22^{d}	5.53±0.24^{cd}	5.20±0.30^{c}
Vanillic	2.85±0.06^{ab}	2.64±0.17^{a}	2.37±0.13^{a}	3.77±0.20^{c}	4.61±0.31^{d}	3.31±0.20^{bc}
p-OH benzoic	2.42±0.07^{a}	3.21±0.16^{c}	2.47±0.00^{a}	2.91±0.17^{bc}	3.61±0.22^{d}	2.77±0.17^{ab}
Caffeic	2.04±0.07^{bc}	1.96±0.19^{b}	0.71±0.07^{a}	2.35±0.16^{c}	2.32±0.16^{c}	1.80±0.10^{b}
Free phenolic acids (mg/g of DM)	5.89±0.24^{b}	3.68±0.10^{a}	3.46±0.13^{a}	5.76±0.15^{b}	5.50±0.17^{b}	5.82±0.02^{b}
Total alkylresorcinols (mg/g DM)	291±^{ab}	261±8^{a}	344±8^{bc}	356±4^{c}	348±18^{bc}	269^{a}
AR 17:0	14.0^{abc}	13.0±0.5^{ab}	13.8±0.1^{ab}	16.3±0.4^{bc}	17.5±1.1^{c}	11.1^{a}
AR 19:0	105^{ab}	94.0±2.1^{ab}	107±2^{bc}	123±2^{c}	124±5^{c}	88.7^{a}
AR 21:0	137^{ab}	129±3^{a}	178±2^{c}	179±4^{c}	168±7^{bc}	139^{ab}
AR 23:0	22.8^{ab}	15.4±0.9^{a}	27.7±2.0^{b}	23.6±1.4^{ab}	22.2±0.7^{ab}	19.2^{a}
AR 25:0	7.9^{a}	5.8±0.13^{a}	13.8±0.85^{c}	8.96±0.43^{ab}	12.3±0.71^{bc}	8.6^{a}
Others	3.97^{ab}	3.21±0.11^{ab}	4.32±0.59^{b}	4.70±0.60^{b}	3.97±0.20^{ab}	2.36^{a}
Total lipids (% of DM)	2.04±0.20^{bc}	1.78±0.01^{ab}	1.47±0.04^{a}	2.37±0.16^{d}	2.15±0.13^{cd}	2.39±0.12^{d}
Fatty acids composition (%):						
C16:0	17.97±0.13^{d}	17.68±0.19^{cd}	17.64±0.11^{cd}	16.96±0.15^{ab}	16.75±0.06^{a}	17.25±0.18^{bc}
C18:0	0.81±0.01^{ab}	0.94±0.00^{b}	0.83±0.13^{ab}	0.72±0.02^{a}	0.86±0.02^{ab}	0.85±0.06^{ab}
C18:1	13.58±0.02^{a}	15.77±0.01^{de}	15.42±0.04^{c}	15.65±0.06^{cd}	15.03±0.25^{b}	16.03±0.02^{e}
C18:2	63.17±0.06^{c}	61.82±0.00^{a}	62.03±0.04^{ab}	63.21±0.33^{c}	63.79±0.33^{d}	62.49±0.09^{b}
C18:3	3.90±0.13^{d}	3.19±0.01^{b}	3.60±0.04^{c}	3.00±0.10^{b}	3.03±0.08^{b}	2.72±0.06^{a}
C20:1	0.58±0.05^{abc}	0.61±0.00^{bc}	0.49±0.04^{a}	0.47±0.04^{a}	0.56±0.04^{ab}	0.68±0.06^{c}
Total sterols (mg/g of DM)	675±7^{b}	522±20^{a}	500±7^{a}	675±30^{b}	638±30^{b}	729±28^{b}
Campesterol	191±2^{c}	132±4^{ab}	115±2^{a}	132±5^{ab}	134±1.3^{ab}	157±8^{bc}
β-sitosterol	455±17^{b}	367±16^{a}	358±5^{a}	509±20^{bc}	467±21^{bc}	538±13^{c}
Stigmasterol	28.7±0.7^{a}	23.3±0.6^{a}	26.0±0.4^{a}	34.4±2^{a}	37.3±0.2^{a}	33.9±0.5^{a}
Total tocols (mg/g of DM)	49.9±1.2^{a}	45.3±1.3^{a}	49.2±4.1^{a}	50.2±3.1^{a}	45.7±0.6^{a}	44.0±4.5^{a}
α-T	19.6±0.4^{b}	16.8±0.3^{a}	17.5±0.9^{ab}	17.2±0.2^{ab}	15.9±0.1^{a}	15.7±2.3^{a}
α-T3	8.34±0.5^{b}	6.97±0.68^{ab}	7.37±0.45^{ab}	6.93±0.72^{ab}	7.19±0.22^{ab}	6.03±0.52^{a}
β-T	5.15±0.21^{bc}	4.85±0.07^{b}	5.54±0.17^{c}	4.75±0.42^{b}	3.99±0.04^{a}	4.70±0.42^{b}
β-T3	15.9±0.2^{a}	15.7±0.8^{a}	17.8±1.25^{ab}	20.2±1.4^{b}	17.6±0.3^{ab}	16.6±1.2^{ab}
δ-T	0.95±0.0^{a}	0.95±0.02^{a}	0.99±0.05^{ab}	1.04±0.03^{b}	0.98±0.01^{ab}	0.96±0.04^{ab}
Total carotenoids (mg/g of DM)	3.51±0.18^{d}	2.22±0.13^{bc}	2.80±0.11^{cd}	1.87±0.15^{b}	1.24±0.10^{a}	2.52±0.05^{c}
Lutein	2.78±0.13^{e}	1.56±0.11^{bc}	2.15±0.08^{d}	1.27±0.09^{b}	0.78±0.06^{a}	1.88±0.01^{c}
Zeaxanthin	0.53±0.03^{b}	0.33±0.01^{a}	0.48±0.02^{b}	0.38±0.04^{a}	0.28±0.02^{a}	0.49±0.04^{b}
β-carotene	0.09±0.01^{b}	0.20±0.00^{c}	0.05±0.00^{a}	0.12±0.01^{b}	0.09±0.01^{b}	0.04±0.00^{a}
9-cis β-carotene	0.02±0.00^{a}	0.08±0.00^{b}	0.01±0.00^{a}	0.04±0.00^{a}	0.04±0.00^{a}	0.01±0.00^{a}
Others	0.11±0.01^{a}	0.06±0.00^{a}	0.10±0.00^{a}	0.06±0.01^{a}	0.04±0.00^{a}	0.10±0.01^{a}

The results are presented as mean values ± standard deviation. Different letters in the same raw indicate significant differences (P≤0.05).

contrast, this fraction was the lowest (3.46-3.68 μg/g) in grain of Arabella and Izera cvs. The content of alkylresorcinols varied from 261 (Arabella cv.) to 356 μg/g (Tybalt cv.). Among them, homologues with an alkyl chain length of AR19:0 and AR21:0 prevailed, reaching 34% and 50% on average, respectively. The determined contents of phenolic acids and alkylresorcinols were relatively low, but still within the ranges given by other authors (Shewry and Ward, 2012; Ziegler et al., 2015; Shewry and Hey, 2015).

The composition of grain lipids in tested samples is presented in Tab. 2. Lipids usually account for approx. 2-3% of wheat grain mass (Konopka et al., 2017). Most of them are triacylglycerols deposited in endosperm and germ in the form of storage oil bodies. They are accompanied by sterols, tocols and carotenoids with various protectant activities against plant stresses during growth. Usually, the overall content of low molecular lipophilic compounds does not exceed 1,000 μg/g of grain (Konopka et al. 2017).

The content of lipids varied from 1.47% in Izera cv. to 2.39% in Łagwa cv. The used wheat cultivars were only slightly differentiated by the shares of fatty acids in grain, with three main compounds: C18:2 (ca. 62%), C16:0 (ca. 17%) and C18:1 (ca. 15%). More variable was the content of sterols, which ranged from 500 μg/g (Izera cv.) to 729 μg/g (Łagwa cv.). Only three homologues were found among this group: β-sitosterol, campesterol and stigmasterol, with average shares of 72%, 23% and 5%, respectively. Tocols content was the least variable feature among the tested cultivars (with a coefficient of variation 5.7%) and varied from 44.0 μg/g (Łagwa cv.) to 50.2 μg/g (Tybalt cv.). In all samples, five homologues were determined in order of participation: β-tocotrienol and α-tocopherol (ca. 36%), α-tocotrienol (ca. 15%), β-tocopherol (ca. 10%) and δ-tocopherol (ca. 2%). The smallest part of lipophilic compounds constituted carotenoids, with an average amount of 2.36 μg/g and high variation from 1.24 μg/g (KWS Torridon cv.) to 3.51 μg/g (Ostka Smolicka cv.). The main homologues of this group were lutein (ca. 72%), zeaxanthin (ca. 18%), and β-carotene (ca. 5%). Shares of minor compounds such as β-carotene and 9-cis-β-carotene were highly variable between the used cultivars. The determined profile of low molecular lipophilic compounds was within the limits given by other authors since bread wheat grain usually contains from 225 μg/g to 959 μg/g of sterols, from 23.3 μg/g to 79.7 μg/g of tocols and from 1.40 μg/g to 4.90 μg/g of carotenoids (Shewry and Ward, 2012; Konopka et al., 2017).

Correlations between biological indices of wheat grain susceptibility to granary weevil infestation and phenolic and lipophilic grain compounds

In summary, a total of 6 biological indices and 37 chemical compounds were analysed. The correlations between biological and chemical properties of the used wheat cultivars are presented in Tab. 3. It has been found, that the percentage of damaged kernels after one-week of *S. granarius* infestation was only positively correlated (r=0.82) with the share of C20:1 fatty acid, number of laid eggs with content of AR 19:0 (r=-0.86) and AR 21:0 (r=-0.90) and with the share of C20:1 fatty acid (r=0.96), while grain weight loss negatively (r in range 0.90 - 0.94) with total phenolic acids and some individuals (ferulic acid and protocatechuic acid) and positively with total carotenoids and lutein and zeaxanthin content (r in range 0.83-0.88). Parent mortality after one-week infestation was correlated with AR 17:0 (r=0.88) and AR 19:0 (r=0.87) and the share of C18:2 fatty acid (r=0.88). In contrast, adult progeny after eight-week of *S. granarius* infestation was mostly correlated with campesterol content (r=0.93) and with the share of C18:1 fatty acid (r=-0.86). In the case of impact of composition of low molecular phytochemicals on the final grain weight loss (after 8-weeks) the significant relationships were determined for the content of free phenolic acids (r=0.83) and campesterol (r=0.91).

As mentioned in the introduction part, the susceptibility of a cultivar to infestation by pests is a result of the cumulative effect of kernel physical features and various chemical compounds. The data about the impact of grain phenolic compounds and grain lipids on resistance or attractiveness of wheat grain to *S. granarius* infestation are relatively scarce. It was previously found, that the grain lipid fraction is attractive for the egg-laying process. Triacylglycerols were recognized as strong attractants of the granary weevil (Nawrot, 1983). Cuticular lipids were determined as crucial for the feeding and oviposition behaviour of this beetle (Niewiada et al., 2005). In general, beetles produced 64-95% less dust and laid 7-16% fewer eggs in kernels from which cuticular lipids had been removed. These cuticular lipids consisted mainly of alkanes: n-heptacosane (C27), n-nonacosane (C29) and n-hentriacontane (C31), wax esters, triacylglycerols, C16:0 and C18:1 free fatty acids, triterpene alcohol - amyrine, and sterols (Niewiada et al., 2005). This implies that these compounds may have a major role in food selection and the search for an oviposition site prior to grain infestation (Nawrot et al., 2010). Fungal volatile metabolites such as 1-octanol and 2-methyl-1-butanol can also increase egg lying on wheat kernels (Niewiada et al., 2005). In the case of infestation of rice grain by *S. zeamais* Maeshima et al. (1985) concluded that oviposition was stimulated by a synergistic action – a mixture of ferulates, diglycerides and free sterols. In the current study, a strong positive correlation was found between indices of long-term infestation and campesterol content. Insects cannot synthesize sterol structures and need cholesterol or phytosterols as precursors from food for the biosynthesis of ecdysteroids, which are insect moulting and sex hormones (Singh and Kaur, 2018). Campesterol is preferentially deposited in some insect tissues (Behmer and Nes, 2003), but specific data for *S. granarius* are not available.

Nwosu (2016) discovered that mechanisms of maize resistance to *S. zeamais* were antibiosis, antixenosis and preference, as the increases in maize varietal crude fibre, phenolic acids and trypsin inhibitor significantly increased the mortality of the maize weevil adults and significantly reduced their survival rate and oviposition by the adult females. At the same time, the percent of grain damage, percent of weight loss and weight of grain flour was also reduced. The significant increase of phenolic acid and trypsin inhibitor in grains resulted in low infestation (Nwosu, 2016). These results were in accordance with earlier findings by Serratos et al. (1993), that there was a significant negative correlation between phenolic content (i.e., hydroxycinnamic acid accumulation in maize grain) and susceptibility of the grain to weevil infestation. Likewise, Arnason et al. (1994) found that the variation in resistance of Mexican landraces of maize to maize weevil was negatively correlated to phenolic and protein content of the variety tested. A detailed analysis of the phenolics revealed the presence of diferulate which may contribute to the mechanical resistance of the kernel by the cross-linking of cell wall hemicelluloses (Arnason et al., 1994). Apart from the impact on the mechanical resistance of the kernel, phenolic compounds are also able to precipitate of proteins, and this effect can affect pest digestive enzymes.

Our study confirmed the only protectant impact of grain phenolic acids on the dietary habit of *S. granarius* manifesting as reduced grain weight loss after one-week infestation. In contrast, these compounds did not affect other indices of infestation. It can be explained by relatively low concentration of these compounds in used grain since wheat grain samples can contain 2-3-fold highest content of phenolic acids (Shewry and Ward, 2012; Ziegler et al., 2015). Positive correlation between free phenolic acids and loss of grain mass after an eight-week infestation indicates the attractiveness of grain with slightly higher degradation of cell walls, which is hypothetically indicated by the increased content of free phenolic compounds.

Tab. 3: Correlations between parameters of development of *S. granarius* and chemical composition.

Parameter	At 1 week				At 8 weeks	
	DK	NE	GWL	MP	AP	TGWL
Total phenolic acids	0.06	-0.17	-0.90*	0.26	-0.41	-0.26
Ferulic acid	0.07	-0.18	-0.91*	0.24	-0.45	-0.29
Sinapic acid	-0.31	0.29	0.48	0.09	0.57	0.41
p-cumaric acid	0.49	0.08	-0.44	0.27	-0.02	0.20
Protocatechuic acid	0.33	-0.01	-0.94*	-0.05	-0.58	-0.40
Vanilic acid	0.08	-0.23	-0.44	0.62	0.05	0.22
p-hydroxybenzoic acid	0.01	-0.08	-0.76	0.24	-0.40	-0.31
Caffeic acid	0.08	0.13	-0.36	0.55	0.37	0.49
Free phenolic acids	0.34	0.19	0.17	0.50	0.68	0.83*
Total alkylresorcinols	-0.42	-0.80	0.07	0.67	0.11	0.17
AR 17:0	-0.67	-0.80	-0.30	0.88*	0.04	0.08
AR 19:0	-0.65	-0.86*	-0.23	0.87*	0.06	0.11
AR 21:0	-0.47	-0.90*	-0.23	0.43	-0.31	-0.26
AR 23:0	-0.49	-0.74	0.41	0.39	0.11	0.08
AR 25:0	0.63	0.64	0.69	-0.19	0.64	0.67
Total lipids	0.52	0.28	-0.23	0.28	0.34	0.55
Fatty acid C16:0	-0.15	0.33	0.68	-0.35	0.34	0.15
Fatty acid C18:0	0.23	0.51	-0.14	-0.53	-0.35	-0.45
Fatty acid C18:1	0.47	0.12	-0.76	-0.62	-0.86*	-0.74
Fatty acid C18:2	-0.17	-0.33	0.04	0.88*	0.52	0.62
Fatty acid C18:3	-0.66	-0.26	0.76	0.26	0.50	0.30
Fatty acid C20:1	0.82*	0.96*	0.10	-0.53	0.13	0.17
Total sterols	0.55	0.36	0.14	0.27	0.58	0.75
Campesterol	0.25	0.54	0.68	0.20	0.93*	0.91*
ß-sitosterol	0.58	0.26	-0.06	0.23	0.38	0.58
Stigmasterol	0.27	-0.15	-0.22	0.51	0.17	0.36
Total tocols	-0.73	-0.67	0.40	0.54	0.40	0.33
α-T	-0.56	-0.17	0.73	0.36	0.71	0.56
α-T3	-0.76	-0.37	0.64	0.59	0.62	0.45
ß-T	-0.24	-0.06	0.55	-0.39	0.06	-0.09
ß-T3	-0.30	-0.74	-0.42	0.42	-0.25	-0.12
δ-T	-0.34	-0.74	-0.43	0.40	-0.26	-0.14
total carotenoids	0.01	0.33	0.86*	-0.27	0.55	0.41
Lutein	0.01	0.31	0.88*	-0.25	0.56	0.42
Zeaxantin	0.23	0.30	0.83*	-0.28	0.51	0.45
ß-Caroten	-0.32	0.04	-0.51	0.03	-0.19	-0.24
9-cis ß-Caroten	-0.24	0.03	-0.66	0.02	-0.33	-0.35

*Correlation coefficient statistically significant (P≤0.05).
Abbreviations: DK – percentage of damaged kernels, NE – number of eggs, GWL – percentage grain weight loss, MP – parent mortality, AP – adult progeny, TGWL – total percentage grain weight loss.

Conclusions

This study showed that the susceptibility of six Polish spring wheat cultivar grains to *S. granarius* infestation varied significantly. This phenomenon cannot be explained by variation in grain protein and hardness since the used cultivars were similar in these factors and had similar overall quality. However, these cultivars differed in the content and composition of specific phenolic and lipophilic phytochemicals. Some of these compounds may act as pest attractants (potential candidate is C20:1 fatty acid) or favour the growth during

long-term infestation (campesterol). The final results of *S. granarius* infestation may have also been affected by phenolic acids, which are responsible for compactness of the pericarp- and aleuronic-layer cell wall structures and can affect dietary habit of this beetle. Further studies are needed using grains from highly differentiated cultivars in terms of the cited compounds.

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