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Superabsorbent Seed Coating and Its Impact on Fungicide Efficacy in a Combined Treatment of Barley Seeds

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Abstract: The technology of seed coating with superabsorbent polymer (SAP) has the potential to mitigate the negative impact of drought on seed germination and crop establishment. However, their application on the seed surface can affect the effectiveness of pesticides used for seed treatment in the protection against phytopathogens. In our work, the influence of the Aquaholder®Seed polymer coating on the effectiveness of fungicides in the protection of germinating seeds of spring barley cv. Bojos and Laudis against the fungal pathogen *Bipolaris sorokiniana* was studied. One-half of the seeds were first treated with fungicides, and then a polymer was applied. Fungicide efficacy was evaluated in a Petri dish test and pot test under the pathogen attack. Seed coating with SAP did not negatively affect fungicide efficacy. The percentage of germinated seeds, seedling emergence, plant height, and symptoms of the disease in the fungicide-treated variants were not significantly changed by the SAP application. Moreover, in cv. Laudis, the application of SAP alone partially protected germinating seeds against pathogen attack. The amount of pathogen DNA in plant tissues of cv. Laudis was not significantly different among seed treatments, while in cv. Bojos, the pathogen DNA increased in seeds coated with SAP alone but decreased in combined treatment with fungicides. These results demonstrated that SAP seed coating does not negatively affect the efficacy of fungicides used for seed protection against fungal pathogens.

Keywords: *Bipolaris sorokiniana*; fungicides; hydrogel; real-time PCR; seed coating; superabsorbent polymer

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1. Introduction

Drought is a major abiotic stress affecting crop production. Water scarcity related to climate change and rising water consumption due to population growth and increased demands in industry, agriculture, and households is expected to worsen. It is predicted that the area of rainfed crop cultivation will continuously decrease while irrigated areas will increase by 12% in the period between 2020 and 2025 [1]. Moreover, the precipitation distribution is expected to be uneven and difficult to predict. To mitigate the impact of climate change on agricultural production, it is necessary to explore new agronomic strategies to cope with the lack of rainwater and to reduce the consumption of water for irrigation.

Superabsorbent polymers (SAP) have the potential to improve soil water-holding capacity and reduce the impact of drought stress on plants [2]. They act as water reservoirs that absorb water from irrigation, rainfall, and soil moisture as well. After water absorption, they form hydrogel—a highly hydrated 3D polymer structure insoluble in water. During dry periods, the water from hydrogel is gradually released for plant uptake. This process of water absorption and desorption enables sustainable water usage [3]. Besides the

improvement of soil water-holding capacity, depending on the soil type, SAP can affect other soil physical or chemical properties, such as soil porosity, bulk density, release and uptake of nutrients, or leaching of pesticides [3,4].

According to a meta-analysis published in 2023 [5], SAPs were applied primarily in growing wheat (39%) and maize (27%). However, the highest increase in crop yield was observed in tuber crops, and the greatest economic benefits were observed in vegetables. SAPs used mostly (77%) were of synthetic origin and applied as granulate; however, studies were also conducted with natural polymers based on peptides, polysaccharides, or other compounds. Seed coating represented only 10% of cases in this meta-analysis. The application of SAP as a seed coating plays a crucial role during the critical period of seed germination and seedling emergence. This form of application serves as insurance for growers in times of drought after sowing. Although the yield increase was the lowest within this form of application [5], nevertheless, its effect in drought conditions during seed germination can be fundamental since the optimal establishment of the crop stand has a definite effect on the harvest. The effectiveness of such application depends on the type of crop and soil properties, with the best effectiveness in sand soil and the soil moisture during this critical period. The application of SAP as a seed coating requires the use of only a small amount of polymer and allows the addition of growth promoters, nutrients, beneficial microorganisms, pesticides, or other protective agents, including bio-pesticides, with minimal losses to the environment [1,3,6].

The positive effects of seed coating with SAP on the establishment of vegetation, plant growth parameters, and yields were confirmed for several crops—tobacco [7], pigeon pea [8], Korshinsk Pea Shrub [9], cowpea [10], maize [11], soybean [12], and sugar beet [13]. In the last-mentioned work, the authors used the same SAP as in our work. They found increased leaf area index, PRI (photochemical reflectance index) in dry conditions at the beginning of the vegetation, as well as higher root and white sugar yields compared to the control without SAP. However, there is still a lack of published papers describing the impact of SAP coating on pesticide performance in combined seed treatment. The laboratory tests confirmed that coating the seeds with polymer reduced the leaching of pesticides from the seed surface [14,15]. In the study focusing on the application of polymers (polyacrylamide + carboxymethyl cellulose) combined with pesticides as a seed coating, it was found that 6% difenoconazole and fludioxonil in the seed coating agent provided effective control of wheat sharp eyespot in a field experiment [16]. Another study using a seed-coat agent containing microencapsulated prothioconazole demonstrated its preventing effect against fusarium head blight, increased crop dry weight, and reduced pesticide injury to wheat seeds compared to commercial prothioconazole [17].

In our earlier study, we compared the efficacy of fungicides in seeds coated with fungicides and fungicides combined with SAP [18]. We found out that the application of SAP coating to fungicide-treated seeds of maize or wheat did not significantly affect the fungicide's effectiveness against *Fusarium culmorum*. In addition, maize plants from seeds treated with SAP alone showed better growth in heavily infected soil compared to untreated seeds. In our other work [19] with the same SAP used for corn seed treatment, we found better growth of plants (root and coleoptile length, plant height, dry matter production, and leaf tip number) from treated seeds at the early development stages with statistically significant differences in the second tested year.

The objective of the current study was to confirm the neutral effect of SAP coating on the fungicide efficacy during seed germination and early plant growth. Unlike our previously published work, we chose another important crop for the study, spring barley (*Hordeum vulgare* L.) and the soil-borne fungal pathogen *Bipolaris sorokiniana* (Sacc.) Shoemaker. *B. sorokiniana* (teleomorph *Cochliobolus sativus*) belongs to the most serious phytopathogens of grain cereals, including barley. It causes foliar spot blotch, root rot, seedling blight, head blight, and black point on grains, resulting in yield losses. Severe root and crown infection can cause plants to die without producing seeds [20]. We hypothesized

that coating the seeds with a layer of SAP after fungicide application would not negatively affect their effectiveness in protecting spring barley seeds against this fungal pathogen.

2. Materials and Methods

2.1. Biological Material

Seeds of spring barley cv. Bojos and Laudis (belonging to the high-quality malting cultivars most cultivated in the region, susceptible to the studied pathogen) were treated with the combined fungicide preparation Raxil Star (Bayer S.A.S., Lyon, France), containing 20 g L^{-1} fluoropyram, 60 g L^{-1} tebuconazole, and 100 g L^{-1} prothioconazole in a dose of 0.5 L t^{-1}. Half of the fungicide-treated seeds and untreated seeds were then coated with SAP Aquaholder®Seed based on potassium salt of acrylic acid and acrylamide (patent no. EP 4007796 B1, PeWaS s.r.o., Bratislava, Slovak Republic). Aquaholder®Seed formulation was applied in the form of a superabsorbent polymer suspension dispersed in an alcohol medium along with a dissolved adhesive additive. For the treatment, a definite amount of spring barley seeds (2 kg) was placed in a batch rotary coater under continuous rotation. The seeds were treated with Aquaholder®Seed formulation in an amount of 2.5% per 1 kg (i.e., 50 g of formulation for 2 kg spring barley batch) and then dried in a rotary fluid bed dryer for 15 s. The overall duration of treatment was approx. 45 s. Finally, 4 variants of seeds were prepared as follows:

- C—control untreated seeds;
- SAP—polymer-treated seeds;
- F—fungicide-treated seeds;
- F + SAP—combined treatment of fungicides and SAP.

The fungal pathogen *Bipolaris sorokiniana* (Sacc.) Shoemaker used in the experiments originated from the collection of fungal pathogens in the Research Institute of Plant Production in Piešťany. The identity of the pathogen was previously confirmed by PCR with specific primers [21]. Three-week-old mycelium grown on potato dextrose agar (PDA; Duchefa Biochemie, Haarlem, The Netherlands) at 22 °C in the dark was scraped from the medium in a Petri dish, resuspended in 50 mL of sterilized distilled water, and filtered through autoclaved cotton gauze to remove mycelial fragments. The concentration of the conidial suspension was measured using a hemocytometer and adjusted to 10^5 mL^{-1}. The suspension was used directly for seed infection or preparation of soil inoculant.

2.2. Petri Dish Test for Germination of Infected Seeds and Manifestation of Disease Symptoms

Ten seeds of each variant of barley cultivars Bojos and Laudis were placed on filter paper moistened with 2 mL of sterile water in a Petri dish (Ø 90 mm). Fifty microliters of prepared pathogen suspension were gently applied under each seed. The seeds were allowed to germinate at a temperature of 22 °C and 12 h photoperiod. After ten days, the percentage of germinated seeds (=seeds germinated/total seeds × 100; the seeds were considered germinated when the radicle and the coleoptile were at least 5 mm long) and the disease symptom development by the 5-point rating scale (0 = no symptoms, 1 = a few light spots on coleoptile, 2 = several isolated brown spots, 3 = numerous brown spots, 4 = dark-colored coleoptile) were evaluated. The experiment was performed in triplicate and repeated twice.

2.3. Pot Test for Plant Growth under Pathogen Attack and Disease Development

Previously published methodology [18] with small modifications was used for soil infection. Barley groats (20 g per flask, approx. 500 grains) were inoculated with 10 mL of conidial suspension of *B. sorokiniana* and cultivated for 1 week at 22 °C in the dark. Pots (Ø 100 mm, 3 pots per each variant) were filled with the autoclaved soil mixture (horticulture substrate Klasmann-TS 3; Klasmann-Deilmann, Geeste, Germany, sand, and arable soil; 1:1:1 by volume). Five pieces of barley groats overgrown with the pathogen mycelium were placed on the substrate in the center of the pots, 10 tested barley seeds were placed in a circle about 15 mm from the edge of the pot, and all were covered with

10 mm of soil mixture. All the pots were regularly watered with the same amount of water according to the plant's needs (80 mL at day 0 and then 40 mL per pot 2–3 times a week) and cultivated while covered with plastic cups. Plants were cultivated in a growth chamber at 18–20 °C under the 16 h photoperiod (light intensity 200 μmol m^{-2} s^{-1}). Plant emergence and height were measured on the 7th day, disease symptoms on coleoptile and subcrown internode were evaluated after 3 weeks by the 6-point rate scale (0 = no symptoms, 1 = light brown spots covering less than 10% of the coleoptile, 2 = 11–25% of the coleoptile area affected by browning, 3 = 26–40% of the coleoptile or subcrown internode browned, 4 = 41–75% of the coleoptile and cubcrown internode browned, 5 = more than 75% of the coleoptile area browned, subcrown internode severely damaged) [22].

After evaluation, the basal part of the stems (coleoptile and subcrown internode) was taken for DNA isolation. Plant material was collected from each plant, and a mixed sample (0.1 g of plant material) was prepared for each pot. A negative control was taken from plants of each variant cultivated without infection, and the pathogen sample consisted of 0.1 g of *B. sorokiniana* mycelium scraped from Petri dishes. Samples were ground to a fine powder in a cooled mortar using liquid nitrogen and homogenized, and pure total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The concentration and quality of isolated DNA were checked spectrophotometrically using NanoDrop1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), and total DNA was quantified by Qubit™ Flex Fluorometer (Invitrogen™, Thermo Fisher Scientific Inc., Waltham, MA, USA).

Quantification of pathogen DNA was carried out by a real-time PCR method using an ABI PRISM® 7000 machine (Applied Biosystems, Foster City, CA, USA) in MicroAmp optical 96-well plates (Applied Biosystems, Foster City, CA, USA). A region of 102 bp with a specific TaqMan probe with corresponding primers for real-time PCR was designed from the region of the Brn1 locus bounded by published COSA F/R primers [21]. New TaqMan™ probe BISOR2 5′FAM 3′MGB (CGGTATCGGGAAGGCCATGG) with forward (CAAGCTGACCAAATCACCTTCA) and reverse (ACGGCGTTGGCGTAGTTG) primer pair BISOR2 specific for *B. sorokiniana* were designed using the Primer Express Software version 2.0 (Applied Biosystems, Foster City, CA, USA). The specificity of this newly designed primer pair and the TaqMan probe was compared to the nucleotide collection contained in the National Center for Biotechnology Information using the program Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi, accessed on 27 June 2022). A match was found only with sequences of the genus *Bipolaris*/*Cochliobolus*. The reaction was carried out in 25 μL reaction volume consisting of 12.5 μL TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 300 nM of each primer, 200 nM of TaqMan MGB probe (labeled with FAM fluorescent dye), and 3 ng of total DNA. As standards, five dilutions of pure *B. sorokiniana* DNA (1, 10, 100, 1000, and 10,000 pg μL^{-1}) were applied. Conditions of PCR were as follows: 95 °C for 10 min, 40 cycles at 95 °C for 15 s, and 59 °C for 1 min. For an evaluation of the results, the ABI PRISM® 7000 software (Applied Biosystems, Foster City, CA, USA) was used, and the content of pathogen DNA in the analyzed samples was expressed in pg per 1 ng of the total DNA.

2.4. Statistical Analysis

Experimental data were analyzed by ANOVA followed by the LSD test (the least significant difference) at $\alpha = 0.05$; pairwise comparisons of plant height between control and infected soil of each seed variant were performed by *t*-test, all using the statistical software STATGRAPHICS XVII–X64 (Statpoint Technologies, Inc., Warrenton, VA, USA). Percentage data were transformed by square root transformation before analysis. Principal component analysis (PCA) by using a correlation matrix and grouping between treatments with the first two principal components was performed using the PAST (PAleontological STatistics) software version 3.19 [23]. Scores from all principal components from PCA were used for the Permutational multivariate analysis of variance (PERMANOVA) with Euclidean distance measure to determine the significant effects among variants.

3. Results

3.1. Petri Dish Test for Germination of Infected Seeds and Manifestation of Disease Symptoms

The percentage of germinated barley seeds infected with the conidial suspension of *B. sorokiniana* in this test was not statistically affected by seed treatment (p = 0.083; ANOVA), cultivar (p = 1.00), or their interaction (p = 0.66). In all variants, 93.3–100% of the seeds germinated in 10 days. There were no disease symptoms on plants germinated from fungicide-treated seeds (Figure 1), regardless of SAP treatment. Frequency of disease symptoms on plants of cv. Bojos was similar for untreated seeds and SAP-treated seeds (90.0 or 92.9%, respectively), while in cv. Laudis, it was 96.7% for untreated seeds and 78.6% for SAP-treated seeds. The average disease symptom intensity measured by a 5-point rate scale was similar for control and SAP-treated seeds in cv. Bojos, however, for cv. Laudis, the symptoms were statistically stronger in plants grown from control seeds compared to SAP-treated seeds (Figure 2a).

(**a**) (**b**)

Figure 1. (**a**) The symptomless (F + SAP treatment) and (**b**) symptomatic (SAP treatment) plants in the Petri dish test of cv. Bojos after the infection with *B. sorokiniana*.

3.2. Pot Test for Plant Growth under Pathogen Attack and Disease Development

In the pot test, plants grew for 3 weeks in soil infected by barley groats overgrown with the mycelium of *B. sorokiniana*. Germination of seeds was not influenced by the seed treatment and reached 96.7–100% in all variants. The height of plants was significantly affected by cultivar, seed treatment, and soil infection (p = 0.000 for all factors; ANOVA). The plant growth was reduced in the infected soil, and the difference was statistically significant in all variants of both cultivars (t-test; Figure 3). The application of fungicides also significantly affected the growth of plants, especially in cv. Bojos. The application of SAP did not significantly affect growth in infected soil, comparing variants without fungicides with variants treated with fungicides (Figure 3).

Disease symptoms were observed on 76.3 and 76.6% of untreated plants of cv. Bojos and Laudis, while for SAP-treated seeds, it was 75.5 and 62.6% of plants, respectively. In pesticide treatments (F or F + SAP), 30–36.6% of Bojos and 53.3% of Laudis plants were symptomatic. The average disease symptom intensity evaluated by the 6-point rate scale (Figure 4) was weaker on fungicide-treated seeds without a statistically significant impact of SAP in both cultivars (Figure 2b). Seeds not treated with fungicides were more severely affected. In cv. Bojos, the differences between fungicide-treated seeds and seeds without fungicides were statistically significant; in addition, disease symptoms were also observed on plant leaves (1–3 small spots) in variants not treated with fungicides (F or F + SAP). More plants with symptoms on leaves (46.42%) were found in the control variant compared to those treated with SAP (24.14%). In cv. Laudis, plants from seeds treated with SAP alone did not show statistically different symptom intensity from untreated (control) seeds or

fungicide-treated seeds (Figure 2b). Thus, plant infestation here was slightly lower than in control seeds but higher than in fungicide-treated seeds (Figure 2b).

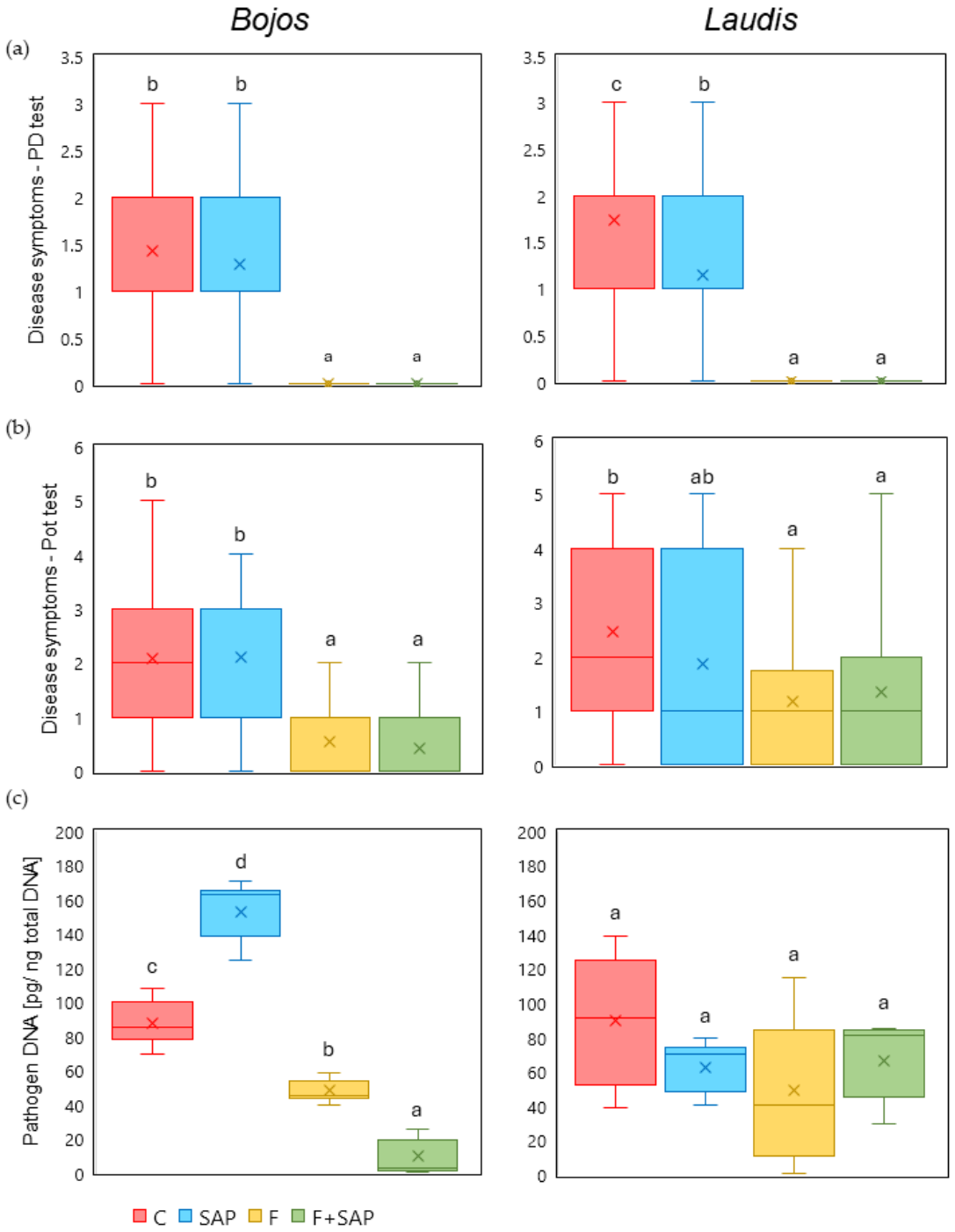


Figure 2. Disease symptoms of *B. sorokiniana* in (**a**) Petri dish test with seeds of cv. Bojos and Laudis infected by conidial suspension of the pathogen, evaluated 10 days after infection by the 5-point rate scale (0 = no symptoms, 4 = full infection), (**b**) disease symptoms on juvenile plants grown 3 weeks in infected soil in the pot test, evaluated by the 6-point rate scale (0 = no symptoms, 5 = full infection), and (**c**) amount of the pathogen DNA in pg per 1 ng of the total DNA isolated from plants in the pot test; C—untreated seeds, SAP—polymer-treated seeds, F—seeds treated with fungicides, and F + SAP—combined treatment. Data marked with the same letter were not statistically different by the LSD test at $\alpha = 0.05$.

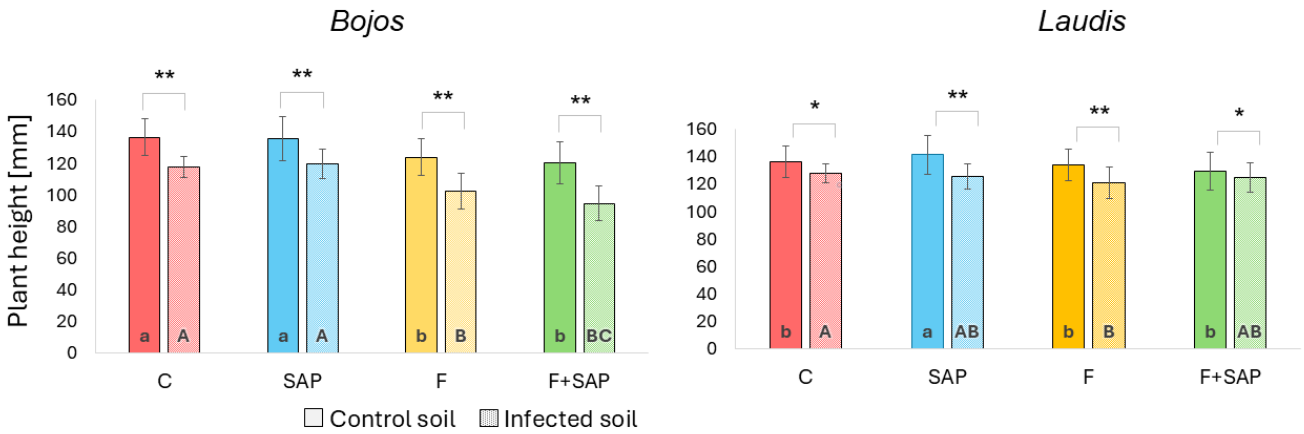


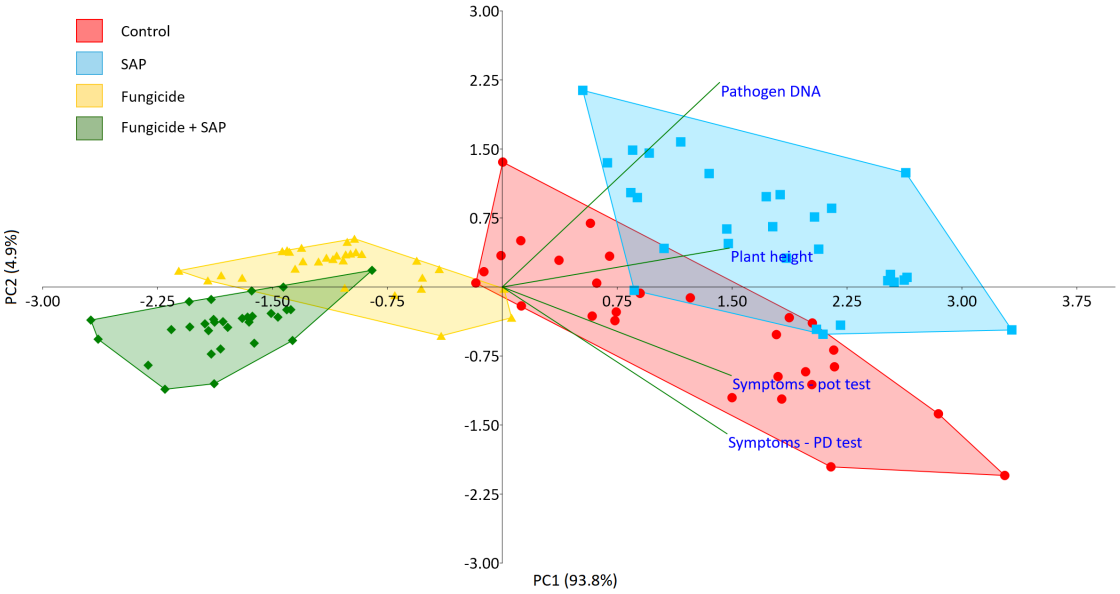
Figure 3. Plant height of cv. Bojos and Laudis in control soil and soil infected by *B. sorokiniana*; C—untreated seeds, SAP—polymer-treated seeds, F—seeds treated with fungicides, and F + SAP—combined treatment. Columns marked with the same letter were not statistically different by the LSD test at $\alpha = 0.05$; the test was performed separately for the control (lowercase) and infected soil (uppercase). Pairwise comparisons of plant height between the control and infected soil of each seed variant were made by *t*-test, $\alpha = 0.01$ (*) or 0.001 (**).

Figure 4. Symptoms of *B. sorokiana* on leaf sheath and subcrown internode of barley plants cultivated in infected soil according to rate scale (0 = no symptoms–5 = the highest damage) and spots on the leaves of cv. Bojos.

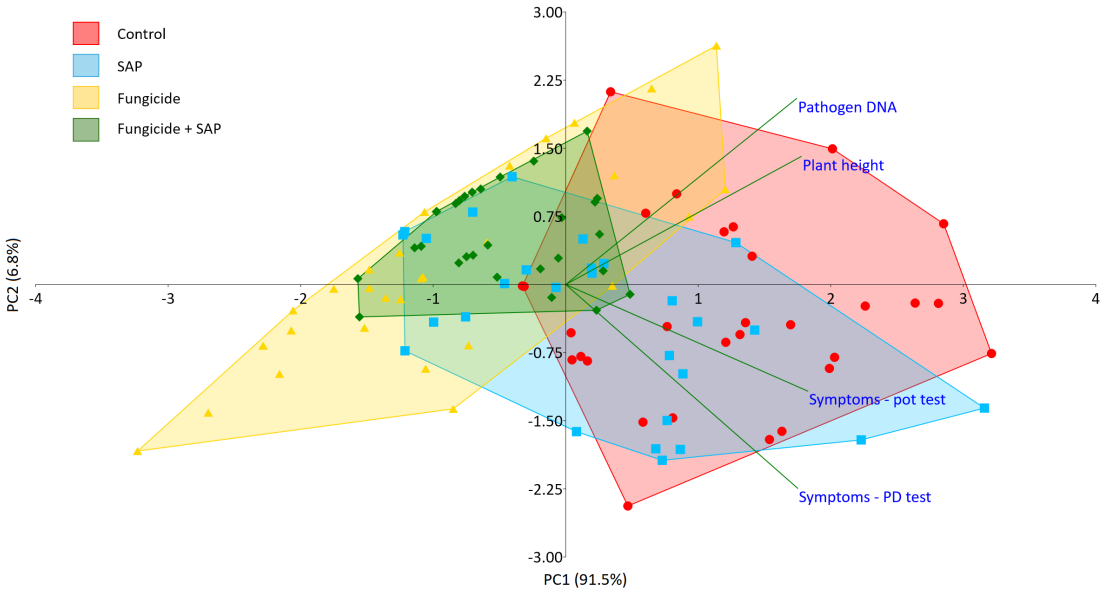
A similar trend of pathogen attack in this cultivar and the highest infection rate in the control variant was also confirmed by measuring the amount of pathogen DNA using real-time PCR (Figure 2c), although no statistical differences among these variants were found. On the other hand, in cv. Bojos, seed treatments significantly differred from each other. The lowest amount of pathogen DNA was measured in the combined treatment with fungicides and SAP; the highest amount was not measured in the control variant but in the SAP treatment (Figure 2c). Application of SAP here showed the opposite effect compared with cv. Laudis.

Based on PCA and principal component 1 (PC1), evaluated parameters such as plant height, disease symptoms in Petri dish and pot tests, and the amounts of pathogen DNA were positively correlated with samples of control and SAP variants in cv. Bojos (Figure 5a). In contrast to the previous two variants, the samples from fungicide and fungicide + SAP variants of this cultivar were negatively correlated with these parameters based on PC1 and

showed lower variability within their treatment. However, there is a clear differentiation of individual variants and subtle overlaps of a few samples between pairs of control and SAP variants, as well as fungicide and fungicide + SAP variants. In the case of the cv. Laudis, the distribution of samples according to variants was ambiguous (Figure 5b). Only the samples of the control variant (except for one sample) had a positive correlation with the evaluated parameters according to PC1. The samples from other variants were located in the middle of the PCA plot without a clear distinction. Using PERMANOVA, a statistical comparison of the values from all principal components from PCA showed strong significant differences between all variants in the cv. Bojos. In the cv. Laudis, PCA shows no statistically significant difference between fungicide and fungicide + SAP variants (Table 1).



(**a**)



(**b**)

Figure 5. The principal component analysis constructed from evaluated parameters of cv. (**a**) Bojos and (**b**) Laudis. Both graphs with PC1 and PC2 explained over 98% of the variability in the data. C—untreated seeds, SAP—polymer-treated seeds, F—seeds treated with fungicides, and F + SAP—combined treatment.

Table 1. Pairwise comparisons among variants analyzed using PERMANOVA with Euclidean distance; *p*-values below the diagonal correspond to cv. Bojos and above diagonal to cv. Laudis. C—untreated seeds, SAP—polymer-treated seeds, F—seeds treated with fungicides, and F + SAP—combined treatment.

	C	SAP	F	F + SAP
C	-	0.0013	0.0001	0.0001
SAP	0.0002	-	0.0001	0.0001
F	0.0001	0.0001	-	0.0750
F + SAP	0.0001	0.0001	0.0001	-

4. Discussion

B. sorokiniana is a seed-borne pathogen, but the infection of germinating plants also takes place through infected post-harvest residues in the soil [20]. Testing of germinating plant infections can be performed in laboratory conditions in Petri dishes or in soil. Infection of seeds with a fungal pathogen can be simulated by briefly soaking the seeds in a suspension of the pathogen [24], and the evaluation of the development of disease symptoms is then carried out during seed germination on filter paper. In our case, the methodology was slightly adjusted. SAP-coated seeds absorb liquid extremely quickly, which could cause seeds to adhere much more inoculum to their surface than uncoated seeds. Therefore, the seeds were infected by pipetting suspension directly under the seed; hence, all types of seed treatment were infected with the same dose of conidia. This method of application was proven to be suitable, with a high frequency of infected plants in the test. Soil infection can be simulated by mixing the inoculum in the form of ground grains of wheat or barley [25], millet seeds, buckwheat, or pearl barley [26] overgrown with mycelium of the pathogen. In the current experiment, the previously developed and confirmed method of wheat infection with *Fusarium culmorum* [18] was used. Therefore, the soil infection was simulated by applying barley groats overgrown with mycelium in the center of the pot and placing the tested seeds in a circle around its edge.

In the Petri dish test, we found that the application of SAP alone to the seed of cv. Bojos did not affect the disease symptoms in plants. On the contrary, symptoms were statistically significantly lower in SAP-treated seeds compared with untreated seeds in cv. Laudis (Figure 2a). The same response was observed in plants of both cultivars in the pot experiment; however, the differences were not statistically significant for either cultivar. Although, cv. Laudis, treated with SAP again, showed weaker disease symptoms compared to the control (Figure 2b). In the Petri dish test, fungicide-treated seeds (F or F + SAP) were asymptomatic (Figure 2a). In the pot test, plants treated with fungicides, with or without SAP, showed the same level of infection, i.e., SAP did not significantly affect the efficacy of fungicides (Figure 2b).

Disease symptoms of cv. Laudis corresponded with the content of pathogen DNA in the tested plants. In cv. Bojos, the highest amount of pathogen DNA was measured in the variant with SAP alone, although visual symptoms did not indicate this. However, the spread of the pathogen in the plant tissue continues gradually, and confirmation of the infection using DNA analysis can precede visual manifestations of the disease on the plant [27]. A probable reason for the higher amount of pathogen DNA may be that the SAP applied as a seed coating has a role at the time of germination and its potential protective function, confirmed in cv. Laudis, gradually weakens. And SAP, even in such a small amount, retains the ability to maintain moisture around plant roots, thereby improving conditions not only for the plant itself but also for the development of the fungal pathogen. This may point to the fact that SAP applied to seeds plays a protective function against pathogens only at a very early stage, during seed germination; later, its effect may vary, depending on the crop, pathogen, and cultivation conditions. However, this negative effect was observed only in this one case, and further verification is needed. For agricultural practice, however, this observation may not be significant at all since this experimental

variant is not standardly used in crop growing because farmers almost exclusively sow cereal seeds treated with fungicide. However, to investigate its influence, we also included a variant with SAP alone in the experiments because there was demonstrated a possible positive effect of the polymer in protection against the fungal pathogen *F. culmorum* in the previous study [18]. In the present experiment, we observed this phenomenon only in the cv. Laudis. If SAP was applied to seeds treated with fungicides, the amount of pathogen DNA was statistically the same in cv. Laudis and even lower in cv. Bojos than in variants with fungicides alone (Figure 2c).

Summarizing all evaluations through PCA analysis, variants in the cv. Bojos were separated more significantly based on fungicide application than SAP, while in the cv. Laudis, all seed treatments partially overlapped (Figure 5). By using PERMANOVA, treatments in the cv. Laudis differed from each other, except for treatments F and F + SAP. In the cv. Bojos, these two variants were also separated from each other, and this difference was probably caused by a statistically significant difference in the amount of the pathogen DNA, where the content was lower in the variant with combined treatment (F + SAP). These results support the hypothesis that SAP does not negatively affect the efficacy of fungicides applied to the seed surface. If a difference was detected, it was in favor of the combined treatment of SAP with fungicides. The neutral effect of SAP application on the effectiveness of fungicides aligns with findings from our previous work, which investigated the attack of wheat and corn plants by the pathogen *Fusarium culmorum* [18]. In that case, even SAP alone partially protected maize seeds against pathogen attack in the case of strong soil infection.

Despite intensive attention to preparation and evaluation of the efficacy of smart pesticides with controlled release, reduced mobility, and phytotoxicity for crops and the environment using naturally and synthetic-based superabsorbents [17,28–34], the lack of scientific works evaluating the impact of seed coating with SAP after pesticide treatment on their effectiveness in plant protection is still observed. Coating seeds with protective compounds enables their accurate application and represents the first line of protection against adverse biotic and abiotic factors [1,35]. Currently, in the era of changing climate and deepening drought, combined seed treatment with pesticides and SAP provides protection against plant diseases or pests, along with improving water availability for seed germination and crop establishment.

In our experiments, the synthetic polymer Aquaholder®Seed was applied on the seed surface. It was shown that this seed treatment had no negative impact on fungicide effectiveness in controlling the pathogen attack of *B. sorokiniana*. This also provides prerequisites for the use of such an approach in the application of alternative protective agents, as was already confirmed in the study applying oregano essential oil combined with natural polymers in the protection of faba bean against a fungal pathogen [6] or maize seeds coated with bacteriophages in polyvinyl polymer to protect them against Goss's wilt [36].

Based on legislative changes in the EU, synthetic hydrogels will have to be replaced by more ecological polymers in the next few years due to their limited biodegradability, which may be further aggravated by prolonged dry periods due to climate change [37]. According to the Regulation of the European Parliament (EU) 2019/1009, the polymer used to regulate the release of nutrients or increase the water retention capacity must meet the criteria of biodegradability from 2026, i.e., they must be capable of physical and biological decomposition in natural soil conditions and must not lead to the accumulation of plastics in the environment [38]. From the perspective of plant protection, hydrogels based on polysaccharides, especially those containing chitosan, are seen as promising [1]. The demand for biodegradable polymers will certainly continue to grow. To become widely applicable in crop cultivation, biodegradable polymers are still under development to improve their biocompatibility, renewability, mechanical strength, and water retention capacity [3,39]. One approach involves the preparation of biodegradable semi-synthetic SAPs based on natural polymers with grafted chains of synthetic polymers, combining the benefits of both synthetic and natural polymers [40,41]. Such polymers appear promising

in agricultural practice as hydrogels retaining water and promising carriers of fertilizers, pesticides, and microorganisms [42]. Their intensive development is based on the previous results of the study of synthetic and natural polymers. However, some aspects, including the effect of SAP on pesticide efficacy, have still not been sufficiently investigated from a scientific point of view.

5. Conclusions

This study aimed to evaluate the effect of SAP coating on the efficacy of fungicidal seed treatment in the protection of emerging plants of two barley cultivars against the fungal pathogen *B. sorokiniana*. The seeds were first treated with a combined fungicide preparation, followed by applying the SAP coating. The results obtained in the Petri dish test and the pot test after the infection with the pathogen showed that SAP does not have a negative impact on fungicides. SAP did not affect the frequency of seed germination, seedling emergence, plant height, and visual symptoms of the disease in the fungicide-treated variants. Moreover, in cv. Laudis, the application of SAP alone was shown to partially protect emerging plants, confirming the results of our previous study with maize infected with *F. culmorum*. Based on these results, it is possible to use superabsorbent polymer seed coating technology to mitigate the impact of climate change, especially drought, on seedling emergence without negatively affecting the effectiveness of fungicides applied to seeds to protect them against fungal pathogens.

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