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The effect of hull hardness on sunflower germination and susceptibility to Indian meal moth

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Summary: Sunflower hull hardness significantly affects seed performance in the field, but its effect on seed susceptibility to major seed pests during storage has not yet been assessed. The aim of this research was to determine the effect of sunflower hull hardness (based on a puncture force) on seed germination, and suitability/susceptibility to Indian meal moth (*Plodia interpunctella*), a major sunflower pest in storages. We tested the hull hardness of 14 sunflower genotypes in a needle penetration test. Germination was assessed daily, up to day 10. The susceptibility of genotypes to *P. interpunctella* attack and suitability for the pest's development was assessed in a biotest that encompassed artificial infestation with the moth eggs. The suitability was assessed based on developmental duration (days) and seed damage (%) after the termination of the moth life-cycle. Statistically significant differences in hull hardness were detected among sunflower genotypes and extreme values were registered for Neoplanta (3.83 N) and NS Romeo (2.24 N), as well as NS Oskar (0.62 N) and NS Kruna (0.84 N). Neoplanta seeds had the lowest germination on the first day (9%), followed by NS Ronin (17%) and NS Romeo (28%). During the final observation, Neoplanta and NS Horizont seeds expressed the lowest germination potential (91 and 92%, respectively), while germination of NS Kruna and Sumo 1 PR seeds was 100% after two days. The developmental duration of the moth did not differ statistically between genotypes as it ranged from 28-32 days. Also, seed damages ranged from 18-21.5%, depending on the genotype, but the values did not differ statistically.

Keywords: genotypes, germination, hull, Indian meal moth, *Plodia interpunctella*, puncture force, sunflower

Introduction

Seed quality is one of the major preconditions of successful plant production, because fast germination and a uniform field emergence are essential for achieving high yields (Mrđa et al., 2012; De Ron et al., 2016). Many studies are based on examining the physical and mechanical properties of seeds, as well as their chemical composition, all with the aim of improving seed production while maintaining high quality (Mostafa & Afify, 2022). Based on these properties, seed efficiency during sowing, as well as seed manipulation and post-harvest processing can be predicted (Seifi, 2011). Physical properties of seeds are usually stable and observable and thus are considered a reference indicator for screening high-vigour seeds (Li et al., 2022). The quality of sunflower seeds is primarily influenced by genetic traits inherited from parental lines and by environmental conditions during production (Krstić et al., 2023), while the post-harvest processing and storage conditions play a major role in preserving the germination and viability of the produced seeds (Zambolim, 2005; Prole et al., 2010). In common practice, sunflower seeds are used for sowing for several years and thus are stored for shorter or longer period, namely as long as the quality conditions are fulfilled (Mrđa et al., 2011). During storing, seed vigour gradually declines due to physiological, biochemical, physical, and cytological changes (Ebone et al., 2019; Morais et al., 2021). Unfortunately, a reduction of seed vitality (vigour) during storage can also be a result of stored product pest infestations (Lucca Filho, 2003).

Sunflower seeds are often threatened by different pests during post-harvest period, but Indian meal moth (*Plodia interpunctella* Hübner) has recently become the most significant pest, that is more frequently found in storages. This pest causes seed deterioration (firstly consuming the germ) which is responsible for high economic losses (Atanasov, 1974; Beratlić & Iliescu, 1990; Gvozdenac et al., 2018). It is well known that the quality and nutritive characteristics of the available food source influence development of *P. interpunctella* (Locatelli & Limonta, 1998; Vukajlović & Pešić, 2012; Predojević et al., 2017; Vukajlović et al., 2017). The majority of so far published studies are dealing with the development of the *P. interpunctella* on different durable commodities such as cereals and cereal products, while information on sunflower and other oil crops is scarce. Several facts inspired this research. Primarily, a steady and continuous increase in sunflower production from 167,000 ha to 231,000 ha in 2011-2018, and a further increase of 15.9% in 2024 compared to the average of the previous ten years (Chamber of Commerce and Industry of Serbia, 2024). Also, in the 2020-2024 period, the production area increased from 221,149 ha (2020) to 248,607 ha in 2024, which is 3.4% more than in 2023, while the export of sunflower seeds increased from 15,671 t to 136,580 t in the 2011-2018 period and increased significantly up to 307,000 t in 2024 (Chamber of Commerce and Industry of Serbia, 2024). The second fact that inspired this research is confirmed annual increase of this moth population in storages all around Serbia, based on multiannual monitoring with pheromone-based traps results (Gvozdenac, unpublished data). Mentioned indicates the growing importance of *P. interpunctella* as sunflower seed pest. The most comprehensive studies on *P. interpunctella* as a pest of sunflower in Serbia date back to 1980s (Almaši, 1984). However, recent work published by Gvozdenac et al. (2018) emphasizes the increased importance of this pest, as well as the influence of different types of sunflower seeds (oily and protein) and the level of the initial seed damage, on moths life history parameters. The increase of economic importance and damages caused by this pest impose a need for a more detailed study in terms of defining conditions that contribute to this trend, to be able to create more sustainable pest management strategies.

Seed germination depends on many environmental (Haj Sghaier et al., 2023; Šerá & Hnilička, 2023) and endogenous factors (Ali et al., 2021; Carrera-Castaño et al., 2020). Several authors confirmed that the hull hardness affects seed parameters and yield of sunflower (Gupta & Das, 2000; Babić et

al., 2012; Selvam et al., 2014; Mohammadi-Moghaddam et al., 2021), however, its relation with germination and susceptibility of seeds to *P. interpunctella* was not yet assessed. The aim of this research was to determine the effect of sunflower hull hardness on seed germination of divergent sunflower genotypes, and their susceptibility to *P. interpunctella* attack and to correlate them with moths' bionomy traits.

Materials and methods

Seed Samples: In this experiment, we used 14 divergent sunflower genotypes, namely 13 sunflower hybrids (NS Oskar, NS Kruna, NS Sumo Sjaj, NS Ronin, NS Pegaz, NS Sumo Sun, NS Horizont, Sumo 1 PR, NS Konstantin, NS Smaragd CLP, NS Kiril, NS Romeo and Neoplanta) obtained from a rich sunflower gene collection of the Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, and seeds of the public variety named "Kolos". Seeds of each genotype were mechanically cleaned, uniformed, and stabilized at the moisture content of 9%. According to Škorić et al. (1989), this level of seed moisture enables the stabilization of biochemical processes and the maintenance of seed quality. Also, by maintaining moisture at a constant level, the influence on the mechanical properties of sunflower seeds and kernels is avoided (Gupta & Das, 2000; Selvam et al., 2014).

Hull hardness test: The hull hardness of 14 sunflower genotypes was evaluated in a needle penetration test and estimated based on external force (N) applied on seeds that managed to puncture the hull. These tests were performed at the Laboratory for Biosystem Engineering, Faculty of Agriculture University of Novi Sad. Needle was applied to fifteen (15) whole seeds in the horizontal orientation, for each genotype. The test was performed on an FTC TMS-pro device. A cell with a load capacity of 50 N was used in the experiment. The speed of the pre-test was 60 mm/min, and the test was 30 mm/min. The probe displacement is set to 2 mm.

Germination test: Seed germination was tested in a germination chamber at 25°C by a standard laboratory test according to ISTA Rules (2021). Four replicates of 100 seeds were germinated in moistened filter paper rolls. Germination was assessed daily for 10 days, when the total germination (%) was calculated.

Insect bioassay: *P. interpunctella* parenteral population originates from a population reared under controlled conditions, on artificial diet (Silhacek & Miller, 1972) for at least ten generations in the Laboratory for Plant Stress at Sunflower Department at the Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia. *P. interpunctella* bioassay was carried in glass jars that contained 300 g of seeds of each sunflower genotype, artificially infested with two-day old moth eggs, in a climate chamber under controlled conditions (27 ± 2 C°, $65\% \pm 5\%$ RH, photoperiod 14:10 L:D). We monitored developmental duration (days elapsing from egg till moth death), and recorded seed damage (%) on each genotype, after the termination of the moth life cycle. The experiment was carried out in four replicates.

Data analysis: Hull hardness was presented as a force (N) needed for a needle to puncture the sunflower seed hull, seed germination was expressed in %, while the data from insect bioassays was presented as mean a number of days that represented mean developmental duration *P. interpunctella*. Data was subjected to one-way ANOVA, and differences between all mentioned traits were analyzed using Duncan's multiple range test, also enabling to formation of homogenous groups that are not statistically significant. Additionally, correlation analysis was also performed to test the influence of hull hardness on sunflower seed germination and life cycle of *P. interpunctella* and the level of damages caused by this pest.

Results and discussion

Statistically significant differences were determined between the hull hardness among 14 tested sunflower genotypes, as well as in their germination in different observation periods (Table 1). Only the germination values for the 1st, 2nd and 3rd days are presented since the germination did not change until day ten, which is also presented in Table 1. The highest value of hull hardness (based on puncture force) was employed for Neoplanta seeds (3.83 N), followed by NS Romeo (2.24 N), NS Kiril (2.04 N) and NS Konstantin (2.00 N). The lowest hull hardness was registered for NS Oskar (0.62 N) and NS Kruna (0.84 N).

Table 1. Puncture force (N) and germination (%) of sunflower seed in relation to genotype

Genotype	Puncture force (N)	Germination (%)			
		1 st day	2 nd day	3 rd day	4 th -10 th day
NS Oskar	0.62 ± 0.2 ^g	74.0 ± 0.5 ^d	98.0 ± 0.0 ^a	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a
NS Kruna	0.84 ± 0.3 ^f	62.0 ± 2.0 ^e	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a
NS Sumo Sun	0.98 ± 0.3 ^f	86.0 ± 3.0 ^b	99.0 ± 0.0 ^a	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a
NS Ronin	0.99 ± 0.2 ^f	17.0 ± 5.4 ^h	99.0 ± 0.0 ^a	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a
NS Pegaz	1.09 ± 0.2 ^f	63.0 ± 1.0 ^e	99.0 ± 0.0 ^a	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a
NS Sumo Sja	1.20 ± 0.4 ^{ef}	56.0 ± 2.0 ^f	97.0 ± 1.0 ^a	98.0 ± 0.0 ^a	100.0 ± 0.0 ^a
Kolos	1.37 ± 0.4 ^{def}	79.0 ± 2.0 ^c	92.0 ± 2.0 ^b	93.0 ± 3.0 ^b	95.0 ± 1.0 ^b
Sumo 1 PR	1.47 ± 0.6 ^{de}	93.0 ± 0.6 ^a	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a
NS Horizont	1.60 ± 0.4 ^{de}	80.0 ± 1.0 ^c	90.0 ± 4.0 ^b	91.0 ± 3.0 ^b	92.0 ± 4.0 ^b
NS Smaragd CLP	1.61 ± 0.5 ^{de}	91.0 ± 2.0 ^a	95.0 ± 2.0 ^{ab}	97.0 ± 1.0 ^{ab}	98.0 ± 1.0 ^a
NS Konstantin	2.00 ± 0.6 ^c	57.0 ± 4.0 ^f	99.0 ± 0.0 ^a	99.0 ± 0.0 ^a	100.0 ± 0.0 ^a
NS Kiril	2.04 ± 0.6 ^c	63.0 ± 4.0 ^e	89.0 ± 2.0 ^b	91.0 ± 2.0 ^b	93.0 ± 3.0 ^b
NS Romeo	2.24 ± 0.6 ^b	28.0 ± 2.5 ^g	99.0 ± 0.0 ^a	100.0 ± 0.0 ^a	100.0 ± 0.0 ^a
Neoplanta	3.83 ± 0.9 ^a	9.0 ± 2.5 ⁱ	86.0 ± 4.0 ^c	86.0 ± 4.0 ^c	91.0 ± 2.0 ^b
F value	28.44 ^{**}	1047.95 ^{**}	321.31 ^{**}	66.32 ^{**}	166.23 ^{**}

The germination test showed significant differences in % of germinated seeds on the first day. The lowest germination potential was recorded for Neoplanta (9%), followed by NS Ronin (17%) and NS Romeo (28%). On the second day, the germination of all genotypes was higher than 85%, which exceeds the minimal germination (80%) stipulated by the national regulation for the sunflower (Republic of Serbia, 2025). However, there were significant differences in germination between the genotypes, after each day of observation. Seed germination on day 10 remained the lowest in the case of Neoplanta, NS Horizont and NS Kiril (91, 92 and 93%, respectively) while NS Kruna and Sumo 1 PR reached 100% germination on the second day.

The results are following the report from the Babić et al. (2012), noting that the applied force was influenced by the sunflower genotype, but also by the seed moisture content. Namely, the elasticity of seeds is affected by moisture content, i.e. with higher moisture content, the elasticity increases, and thus the required rupture force. Gupta and Das (2000) and Khodabakhshian et al. (2011), examining the influence of force on the seeds of a local sunflower varieties, concluded that in addition to the seed moisture content, the orientation of the seeds in space has a significant influence, so the force used for seeds in a horizontal position was lower. According to the results of this research, where the moisture was reduced to the same level and where the force was applied to the seeds in a horizontal position, the differences that appeared were conditioned by genetic traits, which is also indicated by different studies related to hull characteristics (Marinković, 1992; Denis et al., 1994; Dijanović et al.,

2003; Dauguet et al., 2016). Seed hull is only one of more than 100 traits that characterize seeds, and on which hereditary factors have a crucial influence. Many of them, such as seed weight, size, shape, oil and protein content, essential amino acids, and hormones, are significantly influenced by environmental conditions. According to the research of other authors, these characteristics significantly affect seed germination. Namely, the completion of the germination process is considered to be the rupturing of the seed coat and the emergence of a radicle, which is affected by water uptake and the activation of physiological and metabolic processes (Ali et al., 2021). The different amounts of water, as well as the rate of absorption, caused differences in the time required for the germination of seeds of divergent sunflower genotypes. Water affects the processes in the seed that break down the accumulated nutrient reserves, enabling the growth and development of seedlings (Kibinza et al., 2006; Han & Yang, 2015; El-Maarouf-Bouteau, 2022; Khaeim et al., 2022).

The correlation analysis showed a strong negative correlation between the hull hardness (based on puncture force) and sunflower seed germination on the 1st, 2nd, 3rd and 10th day, as the Pearson correlation coefficients are: -0.617^{**} , -0.53^{**} , -0.716^{**} and -0.648^{**} , respectively). Namely, the higher the puncture force (i.e. harder hull), the germination will be lower and slower, especially in the first days. According to Kaya (2009) sunflower seeds may take longer to complete the germination process than cereal crops because the seed coat slows down the movement of water. Seed hull influence germination and seedling growth by preventing or slowing down water absorption.

In insect biotest, statistically significant differences were recorded between the insect developmental duration (MDD) among hybrids ($F_{13}=16.5^*$, $p>0.01$), ranging from 28 days on NS Oskar and NS Smaragd CLP to 32 days on Neoplanta. The mean developmental duration (MDD) of *P. interpunctella* ranged from 28 to 32 days, depending on the genotype (Figure 1). Also, the correlation analysis showed a strong positive correlation between the hull hardness and the MDD *P. interpunctella* indicating a longer mean developmental duration of the moth life-cycle on genotypes with the harder hull (Pearson correlation coefficient was 0.867^{**} ; $p<0.000$). The coefficient of determination was 0.75.

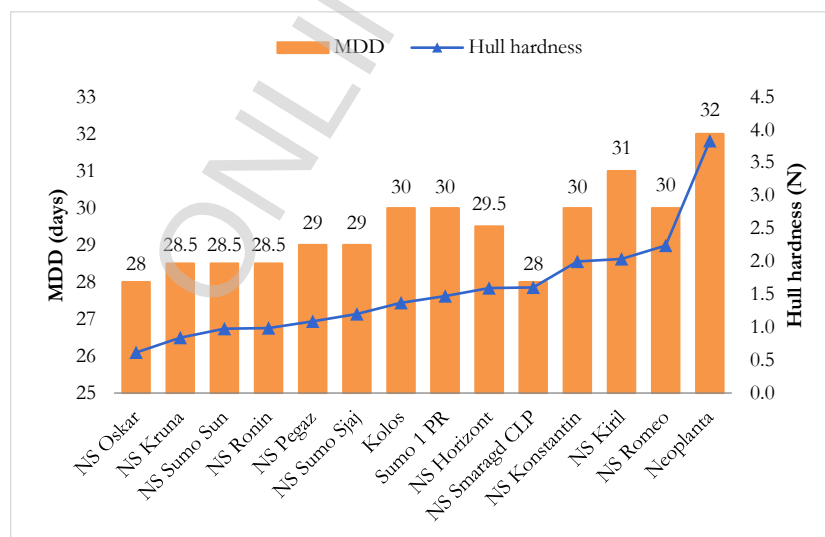


Figure 1. Mean developmental duration (MDD) of *P. interpunctella* on different sunflower genotypes in relation to hull hardness (i.e. puncture force)

Statistically significant differences were recorded between the seed damages that ranged from 18.0 to 21.5%, depending on the genotype ($F_{13}=2.45^*$, $p>0.01$), as presented on Figure 2. Statistically the highest damages were recorded on NS Oskar, NS Kruna, NS Ronin and Kolos (20.5-21.5%), while

the lowest was on Neoplanta (18.0%). A strong negative correlation was recorded between the puncture force (observed as hull hardness) and the percent of seed damages indicating a lower damage on sunflower genotypes with harder hulls (Pearson correlation coefficient was -0.786^{**} ; $p < 0.000$). The coefficient of determination was 0.62, therefore the percentage of variance was 62%.

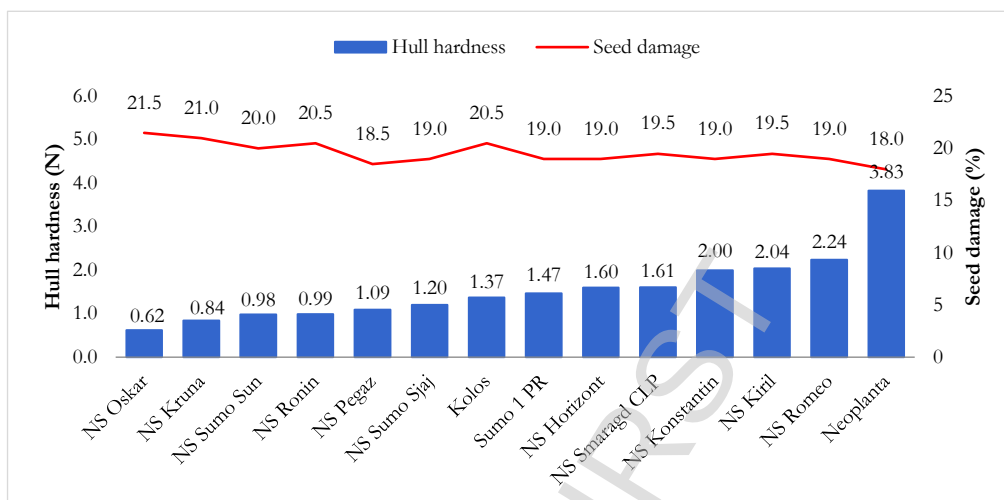


Figure 2. Sunflower seed damage (%) on different genotypes

Recently, there is an increase in number of studies on different seed priming treatments because priming can enhance germination and seedling emergencies in field conditions, especially in the first days. This is especially important for seeds with a hard seed coat, or with hull, like sunflower. Therefore, this work provides valuable information about the genotypes that should be subjected to seed priming. Also, the work provides valuable information about the susceptibility of specific genotypes to *P. interpunctella* attack, but also indicates the plasticity of this pest species.

Conclusions

This study demonstrates that sunflower genotypes significantly differ in hull hardness (based on the puncture force) as well as in germination after each day of observation. A strong negative correlation was confirmed between the hull hardness and sunflower seed germination, especially in the first days. Genotypes with the strongest hull hardness had lower and slower seed germination. Statistically, no significant differences were recorded between the moth's developmental duration although it was strongly positively correlated with the hull hardness. Also, a strong negative correlation was recorded between the hull hardness and % of seed damage caused by *P. interpunctella*.

Author contributions: conceptualization SG and JO; data curation MK; formal analysis SG and MK; investigation SG, JO, MR, IP; methodology SG, JO, MR and IP; project administration SC, SJ and VM; resources SG, SC and SJ; supervision SG and JO; validation SJ and VM; visualization SC; writing – original draft SG, JO, MK and MR; writing – review & editing SC, SJ, VM and IP.

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Data availability statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Ethical issues statement: No assistance from AI tools has been used for the writing of this manuscript.

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Uticaj tvrdoće ljuske suncokreta na klijavost i osetljivost semena prema bakrenastom plamencu brašna

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Sažetak: Parametri kvaliteta semena uključujući i tvrdoću ljuske kod suncokreta imaju značajnu ulogu u performansama semena u polju, nakon setve. S druge strane, uticaj ovog parametra (tvrdoće ljuske) na osetljivost semena prema skladišnim štetočinama do danas nije utvrđen. Cilj rada zasnovan na ovim činjenicama bio je da se utvrdi uticaj tvrdoće ljuske semena suncokreta (baziranoj na sili probijanja FTC TMS-Pro uređajem) na klijavost i na osetljivost na napad bakrenastog plamenca brašna (*Plodia interpunctella*). Za ispitivanje tvrdoće ljuske semena 14 genotipova suncokreta, korišćen je test „probijanja igle“. Klijavost je ispitivana svakodnevno do desetog dana, standardnim laboratorijskim testom. Podložnost genotipova suncokreta na napad plamenca i pogodnost za razvoj ove štetočine procenjeni su u biotestu koji je podrazumevao veštačku infestaciju genotipova jajima štetočine. Pogodnost za razviće je procenjena na osnovu dužine razvića (dani), i oštećenja semena (%) nakon završetka životnog ciklusa moljca. Utvrđene su statistički značajne razlike u tvrdoći ljuske između genotipova suncokreta i ekstremne vrednosti su registrovane za semena genotipa Neoplanta (3,83 N) i NS Romeo (2,24 N), kao i NS Oskar (0,62 N) i NS Kruna (0,84 N). Semena Neoplante (9%), NS Ronina (17%) i NS Romea (28%) su imala najnižu klijavost prvog dana. Tokom završne ocene (10. dana) klijavost semena Neoplante (91%) i NS Horizonta (92%) ostala je najniža, dok su NS Kruna i Sumo 1 PR dostigli 100% klijavosti već drugog dana. Dužina razvića *P. interpunctella*, ekonomski visoko značajne štetočine uskladištenih proizvoda, nije se statistički razlikovala između genotipova (28-32 dana). U zavisnosti od genotipa, % oštećenja semena se kretao od 18-21.5%, ali se vrednosti nisu statistički razlikovale međusobno.

Ključne reči: bakrenasti plamenac brašna, genotipi, klijavost, ljuska, *Plodia interpunctella*, sila probijanja, suncokret