

Susceptibility of naked oat cultivar seeds to mechanical damage

Research Article

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Received 13 February 2013; Accepted 05 August 2013

Abstract: Mechanical damage to seeds occurring during harvesting and threshing, especially in naked cultivars, is one of the main factors decreasing the size and quality of yield. The objective of this study was to assess the susceptibility of naked oat cultivars to mechanical damage, considering the biometric parameters of seeds determined on the basis of computer image analysis and weight tests. The testing was carried out on eight cultivars harvested between 2008 and 2010 at 15% moisture and threshed at either 1.6 or 2.4 m·s⁻¹ threshing drum speed. A 50% increase in the threshing speed caused an average 19% increase in the frequency and a 29% increase in the area of microdamage to seeds. There was a corresponding 4.1 mm² change in the microdamage area when using the threshing speed of 1.6 m·s⁻¹, and a 6.1 mm² change when using the threshing speed of 2.4 m·s⁻¹ was 53% and 68%, respectively, determined by a decreasing seed shape coefficient indicating seed elongation. The greatest resistance to mechanical damage was found on the Bullion cultivar, which was also characterized by the largest total projected area of seeds (7.14 mm²), as well as the greatest seed density (63.8 kg·hL⁻¹) and thousand kernel weight (TKW) (28.2 g).

Keywords: *Hulles oat • Mechanical microdamage • Shape coefficient • Computer image analysis*

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Abbreviations:

TKW - thousand kernel weight;
LTS - low threshing speed;
HTS - high threshing speed;
F_M - frequency of microdamage;
A_M - area of seed microdamage;
K_m - shape coefficient.

1. Introduction

The sowing material of new cultivars is considered to be a fundamental element of the continual biological progress. In the seed industry, the basic problems associated with the production of high quality cereal seed intended for sowing or consumption are susceptibility to mechanical damage [1] and preharvest sprouting [2,3]. Financial losses caused by these phenomena are difficult to assess since both

are strongly modified by the use of agrotechniques and the environment. However, some estimates have put the losses for producers during unfavorable years as much as 10-50% due to sprouting [4] and 50-90% due to mechanical damage [5]. In 2003, a report was published indicating that preharvest sprouting can occur in traditional oat cultivars [6]. According to this report, the sprouting seeds of husked oat show increased susceptibility to mechanical damage occurring during the husking process. However, little is known about the susceptibility to sprouting and mechanical damage in naked oat cultivars, which have lately been more and more commonly grown. The multi-flowered inflorescence, which is specific for naked oat, as well as long pedicels and weaker lignification of lemma and palea [7], all these factors may lead to varied susceptibility of seeds to mechanical damage and sprouting if the maturation process takes place under changing weather conditions. The more delicate structure of naked oat seeds, compared to traditional

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cultivars, is results from a lack of husks as well as protruding seed germs [8], which are more exposed to the action of static and dynamic forces. Doehlert and McMullen [6] report that the increased activity of alpha-amylase, which causes the decomposition of starch in oat seeds, is accompanied by a decrease in the concentration of beta-glucans, which are integral in determining the hardness of seed coats. In cultivars with increased susceptibility to sprouting, there is an increase in alpha-amylase activity during seed maturation, resulting in acceleration of starch hydrolysis [9]. As a consequence, the loosened structure of the endosperm negatively affects the filling and weight of seeds, which may also increase seed susceptibility to mechanical damage during harvest or threshing.

The aim of this research was to assess the susceptibility of naked oat cultivars to mechanical damage during threshing using on a variety of seed parameters determined using computer image analysis and weight tests.

2. Experimental Procedures

2.1 Plant material

The study was carried out on seeds from the following naked oat cultivars: Polar (HR Strzelce sp. z o.o., Poland), Cacko (HR Strzelce sp. z o.o., Poland), Abel (Selgen a.s., the Czech Republic), Avenida (Selgen a.s.u. Prestic, the Czech Republic), Bullion (IGER, United Kingdom), Izak (Selgen a.s., the Czech Republic), Pikant (Toft Planteforædning Roslev, Denmark), Saul (Selgen a.s., the Czech Republic). These cultivars were selected randomly from the Common Catalogue of Varieties of Agricultural Plant Species (CCA). Plant material was propagated in 2008-2010 during field experiments carried out at the Experimental Station of the Department of Plant Breeding and Seed Science in Prusy near Krakow (N 50°07'03" and E 20°05'13").

Plants were harvested when the seed moisture content was 14.5–16.2%. Directly after harvesting, the panicles were threshed using a laboratory threshing machine and speeds of either 1.6 m·s⁻¹ (low threshing speed; LTS) or 2.4 m·s⁻¹ (high threshing speed; HTS). Plant material was cleaned using a Petkus Labor Windsichter K293 pneumatic separator. Seeds were then sieved through a 1.75 mm diameter slotted screen. The initial characterization of the obtained material was done following ISTA regulations [10]. We determined seed moisture content using the dryer method, the weight of 1,000 seeds (TKW), and seed density (hectoliter weight).

2.2 Mechanical damage assessment and measurements

Mechanical damage was assessed using the computer image analysis. The samples of each combination of threshing speed and cultivar, containing 200 seeds (four replications of 50 seeds each) without visible macrodamage were immersed individually in 2% Lugol's solution for 15 min. After rinsing and drying on a sheet of blotting paper, the seeds were laid out with their tops towards the scanning surface. Digital images were obtained using the scanner Epson Perfection V750 Pro, and analysis of the images was done using the software Multiscan 12.07. The frequency of microdamage (F_M) was measured as:

$$F_M = \frac{L_u}{L_z}$$

where:

L_u – number of the detected microcracks

L_z – number of seeds

The projected area of seed microdamage (A_M) was determined as the average area of microdamage per one seed. The seed shape coefficient (K_m) was determined from values of the total projected area of seeds, seed length, width and perimeter, obtained from samples experiencing low threshing speed, and was measured following Grochowicz [11]:

$$K_m = \frac{b}{c}$$

where:

b – seed width (mm)

c – seed length (mm)

2.3 Statistical analysis

Two- and three-factor analyses of variance (independent variables) were done, in which the analyzed factors were years, threshing speed and cultivars. In the case of a random variable, *i.e.* harvest years, the testing was performed according to the mixed model. The share of the selected sources of variation in the total variability of the tested properties was determined on the basis of the estimated variance components [12] and their percentage was given. Where the values were expressed as percentages, the Bliss transformation $y = \arcsin \sqrt{x}$ was used. Alpha significance thresholds for ANOVA's was $P \leq 0.05$ and $P \leq 0.01$ marked as */ or **/, respectively. Confidence intervals for each factor were calculated using Fisher's Least Significant Difference Test (LSD). The evaluation of interrelationships between the properties was carried out on the basis

of the significance of Pearson's linear correlation coefficients and linear regression analysis. All statistical tests were conducted using Statistica 9.1 (version 9.1, www.statsoft.com.).

3. Results and Discussion

3.1 Frequency and area of microdamage

Susceptibility of seeds to mechanical damage is determined by genetic factors, climatic conditions during maturation, as well as harvest and storage conditions [13]. According to Grundas *et al.* [13] the properties of biological materials are formed by temperature and water content. Sudden increases in tissue moisture content may result decrease a seeds' resistance to internal damage (including microdamage). The plant material exhibit considerable variability in construct. Thus, the mechanical properties of seeds depend on their structure and interactions with shape and size, moisture content, state of the surface, temperature and external factors [14].

The three-way ANOVA performed to estimate the frequency (F_M), as well as the area of microdamage (A_M) for naked oat seeds showed a highly significant effect of the tested sources of variation, *i.e.* years, threshing method and cultivars. The same effect was found for the majority of the interactions occurring between them. The frequency of seeds with microdamage was most affected by harvest year, which accounted for 34% of the variation (Table 1). The share of cultivars in the total variability of frequency and area of microdamage did not exceed 4% and 3%, respectively, which indicate similar reaction of tested cultivars to the experimental conditions.

With regards to the area of microdamage, most variability was explained by threshing speed (38%). Harvesting at the optimal moisture content (14-16%) established in earlier studies [15] made it possible to compare the genotypic differences among naked oat cultivars. The results indicate that the greatest average area (0.25 mm²), and simultaneously the lowest frequency of seed microdamage for all the naked oat cultivars was in 2008. During this year, seed maturation in July was the most intense (143 mm) compared with other years. Rainfall also occurred, and the average temperature was 20.8°C (Figure 1).

In the same year also the smallest increase in F_M and A_M values when using the high threshing speed (2.4 m·s⁻¹) was noted (Figure 2).

According to Doehlert and McMullen [16], the most important period for oat development is between heading and harvesting, and the best conditions occur in years

with both moderate rainfall and low temperature. In the analyzed three-year period, such conditions occurred in 2009, where at the end of July and the beginning of August, total rainfall was below 72 mm and temperature was 19.1°C. In that year, the area of microdamage when using low threshing speed was the smallest (0.08 mm²), and the high threshing speed resulted in a significant increase in both F_M and A_M (24% and 41% increase, respectively). Low rainfall during the seed-filling period likely contributed to the increased susceptibility of seeds to damage, which has also been reported by Doehlert *et al.* [17], who studied damage occurring when harvesting traditional oat cultivars. According to Grundas *et al.* [18], dry seeds moistened by dew causes an increase in the moisture gradient and, consequently, the occurrence of critical stresses in the endosperm structure, which lead to transverse cracks in that tissue.

Source of variability	df	Frequency of microdamage	Area of microdamage
Years (A)	2	34.4**	35.9**
Threshing method (B)	1	26.9**	38.2**
Cultivars (C)	7	4.1**	2.8**
AxB	2	2.2**	0.6 ^{ns}
AxC	14	14.8**	2.9**
BxC	7	1.1**	1.4*
AxBxC	14	10.4**	7.1**
Error	144	6.2	11.2

Table 1. Significance of variation and percentage of the components of variance for the frequency and area of microdamage in naked oat seeds.

* - significant at $P \leq 0.05$; ** - significant at $P \leq 0.01$; ns – insignificant

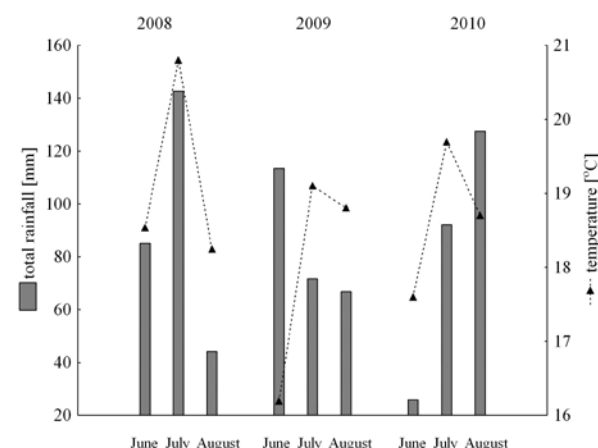


Figure 1. Total rainfall and average temperature in successive months from heading to harvesting of naked oat cultivars in the years 2008-2010.

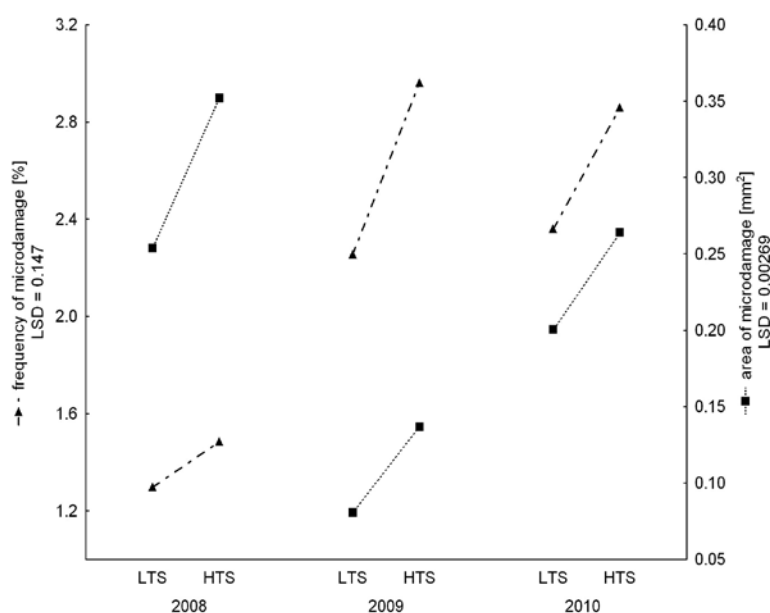


Figure 2. The frequency and area of microdamage per seed for naked oat cultivars using low (LTS) and high (HTS) threshing speeds in the years 2008-2010.

A seed contains unique, visco-elastic material, the physical properties of which indicate great ability to reverse deformations within a specified moisture content range directly after removing the loading factor [19]. Our results suggest there are significant differences in these properties among naked oat cultivars, which results in varied susceptibility of seeds to mechanical damage. The greatest average frequency of microdamage was in the cultivar Avenuda (Table 2).

At the same time Avenuda and Cacko seeds had the greatest microdamage surface area (0.25 mm^2) as well as the greatest increase in this parameter (0.14 and 0.11 mm^2 increase, respectively) at the threshing speed of $2.4 \text{ m} \cdot \text{s}^{-1}$ (Table 3).

According to Greffeuille *et al.* [20], the resistance of seeds to mechanical damage depends on their hardness, which is largely determined by the endosperm properties. Maghirang and Dowell [21] suggest that moisture content can considerably modify the plasticity and resistance of seed coats to mechanical damage. In the case of cereal seeds, there is range of optimal moisture contents that produce the lowest loading forces and highest seed. Seed hardness is affected by the endosperm structure, especially with the continuity of the protein matrix in which starch grains are immersed. Endosperms are highly affected by the environmental conditions experienced during seed development [22,23]. Stenvert and Kingswood [23], carrying studies on wheat, concluded that the endosperms of softer seeds are flourier, with loose, irregular arrangements of starch grains and proteins. As a result seeds are filled with less

nutritive tissue. This may be caused by external forces, which decrease the mechanical strength of seeds and cause tissue breakage (including microdamage). We observed a significantly lower share of F_M in the Pikant, Izak and Bullion cultivars, despite different susceptibilities of their seeds to increasing dynamic loading (HTS). The smallest changes in A_M at various threshing speeds were observed in Polar and Abel, although the lowest average value ($<0.20 \text{ mm}^2$) was found in Saul and Bullion seeds. Engleson and Fulcher [24], studied the mechanical properties of seeds and oat husks and concluded that acids and their derivatives present in seed coats may increase (e.g. ferulic acid) or decrease (e.g. syringic acid) seed stiffness, which influences their susceptibility to mechanical damage during husking. According to Engleson and Fulcher [24], beta-glucans and proteins are the main polymers in plant cell walls, which make them hard and strong. In traditional oat cultivars, Doehlert *et al.* [17] observed less damage in seeds with a greater concentration of proteins, fat and beta-glucans.

3.2 Parameters of seeds and the coefficient of shape

Computer image analysis made it possible to perform biometric measurements of naked oat seeds and complete the characterization based on weight tests. The components of variance estimated on that basis indicate highly significant differences among all the tested sources of variation (Table 4).

Year influenced seed density ($\approx 82\%$), TKW (59%), and seed width (51%). The highest values of

Cultivars	2008		2009		2010		Average	
	LTS	HTS	LTS	HTS	LTS	HTS	F _M	HTS - LTS
Abel	1.29	1.43	2.99	3.05	2.37	2.93	2.34	0.25
Avenida	1.98	2.04	1.81	3.51	2.97	3.44	2.63	0.74
Bullion	1.42	1.75	1.80	2.17	1.86	2.52	1.92	0.45
Cacko	1.16	1.84	3.25	3.43	2.42	2.83	2.49	0.42
Izak	1.22	1.48	0.91	2.28	2.17	2.58	1.78	0.68
Pikant	0.66	1.23	1.20	1.59	2.26	2.56	1.58	0.42
Polar	1.15	1.50	3.18	4.11	2.18	2.75	2.48	0.61
Saul	0.75	1.06	2.22	3.13	2.62	3.26	2.17	0.62
LSD _{0.05}	0.878						0.358	

Table 2. The frequency of microdamage F_M [%] per seed using low (LTS) and high (HTS) threshing speeds.

Cultivars	2008		2009		2010		Average	
	LTS	HTS	LTS	HTS	LTS	HTS	A _M	HTS - LTS
Abel	0.32	0.34	0.13	0.14	0.20	0.28	0.24	0.04
Avenida	0.27	0.45	0.07	0.17	0.26	0.30	0.25	0.11
Bullion	0.29	0.33	0.05	0.09	0.14	0.24	0.19	0.06
Cacko	0.20	0.51	0.13	0.17	0.22	0.28	0.25	0.14
Izak	0.25	0.25	0.05	0.11	0.18	0.28	0.19	0.06
Pikant	0.28	0.41	0.07	0.10	0.24	0.27	0.23	0.06
Polar	0.31	0.28	0.09	0.17	0.19	0.23	0.21	0.03
Saul	0.11	0.24	0.06	0.15	0.17	0.23	0.16	0.09
LSD _{0.05}	0.076						0.031	

Table 3. The area of microdamage A_M [mm²] per seed using low (LTS) and high (HTS) threshing speeds.

Source of variability	df	TKW	Density	Surface area	Length	Width	Circumference	Coefficient of shape
Years (A)	2	59.3**	81.8**	37.2**	17.2**	51.0**	22.6**	57.8**
Cultivars (B)	7	17.3**	10.9**	20.8**	31.4**	7.1**	27.9**	16.0**
AxB	14	10.0**	2.5**	35.1**	46.3**	39.0**	46.1**	14.0**
Error	72	13.4	4.8	6.9	5.2	3.0	3.4	12.1

Table 4. Significance of variation and percentage of the components of variance for the basic parameters of naked oat seeds.

* - significant at $P \leq 0.05$; ** - significant at $P \leq 0.01$; ns – insignificant

interaction between cultivars and years (from 35% to 46%) were found for the surface area, length, width and circumference of seeds. Seed shape, size, volume, area, density, porosity, colour and weight are only some of several dozen variables considered when designing harvesting or processing machines, and used to determine physical and chemical properties of seeds [25-27]. Many researches believe that the size of seeds has a significant effect on their susceptibility to mechanical

damage [28,29] also in naked oat cultivars [30,31]. Thus the use of oat seeds fraction in our study made it possible to eliminate in part the influence seed size on mechanical damage within individual cultivars. This was important because of the morphology of oat panicles, the multi-flowered inflorescence which (3-12 florets in a spikelet) is connected pleiotrophically or strongly linked to seed nakedness and causes a decrease in weight as seed order increases [32]. The two basic dimensions

determined in our studies, *i.e.* length and width of the seeds, were used to calculate a simple shape coefficient, which we related to seed microdamage detected using computer image analysis. Tadeusiewicz and Korohda [33] recommend that every shape coefficient should be sensitive to the variability in the dimensions of the described figure and easy to define, which is especially important in vision systems that provide data in real-time. Relating the obtained microdamage results to seed parameters it turns out that Avenuda and Cacko seeds, which were the most susceptible to mechanical damage, were characterized by the lowest values of the shape coefficient (0.311 and 0.315, respectively), determined by the greatest seed length (5.08 mm) (Table 5).

The Saul seeds, with the smallest A_m and the significantly smallest length (4.45 mm), were characterized by high values of K_m , which corresponds to a two-dimensional "oval" shape. Longer objects are more susceptible to damage not only because of increased risk of surface cracking, but also because of their volume and the accumulation of straining forces in areas where cracks have formed [34]. This creates favourable conditions for further breaks in the continuity of tissues or the appearance of new defects. According to Peltonen-Sainio *et al.* [8], the greatest resistance to mechanical damage was long seeds with smaller diameters, less weight and lower hardness values. In the studies on technological properties of wheat [35] or oat [36], the susceptibility of seeds to mechanical damage was found to decrease as seed density increased, with density determined by a greater share of endosperm in relation to the seed coat. In our study, Bullion was found to be most resistant to mechanical damage. The seeds of that cultivar, characterized by the largest total projected area (7.14 mm²), were also the most dense (63.8 kg·hl⁻¹) and had the highest TKW (28.2 g). Thus, it seems

that naked oat cultivars harvested at approximately 15% moisture content have seeds with greater filling and densities that are less susceptible to mechanical damage. However, we did not find this relationship in the other resistant cultivar, Saul, which similarly to Avenuda was characterized by the lowest seed density of about 60 kg·hl⁻¹. In the case of cultivars with low seed density and weight, shape plays a decisive role in developing resistance to mechanical damage. Observations made with a magnifying glass (unpublished data) showed that Bullion and Saul seeds, in contrast to Avenuda, were characterized by a small number of short hairs gathered at the beard. However, Górný [37] reports that this dominant trait is not recommended for identification of oat cultivars due to its high variability and strong dependence on exogenous factors.

3.3 Relationship between analyzed traits

The correlation coefficients revealed significant linear relationships between the majority of the analyzed variables (Table 6). Considering the performed tests, only TKW significantly correlated with both the frequency and the area of microdamage (r from -0.587** to 0.342*), and seed width (r from -0.453** to 0.599**).

The coefficient of shape was significantly correlated with the area of microdamage measured on samples threshed at both threshing speeds of 1.6 and 2.4 m·s⁻¹ (r =-0.726** and r =-0.824**, respectively), and with the frequency of microdamage at high threshing speed (r =0.301*). Regression equations showed a 4.3 mm² (LTS) and a 6.2 mm² (HTS) increase in the area of microdamage, 53% and 68%, respectively, determined by the decreasing coefficient of shape, thus indicating elongation of the seed shape (Figure 3).

In addition, we found that at both high and low threshing speeds, the share of F_m increased by 0.11

Cultivars	TKW [g]	Density [kg·hl ⁻¹]	Surface area [mm ²]	Length [mm]	Width [mm]	Circumference [mm]	Coefficient of shape
Abel	26.5	61.7	6.65	4.89	1.60	11.24	0.327
Avenuda	27.2	59.3	6.83	5.08	1.58	11.76	0.311
Bullion	28.2	63.8	7.14	5.05	1.62	11.60	0.321
Cacko	28.0	61.1	6.90	5.08	1.60	11.64	0.315
Izak	25.0	60.5	6.27	4.84	1.55	11.09	0.321
Pikant	25.6	61.8	5.22	4.34	1.40	9.97	0.323
Polar	26.0	59.9	6.54	4.92	1.59	11.48	0.323
Saul	26.7	60.0	5.85	4.45	1.49	10.28	0.334
LSD _{0.05}	0.820	0.887	0.283	0.094	0.026	0.183	0.0054

Table 5. Basic parameters of seeds and the coefficient of shape for naked oat seeds.

Correlated parameter		SD	SA	SL	SW	SC	K _m	F _M		A _M	
								LTS	HTS	LTS	HTS
Thousand kernel weight TKW [g]		0.444**	0.548**	0.314*	0.555**	0.231 ^{ns}	0.601**	0.342*	0.432**	-0.587**	-0.535**
Seed density SD [kg•hL ⁻¹]			-0.014 ^{ns}	-0.105 ^{ns}	0.029 ^{ns}	-0.254 ^{ns}	0.264*	-0.397**	-0.445**	-0.061 ^{ns}	0.001 ^{ns}
Seed surface area SA [mm ²]				0.929**	0.971**	0.891**	0.350*	0.557**	0.613**	-0.386**	-0.399**
Seed length SL [mm]					0.886**	0.981**	0.034 ^{ns}	0.528**	0.518**	-0.137 ^{ns}	-0.154 ^{ns}
Seed width SW [mm]						0.852**	0.493**	0.569**	0.599**	-0.453**	-0.517**
Seed circumference SC [mm]							-0.005 ^{ns}	0.593**	0.564**	-0.110 ^{ns}	-0.158 ^{ns}
Coefficient of shape K _m								0.220 ^{ns}	0.301*	-0.726**	-0.824**
Frequency of microdamage F _M [%]	LTS								0.794**	-0.146 ^{ns}	-0.285*
	HTS									-0.624**	-0.422**
Area of microdamage A _M [mm ²]	LTS										0.773**

Table 6. Matrix of correlation coefficients for biometric measurements of seeds and mechanical damage, determined for naked oat seeds (n=48).

* - significant at $p \leq 0.05$; ** - significant at $p \leq 0.01$; ^{ns} - insignificant
LTS – low threshing speed; HTS – high threshing speed

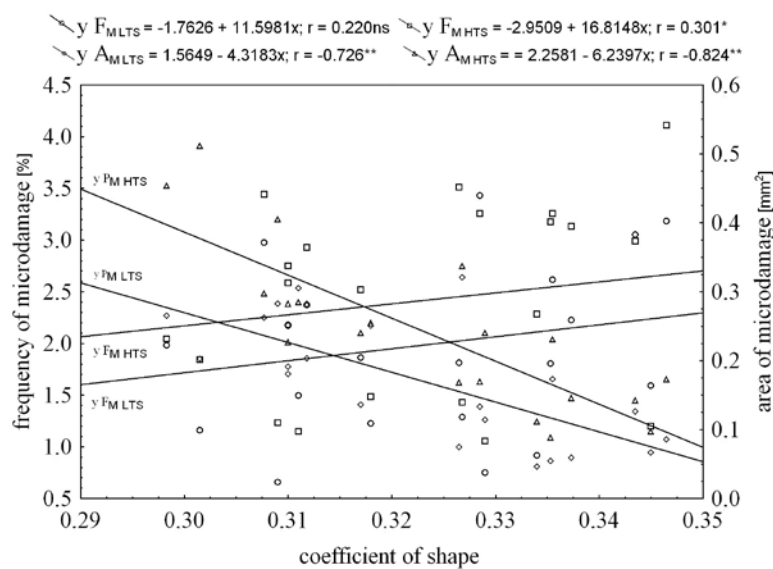


Figure 3. Relationships between the area (A_M) and frequency (F_M) of microdamage for seeds obtained using low (LTS) and high (HTS) threshing speeds, and the coefficient of shape in naked oat cultivars.

to 0.17% and A_M decreased by 0.024 to 0.026 mm² as TKW increased by one gram (Figure 4).

4. Conclusions

Naked oat cultivars, as a source of variation, did not exceed 4% of variability of frequency and area

of microdamage which was highly modified by the years of harvest (>34%) and threshing method (27%). We found varied responses of naked oat cultivars harvested at approximately 15% moisture content to dynamic loading in successive harvest years. The most favourable conditions for developing the highest resistance to mechanical damage were in the year 2009, in which total rainfall in the month

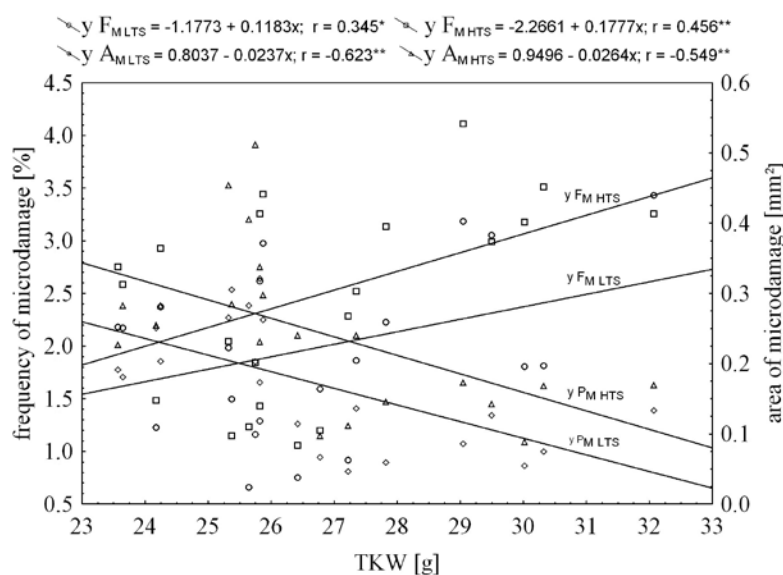


Figure 4. Relationships between the area (A_M) and frequency (F_M) of microdamage determined for seeds obtained using low (LTS) and high (HTS) threshing speed and the thousand kernel weight (TKW) in naked oat cultivars.

preceding the harvest did not exceed 72 mm and the temperature was 18.2°C. The analyses of variance showed a significant effect of threshing speed on seed damage. Increasing the threshing speed from 1.6 to 2.4 m·s⁻¹ increased the frequency of microdamage by 19%, on average, and the area of microdamage by 29%. Computer image analysis showed variation in the basic biometric (morphological) parameters of seeds among naked oat cultivars. Avenuda seeds, which were most susceptible to mechanical damage, were characterized by the coefficient of shape, which indicated the greatest length and the lowest density of seeds (59.3 kg·hl⁻¹). The highest resistance to mechanical damage, determined on the basis of both the frequency and area of microdamage, was shown by Bullion. This cultivar was also characterized by the largest total projected area of seeds (7.14 mm²), the greatest seed density (63.8 kg·hl⁻¹) and TKW (28.2 g). The coefficients of correlation suggest seed width is

the parameter with the strongest linear relationship with area ($r \leq -0.453^{**}$) and the frequency of microdamage ($r \geq 0.569^{**}$). The high coefficient of shape indicates well-rounded seeds accompanied by a decrease in the area of microdamage observed at both low ($r = -0.726^{**}$) and high ($r = -0.824^{**}$) threshing speed. The 4.3 mm² increase in the area of microdamage when using the threshing speed of 1.6 m·s⁻¹, and the 6.1 mm² increase in the area of microdamage when using the threshing speed of 2.4 m·s⁻¹ was 53 and 68%, respectively, determined by the decreasing, by a unit, coefficient of shape, thus indicating elongation of the seed shape.

Acknowledgements

This research was supported by the Polish Ministry of Science and Higher Education - contract number DS/3129/KHRiN.

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