INTERNATIONAL
Agrophysics
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Int. Agrophys., 2024, 38, 165-176
doi: 10.31545/intagr/185216

Effect of different chemical properties of cereal grains on the foraging and microbiome of the rice weevil (*Sitophilus oryzae* L.)**

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Received October 30, 2023; accepted February 22, 2024

Abstract. Due to global warming, the rice weevil is an increasing threat. Various species of weevils coexist with endosymbiotic *Sodalis pierantonius*. This study aimed to check whether different chemical properties of different cultivars of common wheat and barley grain cause disturbances in insect feeding and rearrangements of the rice weevil microbiome. It has been observed that foraging increases in the case of cultivars with low protein content, high steric acid content, and low antioxidant activity. Cultivars with higher antioxidant activity, a tendency was observed to increase the number of adult insects and the intensity of feeding. However, the correlation of number of offspring generations and antioxidants was different depending on species of cereals (R^2=0.9 for barley, R^2<0.1 for wheat). Changes of dominance classes of selected Enterobacteriaceae partially shifted the microbiomes of insects feeding on different barley varieties. In contrast, in some wheat cultivars, displacement of the dominant genus *Sodalis* by genera *Staphylococcus* or *Mammaliicoccus* was observed, severely reducing the rice weevil's foraging ability. Nevertheless, *Sodalis* sp. almost always dominated (from 16.7 to 90.07% for wheat and from 63.4 to 90.9% for barley), and no correlation was observed between species and variety factors of cereals and their abundance.

Keywords: storage pests, *Sodalis* sp., grain properties, symbiotic bacteria, *Sitophilus oryzae*

1. INTRODUCTION

Grain storage is one of the elements of the plant production system in which the food product passes from the field to the consumer's table. It plays a key role, and many studies indicate that the most tremendous losses occur during this stage (Majumder *et al*., 2016). The damage caused during the storage of agricultural products is significant and may be caused by various factors, including insect feeding. It is assumed that storage pests may be responsible for losses of 30-40% (Abass *et al*., 2014; Kumar and Kalita, 2017). These are quantitative and qualitative losses related to grain mass loss or excrement and dead insects appearing in the stored product due to insect feeding (Nietupski *et al*., 2021). The occurrence of insect storage pests contributes to an increase in the temperature and humidity of grain, as a result of which unfavourable biochemical processes occur. Those may contribute to reducing nutrients in stored products and even create toxic substances such as mycotoxins (Padín *et al*., 2002).

One of the insects that is becoming an increasing threat to stored cereal products is the rice weevil (*Sitophilus oryzae* L.). It is a beetle from the weevil family (*Curculionidae*) with a 2.5 to 4.0 mm body length. It is brown with four orange spots on the cover and a head extended into a distinct snout (Longstaff and Evans, 1983; Rita Devi *et al*., 2017). A dozen or so years ago, it did not pose a threat due to too low air temperature, which prevented the increased development of this species. Due to global warming, this species has increasingly better conditions for development in the temperate climate zone and is becoming one of the potentially most dangerous insect storage pests (Kosewska *et al*., 2023).

The threat caused by insect storage pests is significant. Therefore, there is a constant need to protect stored products against destruction, which is particularly important in the increasing demand for food caused by the growth of the world's population (Dubey *et al*., 2008; Smith and Gregory, 2013). Because non-chemical methods do not guarantee

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**This work was supported by a research project of the University of Warmia and Mazury in Olsztyn (no. 30.610.010-110 and 30.610.011-110). The study was supported by the Polish National Science Centre under the project no. UMO-2021/41/N/NZ9/00364 (2022-2024).

complete protection of stored products against the feeding of storage pests, it is necessary to use chemical protection. This is contrary to consumers' feelings and the assumptions of the European Green Deal regarding food safety and its undesirable impact on the natural environment (Dal Bello *et al*., 2000; Klejdysz *et al*., 2017). Therefore, a strategic solution is to reduce pests' harmfulness through advancing plant cultivars and improving their genetic resistance.

Nevertheless, most of the breeding programs implemented so far have focused more on improving seed yield and quality than improving resistance to storage pests (Keneni *et al*., 2011; Kordan *et al*., 2023). The type of food has a significant impact on storage losses. It is one of the most essential biotic factors influencing the development of insects. In grains with lower nutritional value, insects want to avoid laying eggs and feeding on them to a lesser extent. Insects prefer grain with a higher protein content, which does not contain substances they cannot tolerate (Perisic *et al*., 2018). Unfortunately, in the case of storage pests, we cannot determine the characteristics of cereal grains that adversely affect these insects (Keneni *et al*., 2011; Caron *et al*., 2018). Recent achievements in metagenomic research have revealed that the microbiome inhabiting the digestive tract of insects is closely related to insect development, metabolic changes in the body, resistance to undesirable chemicals and pathogens, and is intensely involved in food digestion. Therefore, analysing the composition, activity and functions of the insect digestive tract microbiome is a bioindication method that can precisely determine the impact of factors. Therefore, the food's characteristics influence the development of insects and their intestinal microbiome (Jung *et al*., 2021; Brandon *et al*., 2018; Przemieniecki *et al*., 2020).

This study aimed to check whether different chemical properties of different cultivars of common wheat and barley grain, including antioxidant activity, cause insect feeding disorders and rearrangements in the structure of the rice weevil microbiome. The research has proposed some hypotheses regarding the natural resistance of cereal species and cultivars to *S. oryzae* feeding. According to the hypotheses, grains of diverse cereal species and cultivars have different natural resistance to *S. oryzae* feeding, which is connected to variations in their chemical composition. The chemical parameters of grains also impact the insect symbiotic microbiome composition and its activity and can modify the insect's digestive processes. The digestion of food in insects relies on enzymatic activity and symbiotic microbiome. With the hypotheses in mind, this study attempts to answer the following questions:

- What are the differences in the development of *S. oryzae* on selected wheat and barley cultivars?
- What are the differences in the susceptibility of cereal grain cultivars to degradation during storage pests?
- Which chemical features of wheat and barley grain correlate with their higher natural resistance to feeding *S. oryzae* and reduce storage losses?
- How do the chemical characteristics of cereals affect the population, metabolism, and microbiome of *S. oryzae*?

2. MATERIALS AND METHODS

2.1. Experiment with different feed variants

The specimen of the studied beetle species (*S. oryzae*) used in the experiment came from mass breeding conducted at the Department of Entomology, Phytopathology and Molecular Diagnostics at UWM in Olsztyn. Twenty specimens of 1-2 days old young adult beetles of *S. oryzae* were placed on 500 g of grain in 11 glass containers. For the experiment were used cultivars of wheat and barley. Three cultivars of wheat: Rusałka, Telimena and Impresja, all belonging to the technological group of cereals A, representing high-quality bread cultivars, and three cultivars of barley: Radek, Dante, classified as a fodder type (p) and Ismena, classified as brewing type (b) (COBORU). The selection of cultivars was based on preliminary research, which included ten wheat and ten barley cultivars, which are the subject of a separate study. Based on this study, which analysed the survival data of the rice weevil on the tested cultivars, we classified the Dante barley cultivar as resistant, the Ismena cultivar as having intermediate resistance, and the Radek cultivar as low-resistant. Regarding wheat cultivars, the Telimena cultivar exhibited the highest level of resistance, the Impresja cultivar displayed moderate resistance, and the Rusałka cultivar was low-resistant (personal communication). Insects without feed were a background. Each treatment was performed in three replications. The cultivation was carried out under controlled conditions of temperature (30°C) and humidity (70%) in a breeding chamber (Sanyo MLR – 350 H – Sanyo Electric Co., Ltd., Japan). The incubation parameters were correlated with the best habitat for developing these beetles (Golebiowska, 1969). The inlet was covered with a chiffon mesh for air access and ventilation. The sex of young *S. oryzae* beetles was determined by the proportion of the rostrum (Halstead, 1963). After 20 (first generation; –I) and 40 (second generation; –II), days of breading and starving adult insects were taken for further analysis. Preparation of digestive systems (10 pieces) will take place under the laminar chamber with the use of binoculars and sterile instruments. The collected insects were anaesthetised for 5 min at –80°C, and then surface sterilisation and homogenisation were performed according to the methodology described in the previous work (Kosewska *et al*., 2023).

2.2. Biochemical properties of insects

The biochemical activity and carbon source utilisation in imago digestive tracts were assessed through API ZYM and API 20 NE tests (Biomerieux, France). Approximately 100 mg samples were procured from each variant and then homogenised within vials containing glass beads and 1 ml of peptone water. These samples underwent homogenisation in a TissueLyser LT bead homogeniser (Qiagen, Germany) with 30 oscillations for a duration of 5 minutes. The resultant suspension was subsequently diluted at a 1:10 ratio in peptone water enriched with 1% TSB (Triptic soy broth, Merck, Germany) and then incubated at 30°C for 4 h before being employed in the API tests, following the manufacturer's guidelines. The biochemical and carbon source tests were further incubated at 30°C for 24 h (Przemieniecki *et al*., 2022).

2.3. DNA isolation and molecular analyses

Genetic material was isolated using commercial isolation kits Soil Purification Kit (EURx). Sequencing was performed based on the "16S Barcoding Kit 1-24 (SQK-16S024)". Bacterial 16S rRNA amplification was performed using 24 barcoding primers and HiFi ToughMix repliQa polymerase (Quantabio USA, MA). The library construction and pooling steps were performed according to the instructions provided by the polymerase supplier ("Rapid 16S Metagenomic Library Preparation for Oxford Nanopore Technologies (ONT)® Platform"; www.quantabio.com/product/repliqa-hifi-toughmix/). Sequencing was performed on a flongle flow cell using MinKnown software. The obtained data (~62,000 reads) were analysed by the EPI2ME platform (Metrichor™ Ltd.) using WIMP (What's In My Pot) workflows.

2.4. Chemical analyses of grain

Dry mass was determined using the drying method. It involves drying a crushed 5 g sample at 105°C for 5-8 h until a constant mass is obtained. Then, the lack of change in the mass of the sample, stored in the desiccator until reaching ambient temperature, was confirmed three times. Raw ash was determined by burning an air-dry sample, 5 g weighed, in a muffle furnace at a temperature of 500-550°C for 5-6 h. The total protein content was determined based on the total nitrogen (N) determined by the Kjeldahl method and then on its conversion into total protein (N×6.25), following the Standard: PN-ISO 5983.2000. Crude fat was extracted using a Soxhlet extractor, following standard PN-ISO 6492: 2005 procedures. A 1 g portion of air-dried and homogenised whole

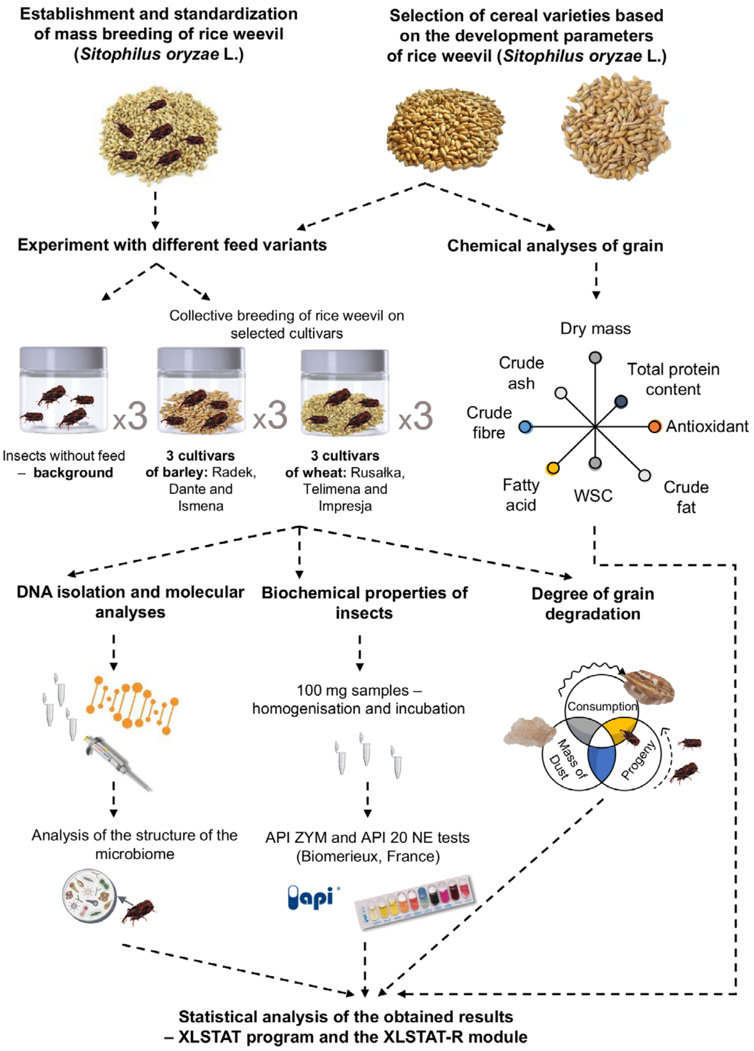

Fig. 1. Scheme of the experiment.

grain samples underwent extraction with petroleum ether in a Soxhlet extractor for 6-8 hours until all fat was removed. The ether was then evaporated from the extract in the same apparatus, and the resulting crude fat residue (ether extract) was dried in an oven and weighed. To determine the fatty acid composition, fatty acid methyl esters were prepared using a modified Peisker method (methanol: chloroform: concentrated sulfuric acid in a 100:100:1, v/v ratio) (Żegarska *et al*., 1991). Gas chromatography, utilising a Varian CP-3800 gas chromatograph with a flame ionisation detector (FID), a 50 m long capillary column (internal diameter: 0.25 mm, film thickness: 0.25 μm), and a 50:1 split dispenser, was employed for the separation and determination of fatty acids. A 1 μl sample was used, with the following operational conditions: detector temperature at 250°C, injector temperature at 225°C, column temperature at 200°C, and helium as the carrier gas (1.2 ml min^{-1} flow). The study utilised individual standards of fatty acid methyl esters (Sigma-Aldrich). The relative content of fatty acids was expressed as a percentage of the total area of all fatty acid peaks in the sample. The anthrone method determined water-soluble sugars – WSC (Thomas, 1977). The crude fibre was determined by the classic Henneberg-Stohmann method for determining the raw fibre content of feed following the PN–EN ISO 6865.2002 standard**.** The antioxidant capacity of samples was determined according to the Benzie and Strain method with some modifications (Benzie and Strain, 1996). The FRAP reagent was prepared by mixing 25 ml of 0.3 M acetate buffer, 2.5 ml of 10 mM TPTZ (2,4,6-tripyridyl-s-tiazine) solution and 2.5 ml of 20 mM $FeCl_3$ $6H_2O$ solution. Then, the mixture was incubated for 10 min in a dark place before use. Ascorbic acid was used as the standard, and distilled water was used as the blank control. A properly diluted sample or standard (0.1 ml) was added to a mixture of 4.0 ml of FRAP reagent. The mixture was incubated for 10 min in the dark, and the absorbance was measured at 593 nm against a blank prepared using distilled water. To prepare the calibration curve, the standards of ascorbic acid were used in the range from 0.01 to 0.2 mg ml^{-1}.

2.5. Statistical calculations

Obtained data from insect biometrics and grain composition after insect feeding were tested firstly for normality of distribution (Shapiro-Wilk test). Then, differences between treatments were determined using Kruskal-Walli's method (Dunn's test). For biometrics, grain chemistry, fatty acids biochemical parameters and bacteriobiomes, dissimilarity analysis was performed based on the Bray-Curtis matrix, and then dendrograms were created using the Ward method. The bacterial community was shown as a percentage abundance. The domination classes were determined according to previous work (Przemieniecki *et al*. 2021), where for more than 10% – Eudominant, 5.01-10% – Dominant, 2.01-5% – Subdominant, 1.1-2.0% – Rare, and less than 1% – Occasional, respectively. The differences between bacteriobiomes were determined using PERMANOVA (Adonis ANOVA). Principal component analysis (PCA) based on the Pearson correlation matrix was performed to determine the relationships between all parameters obtained in the study. Features whose vectors reached the maximum value for precepts other than F1 or F2 were removed from the biplots. Statistical analyses were performed in the XLSTAT program and the XLSTAT-R module (Lumivero, 2023). A detailed diagram of the experiment is presented on Fig. 1.

3. RESULTS

Based on the results of the analysis of variance (Table 1), it was observed that in the case of barley, the Ismena cultivar was more willingly consumed by the rice weevil and was characterised by a significantly higher content of crude ash, crude fat and antioxidants compared to the Dante cultivar. The Radek cultivar had substantially higher total protein content. However, in most cases, it was characterised by an intermediate value of the tested features, which did not differ from the other two cultivars. In the wheat case, the grain's chemical parameters differed significantly. The most significant difference was observed in the case of WSC, which was twice as high in the Rusałka cultivar compared to Impresja.

Table 1. Insect biometric results and grain composition characteristics of the cultivars used in the experiment

Species	Cultivar	Con (g)	MD (g)	NOG	MI (g)	DW (%)	CA (%)	TP (%)	CFa (%)	CFi (%)	WSC (%)	AO*
Barley	Ismena	4.145 a	0.445	228	0.376 a	89.08 ab	2.25 a	10.30 b	2.2 a	4.55 b	2.74 ab	0.177 a
	Radek	3.218 ab	1.115	198	0.295 ab	88.83 b	2.20 ab	10.60 a	1.9 ab	4.85 ab	2.29 b	0.139 b
	Dante	2.342 b	0.220	163	0.233 b	89.14 a	2.15 b	10.30 b	1.8 b	5.00 a	3.62 a	0.126 b
	p-value	0.035	0.369	0.497	0.007	0.004	0.004	0.036	0.003	0.003	0.004	0.002
Wheat	Telimena	2.791 a	0.207	232	0.289	89.11 ab	1.85 a	14.95 a	1.75 a	2.75 a	3.10 ab	0.024
	Impresja	2.316 a	0.199	211	0.285	89.35 a	1.75 b	12.00 b	1.75 a	2.65 b	2.59 b	0.020
	Rusałka	1.845 b	0.146	152	0.195	89.09 b	1.75 b	13.15 ab	1.35 b	2.65 b	4.50 a	0.023
	p-value	0.048	0.686	0.620	0.632	0.004	0.034	0.004	0.035	0.035	0.004	0.102

Con – Consumption, MD – Mass of Dust, NOG – number of offspring generations, MI – Mass of Imago, DW – dry weight, CA – crude ash, TP – total protein, CFa – crude fat, CFi – crude fibres, WSC – water-soluble carbohydrates, AO – antioxidants, *– concentration of antioxidants in ascorbic acid equivalents.

The Telimena and Impresja cultivars had the highest insect consumption, a significantly higher imago number, and crude fat content than the Rusałka cultivar.

Analysing dendrograms showing the general diversity of the examined cereal varieties depending on the groups of tested characteristics (Fig. 2), two different groups were observed for biometric traits. The first one included the Rusałka and Dante cultivars. The second group was formed by Telimena, Ismena, Impresja and Radek cultivars. In terms of chemical characteristics of the grain, two groups were formed: the first included the barley cultivars: Dante, Radek and Ismena. The second group included the Telimena, Rusałka and Impresja wheat cultivars. The tested cultivars were also characterised by three different fatty acid profile groups: the first group included the Impresja, Rusałka, and Telimena cultivars, and the second group included the Radek and Ismena cultivars, while the Dante cultivar has an intermediate profile between the two previously mentioned groups. The first group, in terms of biochemical characteristics of microorganisms inhabiting the digestive system of insects, was formed by the Ismena, Rusałka and Dante cultivars. The second group included the Telimena and Impresja cultivars, while in the Radek cultivar, the biochemical potential differed from each.

The PCA ordination charts (Figs 3 and 4) present the results of the correlation between parameters describing the development of *S. oryzae* and selected factors related to the chemical composition of wheat and barley grains, the microbiome of the beetle's digestive tract and its enzymatic activity. In the case of wheat, a large variability of the characteristics of the tested cultivars was observed in the experiment. The cultivar resistant to feeding by *S. oryzae* – Telimena was placed on the PCA plot in the upper right quadrant of the plot (Fig. 3). The high resistance of this cultivar (low number of the progeny of *S. oryzae*)

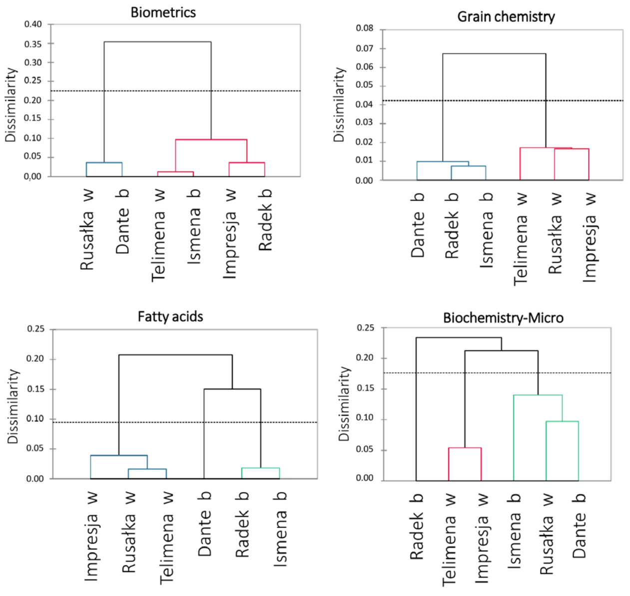

Fig. 2. Dendrograms showing the general diversity of the examined cereal cultivars depending on the groups of examined characteristics; the results were prepared based on the Bray-Curtis dissimilarity matrix (abbreviations: w – wheat, b – barley).

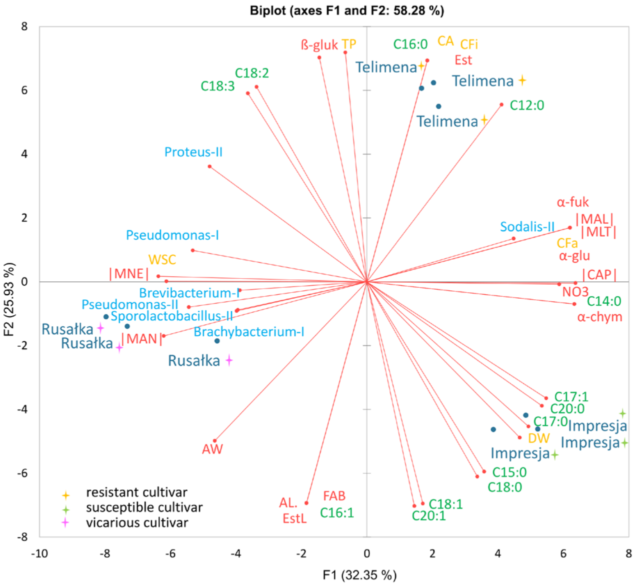

Fig. 3. Relationships between diet (different cultivars of wheat) and enzyme activity in the gastrointestinal tract, grain chemistry and bacterial community determined in PCA (abbreviations: β-gluc – β-glucosidase, AW – valine arylamidase, MNE – D-mannose utilization, MAN – utilization D-mannitol, AL – Leucine arylamidase, EstL – Esterase Lipase (C 8), FAB – Naphthyl-AS-BI phosphohydrolase, α-chym – α-chymotrypsin, NO_3 – nitrate reduction, CAP – decanoic acid utilization, α-glu – α-glucosidase, MLT – malate utilization, MAL – D-maltose utilization, α-fuk – α-fucosidase, Est – esterase, TP – total protein, CA – crude ash, CFa – crude fat, CFi – crude fibres, DW – dry weight, WSC – water-soluble carbohydrates).

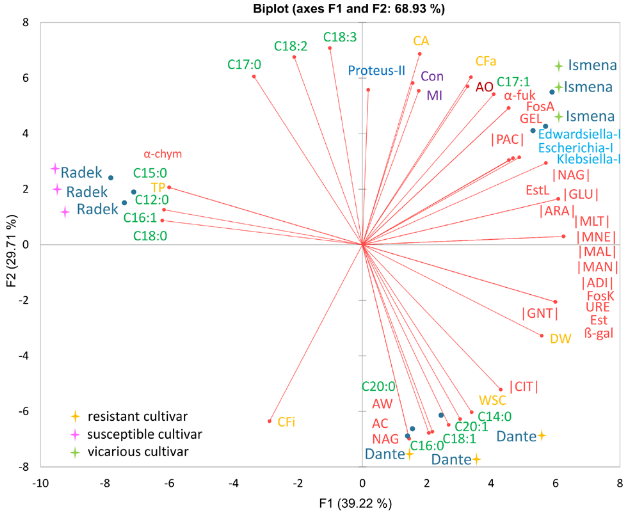

Fig. 4. Relationships between diet (different cultivars of barley) and enzyme activity in the gastrointestinal tract, grain chemistry and bacterial community determined in PCA (abbreviations: α-fuc – α-fucosidase, FosA – Alkaline phosphatase, GEL – protease hydrolysis, |PAC| – utilization phenylacetic acid, |NAG| – N-acetyl-glucosamine utilization, EstL – Esterase Lipase (C 8), |GLU| – D-glucose utilization, |ARA| – L-arabionza utilization, |MLT| – malate utilization, |MNE | – utilization of D-mannose, |MAL| – utilization of D-maltose, |MAN| – utilization of D-mannitol, |ADI| – utilization of adipic acid, FosK – Acid phosphatase, URE – urease activity, Est – Esterase (C 4), β-gal – ß-galactosidase, |GNT| – potassium gluconate utilization, |CIT| – trisodium citrate utilization, α-chym – α-chymotrypsin, NAG – N-acetyl-ß-glucosaminidase, AW – Valine arylamidase, AC – Cystine arylamidase, WSC – water-soluble carbohydrates, DW – dry weight, CA – crude ash, CFa – crude fat, CFi – crude fibres, TP – total protein, Con – consumption, MI – mass of imago).

is associated with a high content of fatty acids (C16:0, C12:0) and higher values of traits describing the content of fibre, crude ash and esterase. The Impresja cultivar was characterised by high contents of seven fatty acids C17:1, C20:0, C17:0, C15:0, C18:0, C18:1, C20:1. For the Rusałka cultivar, characterised by low natural resistance to *S. oryzae* feeding, high abundances of *Pseudomonas*-II, *Sporolactobacillus*-II, *Brachybacterium*-I, D-mannitol utilization, D-mannose utilization and soluble sugars were found. It should be noted that other carbon sources (malate utilization, D-maltose utilization, decanoic acid utilization), nitrate reduction capacity, α-chymotrypsin and α-glucosidase were correlated with a higher proportion of *Sodalis*-II and were characteristic of the Telimena and Impresja cultivars, but not for a change, Rusałka. In the case of barley, a significant difference in the characteristics of the examined cultivars was also observed in the experiment (Fig. 4). The least resistant cultivar, Radek, was characterised by high contents of four fatty acids: C15:0, C12:0, C16:1, C18:0, total protein, and α-chymotrypsin. The Ismena cultivar, as a variety with intermediate resistance, had high values of traits, *i.e.*, raw ash, Imago mass, loss of grain mass, crude fat, antioxidant content, C17:1 fatty acid, α-fucosidase, alkaline phosphatase, protease, *Edwardsiella*-I, *Escherichia*-I, *Klebsiella*-I, esterases (C8) and the ability to utilise 9 out of 14 analysed carbon sources (Fig. 4, Table 1). The Dante cultivar (resistant cultivar) was characterised by high trait values, *i.e.*, water-soluble carbohydrates, five fatty acids C16:0, C18:1, C20:1, C14:0, C20:0, valine arylamidase, cystine arylamidase, N-acetyl-*ß* -glucosaminidase, trisodium citrate utilization.

Analysing the percentage of individual types of bacteria identified in the digestive tract of the rice weevil feeding on various wheat varieties (Table 2), the genus *Sodalis* dominated almost all samples. The exception was Rusałka I-2, where the genera *Staphylococcus* and *Brevibacterium* dominated. In the first generation, co-dominance of *Sodalis* sp. with the genus *Staphylococcus* in the Telimena variant and *Mammaliicoccus* in the Impresja-3 sample was observed. In the second generation, the disappearance of both co-dominant taxa and increased share of the genera *Escherichia* and *Klebsiella* were observed. In the case of barley (Table 3), all variants were dominated by *Sodalis* sp. Only in the case of Ismena-3 co-dominance with *Staphylococcus* sp. was observed in the first generation. This genus disappeared in the second descendant generation of the insect. Apart from Radek of the first generation, a high proportion of *Klebsiella* sp. was also observed in all samples.

Three distinct groups were observed in the dendrogram showing the diversity between the microbiomes of individual samples (Fig. 5). The first group was created from similar wheat-I microbiomes, *i.e.*, two batches of the Telimena and Rusałka cultivars and three batches of the Impresja cultivar. The second group was formed by the cultivars on which the offspring generation of the rice weevil fed, *i.e.*, wheat cultivars Telimena, Impresja 3 batches, Rusałka 1 batch and barley cultivars Ismena 2 batches, Dante 2 batches, Radek 1 batch. The third group was formed by the cultivars on which the parent generation of the rice weevil fed, *i.e.*, wheat cultivars Rusałka 1 batch, Telimena 1 batch, Ismena 3 batches, and Radek 1 batch. Of the cultivars on which

Table 2. Percentage of bacterial genera identified in the digestive tract of a rice weevil feeding on different wheat cultivars

Generation	I									II								
Cultivars	Rusałka			Telimena			Impresja			Rusałka			Telimena			Impresja		
genus / batch	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Sodalis	90.07	2.35	86.62	16.74	68.75	86.99	93.07	90.18	31.60	86.32	83.87	73.36	88.01	87.76	87.07	87.55	86.65	87.32
Staphylococcus	3.52	66.56	5.81	77.38	24.64	2.23	1.41	4.18	5.63	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.11	0.03
Mammaliicoccus	0.62	6.19	0.24	0.46	0.18	0.19	0.08	0.19	60.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Escherichia	1.20	0.03	1.09	0.19	0.80	1.20	1.14	1.10	0.44	3.38	2.34	4.25	2.26	2.40	2.61	2.81	2.21	2.85
Klebsiella	0.51	0.06	0.44	0.07	0.39	0.55	0.54	0.52	0.18	1.32	1.53	2.70	1.85	1.82	1.92	1.79	2.15	2.15
Brevibacterium	0.39	10.39	0.64	0.86	0.79	0.26	0.16	0.36	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Salmonella	0.28	0.02	0.26	0.03	0.29	0.44	0.38	0.36	0.17	0.88	0.97	1.54	0.55	0.66	1.09	1.08	0.84	0.83
Edwardsiella	0.28	0.01	0.19	0.04	0.19	0.24	0.25	0.28	0.09	1.03	0.65	0.39	0.34	0.77	0.54	0.82	0.63	0.69
Enterobacter	0.34	0.03	0.21	0.04	0.19	0.49	0.35	0.24	0.11	0.29	0.81	1.16	0.07	0.61	0.69	0.47	0.63	0.52
Vibrio	0.10	0.02	0.19	0.03	0.18	0.36	0.10	0.12	0.04	0.29	0.65	1.16	0.48	0.52	0.51	0.53	0.58	0.90
Kocuria	0.02	5.97	0.34	0.81	0.66	0.12	0.06	0.20	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacillus	0.15	1.95	0.13	1.00	0.40	0.03	0.02	0.13	0.31	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brachybacterium	0.15	1.09	0.02	0.01	0.02	0.03	0.03	0.06	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Saliniradius	0.00	0.00	0.06	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.04	1.16	0.00	0.00	0.00	0.00	0.00	0.00
Sporolactobacillus	0.00	0.00	0.04	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.16	1.16	0.00	0.00	0.00	0.00	0.00	0.00
	Eudominant			Dominant			Subdominant			Occasional			Casual					
	>10.01			5.1-10.0%			2.1 – 5.0%			1.1-2.0%			<1.0 %					

Table 3. Percentage of bacterial genera identified in the digestive tract of a rice weevil feeding on different barley cultivars

Generation	I									II								
Cultivars	Radek			Dante			Ismena			Radek			Dante			Ismena		
genus / batch	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Sodalis	90.90	87.40	82.60	90.50	84.20	87.40	84.40	76.10	63.40	86.20	87.30	87.00	87.70	88.10	87.40	88.20	86.70	87.60
Staphylococcus	3.39	5.82	1.71	4.00	8.09	0.72	1.50	1.51	18.7	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
Mammaliicoccus	0.35	0.57	3.83	0.17	0.56	0.18	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Escherichia	1.09	1.01	1.22	1.21	1.14	1.73	1.96	2.52	1.63	2.78	2.73	2.73	2.34	2.26	3.37	2.5	2.69	1.67
Klebsiella	0.48	0.45	0.49	0.48	0.42	1.73	2.19	1.76	2.44	2.10	1.57	1.77	2.13	1.75	1.76	1.71	1.75	2.38
Brevibacterium	0.36	0.61	3.83	0.31	1.33	0.07	0.81	1.51	0.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Salmonella	0.37	0.30	0.24	0.28	0.37	0.90	0.92	0.50	0.00	0.91	0.65	0.99	0.95	0.80	0.50	0.73	0.72	1.19
Edwardsiella	0.25	0.26	0.24	0.16	0.25	0.79	1.15	0.76	0.81	1.00	0.78	0.67	0.76	0.54	0.77	0.70	0.98	0.24
Enterobacter	0.23	0.29	0.33	0.33	0.43	0.58	0.69	0.00	0.00	0.65	0.83	0.46	0.54	0.80	0.69	0.46	0.67	1.19
Proteus	0.19	0.18	0.16	0.20	0.10	0.25	0.23	1.26	0.81	0.54	0.65	0.57	0.51	0.40	0.34	0.73	0.54	0.71
Dickeya	0.08	0.09	0.00	0.06	0.07	0.43	0.58	1.26	0.00	0.11	0.31	0.50	0.35	0.29	0.23	0.37	0.13	0.00
Pseudomonas	0.03	0.03	0.08	0.03	0.03	0.18	0.00	0.50	3.25	0.07	0.07	0.07	0.06	0.03	0.04	0.09	0.13	0.00
Brachybacterium	0.05	0.11	1.79	0.04	0.24	0.00	0.00	0.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Latilactobacillus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Eudominant	Dominant	Subdominant	Occasional	Casual
>10.01	5.1-10.0%	2.1 – 5.0%	1.1-2.0%	<1.0 %

insects from the second generation fed, this group included the cultivar Rusałka 2 batches and 1 batch of the Ismena cultivar. There was also some background in this group.

4. DISCUSSION

The development of storage pests, including the number of offspring generation, is influenced by many biotic and abiotic factors (Perisic *et al*., 2018). Breeding insects in laboratory conditions allows for control of some of these factors, for example, by regulating temperature, air humidity and the number of individuals. Limiting the influence of environmental factors is crucial in studying insect feeding mechanisms.

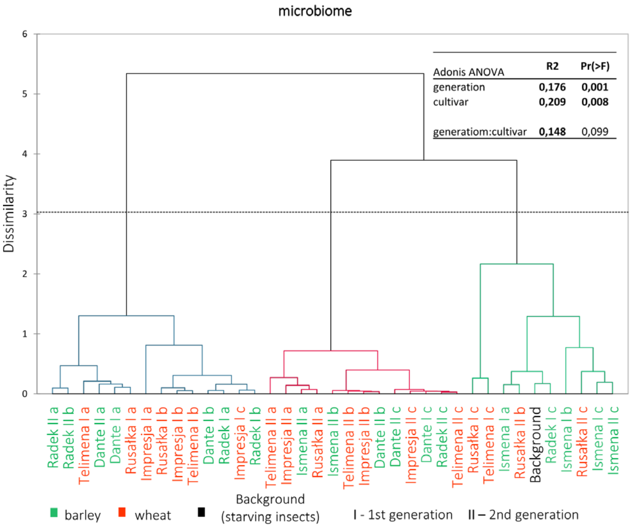

Fig. 5. Dendrogram showing the diversity between the microbiomes of individual samples; the results were prepared based on the Bray-Curtis difference matrix, Adonis ANOVA performed using the R package (Vegan).

Food is an important biotic factor that influences the development of storage insect pests. Boniecki *et al.* (2020) found that the development dynamics of *Sitophilus granarius* L. depend mainly on temperature, seed moisture and type of food. The chemical composition of individual cereal species and even varieties may differ significantly, resulting in increased attraction or deterrence of storage pests (Nawrot *et al*., 2006; Mebarkia *et al*., 2010). In the study by Laszczak-Dawid *et al*. (2011), it was found that in the case of the lesser grain borer (*Rhyzopertha dominica* F.), adults are more likely to feed on grain without covers, which is softer and more prominent in size. This results in easier access for the larvae of this species to food. Other researchers have reached the same conclusions, confirming that the hardness and size of grains have a crucial impact on resistance to feeding by insect storage pests (Bergvinson, 2014; Ahmed *et al*., 2020). As documented in the study of Greffeuille *et al*. (2006), grain hardness depends mainly on genetic factors. However, it may change due to agricultural practices and the variability of climatic conditions. It is particularly important that nitrogen fertilisation significantly impacts wheat's hardness, in accordance with the findings of Dziamba *et al*. (2001). The water content in the grain also plays an essential role in determining hardness. Increasing grain moisture leads to significant changes in its mechanical properties, including a decrease in hardness. Moreover, repeated moistening and drying of grains in field conditions may lead to numerous transverse cracks in the endosperm, decreasing grain hardness (Dziki *et al*., 2011). This factor may significantly increase the susceptibility of the raw material to feeding by storage insect pests. The insect is more willing to eat partially mechanically damaged food (Kavallieratos *et al*., 2012). Some researchers suggest a correlation between grain hardness and its protein content. However, there is evidence that grain hardness depends on the amount of protein and its composition and distribution in the grains (Mikulíková, 2007). This information can be translated into the results we obtained in our research. Insects were more likely to feed on barley cultivars with a lower total protein content than on the tested wheat cultivars and caused a more significant loss of grain weight. Accordingly, the grain weight loss in the Ismena cultivar was 4.145 g, in the Radek cultivar, 3.218 g, and in the Dante cultivar, 2.342 g. In the wheat cultivar variants, the loss was for cv. Telimena 2,791, for cv. Impression 2.316 g, and for cv. Rusałka 1.845 g.

The sugar content and type may influence the development of storage pests, including the rice weevil. Chippendale (1972), in his research examining the effect of carbohydrates in the weevil's diet on its survival, found that the rice weevil developed properly on a diet enriched with starch, dextrins, amylopectin and glycogen while eating food containing amylose, cellulose, inulin, glucose and maltose disappeared. Further research showed that dietary carbohydrates that enabled proper development were nutritional stimulants, while those that caused premature death acted as deterrents to feeding or did not provide adequate physical substrates for feeding. Our research on wheat and barley revealed significant differences in the chemical composition of grain between the varieties tested. Of particular interest is the difference in water-soluble carbohydrates (WSC). In the case of wheat varieties, we observed a significantly higher WSC content than barley varieties, which may affect the availability of sugars for storage pests. The WSC content in wheat and barley may be related to the amylose content. In the case of barley, which often has a higher amylose content than wheat (Li *et al*., 2021; Javid Iqbal *et al*., 2022), differences in WSC content may be due to differences in starch composition, including the ratio of amylose to amylopectin. WSC content may influence the availability of sugars in grains and potentially affect the attractiveness of grains as food for storage pests. Therefore, research on the relationship between WSC content and the amylose to amylopectin ratio in both cereal species may provide important information regarding the preferences and development of these pests.

Mebarkia *et al*. (2010) indicate that the main factor influencing the grain weevil's choice of food is the wheat grain's protein content. According to the observations of Kordan *et al*. (2023), the analysis of the relationship between the intensity of weevil development and the total protein content in the grain of ten selected varieties showed a negative correlation. According to these authors, cultivars considered more resistant to feeding by *S. granarius* are characterised by a higher total protein content. Our research also shows that the rice weevil develops much worse on wheat varieties, especially the resistant Telimena cultivar, with a higher protein content. The situation was different in the case of barley cultivars, where the least resistant cultivar, Radek, was characterised by high contents of four fatty acids, total protein, and an increase of activity of α-chymotrypsin in the insect's gut. This may suggest that the qualitative composition of protein, *i.e.*, the content of individual protein fractions, is as important as its overall quantity (Nietupski *et al*., 2006). In research on the relationship between the diverse chemical composition of proteins in cereal grains and the development of storage pests, Nietupski *et al*. (2006) showed a correlation between the content of individual protein fractions and the feeding of *S. granarius*. Additionally, they found that specific protein fractions, such as albumins, globulins and glutenins, may hinder the development of the grain weevil.

According to Majd-Marani *et al*. (2023), the content of phenols and lipids in the food eaten by insects may also significantly impact development. Significant relationships between the development time, longevity, and fecundity of *S. oryzae* and the content of phenols in the tested grains reveal this factor's important role in the rice weevil's life cycle. During our research, we noticed that the feeding activity of *S. oryzae* tends to increase in plant cultivars with low levels of antioxidant activity. Concerning cereal cultivars showing higher antioxidant activity, we observed fewer adult insects. However, this did not result in a decrease in feeding intensity. It is worth noting, however, that the correlation between these parameters and the level of antioxidant activity did not show a significant correlation.

Based on the results of previous scientific studies, it appears that the impact of the content of fatty acids in grains on the development of insects, available information suggests that the fat content in most cereal plants plays an important role in shaping the storage stability of cereal products and may influence the feeding behaviour of *S. granarius* (Liu, 2011). The research of Majd-Marani *et al*. (2023) correlation analysis showed that the content of glycogen and lipids in *S. oryzae* pupae has significant relationships with development time, fecundity, and longevity of adults. In research conducted by Nietupski *et al*. (2021), no significant relationships were revealed between the level of individual fatty acids in grains of various wheat varieties and parameters related to the development of the grain weevil. However, a positive correlation was observed between the total content of these substances in grains and the number of grain weevil offspring. This indicates the possible influence of fatty acids present in plants on the reproduction process of these insects. Nevertheless, it is possible to find information on the impact of individual fatty acids on storage pests and their development. Research by Kordan *et al*. (2019) indicate that godoleic acid (C 20:1) strongly attracts *S. granarius*. A similar relationship was observed in our studies, where a high C 20:1 content was correlated with wheat cultivars less resistant to rice weevil feeding, *i.e.*, Impresja and Rusałka cultivars (Fig. 3). It should therefore be noted that examining the quantitative and qualitative composition of fatty acids with data on the development of the rice weevil may indicate cereal varieties characterised by more excellent resistance to rice weevil feeding.

Regarding the influence of cereal cultivar characteristics on the microbiome, it was observed that both the variety and the species significantly determined ($p<0.01$) the microbiota structure within a generation. Nevertheless, the determination index was low ($R^2<0.5$) regardless of the factor, which indicates a significant influence of factors other than those used in the experiment and natural randomness resulting from the competition of individual taxa for participation in the bacteriome.

In this study, we observed that cultivars and generation influence the rearrangement of microbiomes. Despite this, random factors and factors not included in this study influenced changes in the taxonomic structure of the bacteriome, as indicated by the low R^2 value.

An important observation is changes in the abundance of certain types. The *Sodalis* genus consistently dominated the insect bacteriomes, regardless of the factor. This symbiont is essential for the development of adult individuals (Heddi *et al*., 1998). Attempting to compete for the bacteriomes, especially in wheat, the *Staphylococcus* genus is responsible for insect infections. Nevertheless, the insect's immune system, including antimicrobial peptides (AMP) and phagocytosis, allows for the decrease of the pathogenicity of undesirable microbiota (El Shazely *et al*., 2019). The results indicate that despite the high presence of *Staphylococcus* and likely breaking of resistance in some insects in the first generation, individuals of the second generation are almost entirely free of this type of microorganism.

In the case of the *Escherichia* and *Klebsiella* genera, their presence is confirmed regardless of the generation, which is natural for insects (Heddi and Ardon, 2005; Wang and Rozen, 2018). However, their presence and stability increase are noticeable in the second generation. It should be noted that the samples of the Ismena cultivar were most readily colonised, characterised by the best development of insects and the broadest spectrum of biochemical activity in the gut, correlated with the raw ash forming the base of mineral substances and raw fat. The activity of *Enterobacteriaceae* bacteria tends to be directed towards facultative symbiosis, fermentative processes resulting in, for example, vitamin production in the digestive system (Gupta and Nair, 2020; Serrato-Salas and Gendrin, 2023).

In our study, it was also observed that all batches associated with the Telimena and Impresja cultivars, which served as the diet for the parental generation of insects, exhibited the formation of distinct microbiomes compared to those of the starving individuals, *i.e.*, those living in the most adverse conditions for their development. The second group primarily comprised samples from insects of the second generation, characterised by a substantial similarity in their microbiome composition. This observation suggests the stabilisation and development of a specialised bacteriome structure. However, it is essential to note that the absence of microbiome clustering based on varieties (sample groups in different categories) indicates a significant influence of randomness, suggesting similar ecological conditions (Fig. 5).

Due to the significant impact of grain characteristics on microbiome rearrangement, unfortunately, symbiotic bacteria of the *Sodalis* genus proved to be resistant. This study has demonstrated that the traits determining the feeding habits of stored grain pests can vary considerably depending on the grain species. The gut microbiome underwent rearrangements depending on grain traits, indicating competition for an ecological niche within the bacteriome. Unfortunately, in the case of *Sodalis* sp., a symbiont essential for the development of the rice weevil, alternative methods that disrupt the functioning of symbionts need to be employed. Future research should focus on the development of antibiotic biopesticides, and the most promising direction for future studies could involve natural volatile organic compounds (VOCs) for fumigating storage facilities (Sidorova *et al*., 2022; Zhou and Jander, 2022).

5. CONCLUSIONS

The general chemical composition of cereal grains plays a crucial role in developing storage pests. The availability of various nutrients can affect the attractiveness of grains as food and the ability of insects to thrive. Understanding these relationships is important in the context of strategies to control storage pests in cereal crops.

Our research shows that the fatty acid profile plays an important role in varietal susceptibility. An important fatty acid that stimulates *S. oryzae* to feed is 20:1 (godoleic acid). However, the content of this fatty acid was negatively correlated with the development of the rice weevil on barley. This observation confirms that the cereal species' different features determine their susceptibility to rice weevil feeding. The role of antioxidants in the development of varietal resistance is of little importance and was observed only in the case of barley.

The structure of the *S. oryzae* microbiome is significantly influenced by both the species and the cultivar of grain on which the species feeds. However, depending on the grain type, other factors influence the structure of the microbiome. It should be noted that the differences between species were much more significant than between cultivars within a cereal species. Higher variability of the rice weevil between microbiome structures was observed in the first generation. The dominant genus in the gastrointestinal tract was *Sodalis* spp., with a high share of *Staphylococcus* spp. It was also observed that the fatty acid profile affects the microbiome and may contribute to its changes. In the second generation, bacteria of the *Staphylococcus* genus were displaced from the microbiome structure. The position of bacteria of the *Escherichia* and *Klebsiella* species strengthened, and at the same time, the weevil became more resistant to factors unfavourable for feeding. The obligate symbiont included in the *S. oryze* bacteriome, *i.e.*, *Sodalis* sp., almost always dominated, and no correlation was observed between species and variety factors of cereals and their abundance. A co-dominance of bacteria of the *Staphylococcus* genus was observed, which may contribute to the death of individuals. However, the functions of *Escherichia* and *Klebsiella* bacteria are not entirely clear; they may be natural intestinal microbiota, but their transmission to the next generation may occur from dead insects, on which these bacteria develop intensively.

In conclusion, the recommendations for integrated methods for protecting stored cereal grains indicate the need to reduce broad-spectrum pesticide use significantly. The use of insecticides raises some food safety concerns. Control of the rice weevil is strategically important as it is a dangerous storage pest. Once pesticide use is restricted, this will be a difficult task. Evidence of symbiotic interactions with microflora among some insects points to the need for significant research into the susceptibility of pests to gut microbial loss. This may lead to new perspectives in storage pest control strategies. Instead of non-selective insecticides in storage and transport facilities, non-toxic substances can be used to destroy the symbiont, preventing insect development. Biological advances in new cereal cultivars may lead to the natural production of bacteriostatic substances against *S. pierantonius*, moderating the problem of cereal storage losses caused by *S. oryzae*.

Conflicts of Interest: The Authors declare they have no conflict of interest.

ACKNOWLEDGMENTS

We would like to express our heartfelt thanks to Katarzyna Smołka from Fluentbe for her invaluable assistance in proofreading and linguistic correction of this manuscript.

FUNDING

The results presented in this paper were obtained from a comprehensive study financed by the University of Warmia and Mazury in Olsztyn, Faculty of Agriculture and Forestry, Department of Entomology, Phytopathology and Molecular Diagnostics.

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