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Potential of Limestonevirus Bacteriophages for Ecological Control of *Dickeya solani* Causing Bacterial Potato Blackleg

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Abstract: Pectinolytic bacteria of the family *Enterobacteriaceae*, specifically *Dickeya solani*, are known to cause potato blackleg. This study aimed to evaluate the effectiveness of a mixture of two bacteriophages from the genus *Limestonevirus* in controlling *Dickeya solani* in both greenhouse and field trials. The potential of bacteriophages for ecological potato control was also assessed. The phages φDs3CZ and φDs20CZ were isolated from soil in the Czech Republic between 2019 and 2021. They were applied preventively and curatively as a solution on artificially wounded and inoculated seed tubers immediately prior to planting. The phage-treated variant showed a highly significant reduction in the extent of *D. solani* infection compared to the untreated control in both the greenhouse and field trial. The effect of the phages depended on the concentration of the solution, the rate of tuber injury, and the sequence of application. When applied preventively, the phages caused a significantly higher reduction in the rate of blackleg symptoms (86.7% and/or 87.1%) compared to the curative application (54.6 and/or 36.6%). Phages φDs3CZ and φDs20CZ showed potential for use in biological potato control against *Dickeya solani*.

Keywords: *Enterobacteriaceae*; *Solanum tuberosum*; *Limestonevirus*; blackleg; biological control

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1. Introduction

Potatoes (*Solanum tuberosum* L.) are a globally significant crop that is susceptible to various pathogens [1]. The most serious pathogens, in terms of production losses, are bacteria belonging to the genera *Pectobacterium* and *Dickeya*, which are part of the *Enterobacteriaceae* family [2]. These bacteria cause bacterial blackleg and potato soft rot [3,4]. During infection, the bacteria produce characteristic extracellular pectinolytic enzymes that degrade the plant cell wall, resulting in typical tissue maceration [1,5]. They can live as epiphytes or facultative saprophytes in soil and groundwater [6]. *Dickeya* species (formerly *Erwinia chrysanthemi*) are Gram-negative, facultative, anaerobic plant-pathogenic bacteria that infect a wide range of economically important crops and ornamental plants worldwide [5]. Most direct production losses in potatoes are caused by quality losses or seed rejection during certification [7]. *Dickeya* spp. have been reported to reduce potato yield up to 25% [8].

D. solani van der Wolf et al. 2014 was already described in 2009 [9]; however, it was established as a new species in 2014 [10]. *D. solani* is a regulated non-quarantine pathogen under European Community law. It is particularly spread by seed and ware potatoes [7] and/or through irrigation water [11]. *D. solani* strains are considered more aggressive than other blackleg-causing bacteria. For disease development, they need lower optimal temperatures and also lower inoculum levels for infection threshold [1,7].

In comparison with other *Dickeya* species, *D. solani* is highly pathogenic on potato plants [12], and has a higher ability to colonize potato roots and spread through the plant vascular system [13]. Effective spreading of an infection, even with a small amount of inoculum and a wider temperature range favorable for the disease development, creates a competitive advantage [14,15]. However, the bacterium is not able to survive in soil for more than three weeks without its host [7]. *D. solani* strains isolated from potato showed to be genetically related, although they were collected from geographically distant places. Within the species, it indicates a very limited diversity. The bacterium spreads from infected seed tubers to the stems of new potato plants [4]. On potato plants, the bacterium induces stem lesions to extensive soft rot and stem dying [16]. Under lower soil moisture, stem bases may soften, turn brown or inky black, and shrivel. Leaves on such stems are stunted, stiff, rolled upwards, and are yellowish, red or could be bronze in color. The affected stems are also stunted, more upright and light green. Affected plants frequently die early and/or a yield reduction occurs [4]. No effective chemical substances are currently available to control *Dickeya* spp. Infection, and this could result in high economic losses [5,17]. No grown potato cultivars are resistant to *D. solani* [18]. Using bacteriophages is an attractive option of biological plant control [5,19,20]. Usually, phages indicate a high specificity to bacterial hosts. Their use against bacteria is quite safe, since they cannot infect eukaryotic cells. For biological plant control, only so-called lytic bacteriophages that lyse a bacterial cell at the end of the infection could be used. Their production in the laboratory is relatively easy and cheap [19,21]. Phages able to infect *Dickeya* species have been isolated and described in several studies [5,8,17,22–28]. In nature, the phages from the genus *Limestonevirus* are dominant [22]. The potential using of phages in biological plant control has been evaluated under both laboratory and field conditions [5].

The aim of the study was to evaluate the efficacy of bacteriophages of the genus *Limestonevirus*, isolated from soil in the Czech Republic against the causal agent of bacterial blackleg, *Dickeya solani*. In greenhouse and field trials, the phages were applied preventively and curatively to potato seed tubers immediately before planting. This paper explores the potential of phages for biological potato control of potatoes against *D. solani*. This research is significant for the advancement of biopesticides.

2. Materials and Methods

2.1. Bacteriophages and Bacterial Strains

From 2019 to 2021, soil samples were collected from potato fields in various locations across the Czech Republic in order to find specific phages for *D. solani* as described by Petrzik et al. [28]. Phages φDs3CZ and φDs20CZ were propagated in *Dickeya* sp. CPPB-050 host, and purified as described in [28]. Bacterial concentration was measured using optical density, where an OD600 = 1 was equivalent to 10^9 CFU/mL [29]. The experimental phage solution consisted of a 50% mixture of φDs3CZ phage and 50% φDs20CZ phage. This mixture was used to prevent the emergence of *Dickeya* mutants that are resistant to either phage.

The bacteria were obtained from the Collection of Plant Pathogenic and Agriculturally Beneficial Bacteria of the Crop Research Institute (CPPB) in Prague-Ruzyně, Czech Republic. The *D. solani* strain CPPB-050, which exhibits high pectinolytic activity and sensitivity to both phages, was isolated from the potato variety 'Laura' grown in the Czech Republic [28]. The bacteria were placed on plates containing King's Medium B Base (HiMedia Laboratories Pvt. Ltd., Mumbai, India) and incubated at room temperature for 48 h prior to the experiments. Then, they were scraped from the plates and suspended in sterile distilled water at a concentration of (5×10^5 CFU/mL).

2.2. Determination of Varietal Sensitivity to D. solani

To test the sensitivity of potato varieties to *D. solani* CPPB-050, 17 genotypes from the breeding company VESA Velhartice a.s. (Czech Republic) were selected (Table 1). In the greenhouse, young plants were infected with a bacterial suspension using a syringe. Healthy tubers from individually evaluated varieties were washed with tap water

to remove soil, disinfected with a 1% solution of sodium hypochlorite (NaClO) (Unilever, Bohumín, Czech Republic) for 15 min, rinsed with sterile water, and air-dried overnight. Eyes (n = 45) were cut from tubers of individual varieties and left to dry and develop suberization at room temperature for one day. The eyes were then planted into a garden substrate in the greenhouse and cultivated at 18–22 °C to grow plants. Only one stem was left for subsequent inoculation. A suspension of *D. solani* (strain CPPB-050) was used for inoculation. The bacterial suspension (5×10^5 CFU/mL) was injected into the plant at 8 cm height using a syringe, and a stem puncture was made at approximately 0.5–1 cm height above the substrate. To ensure uniform plant growth, the greenhouse temperature was maintained between 15–18 °C prior to infection. After infection, the temperature in the greenhouse ranged from 18 to 25 °C. The time interval with the temperature lower than 20 °C had to be as short as possible. Following stem inoculation, a high relative humidity of 70–100% was maintained in the greenhouse. A negative control (without inoculation) was involved in the trials. The tests were conducted in 2019, 2020 and 2021. Plant infection was assessed on the 8th day after infection using a scale of varietal resistance ranging from 9 (uninfected stems) to 1 (dead plants), as described by Zadina and Jermoljev [30].

Table 1. Potato varieties used in the experiment.

Variety	Maturity	Utilization
'Alice'	early	fresh consumption
'Bella'	medium-early	fresh consumption, French fries
'Bohemia'	early	fresh consumption
'David'	medium-early to medium-late	starch
'Dominika'	medium-early	fresh consumption
'Jasmina'	early	fresh consumption
'Jindra'	medium-late to late	fresh consumption
'Katy'	very early	fresh consumption
'Magda'	very early	fresh consumption
'Mariannka'	very early	fresh consumption
'Monika'	early	fresh consumption
'Primarosa'	very early	fresh consumption
'Red Anna'	medium-early	fresh consumption
'Suzan'	very early	fresh consumption
'Verne'	medium-early to medium-late	starch
'Vysočina'	medium-early	fresh consumption
'Westamyl'	medium-late	starch

2.3. Greenhouse Experiments of the Efficacy of Lytic Phages on Dickeya solani

The effectiveness of the phages φDs3CZ and φDs20CZ on *D. solani* was investigated through artificial inoculation of potato tubers (variety 'Red Anna') in a greenhouse pot experiment. The tubers were prepared for the experiments following the method described in [31]. Immediately prior to planting, the tubers underwent severe mechanical wounds through cross-section, while the stem-ends and bud-ends were mildly pricked with a sterile steel needle (diameter: 3 mm, length: 10 mm). A phage suspension was applied either preventively before bacterial inoculation or curatively after bacterial inoculation. When applied preventively, 10 µL of phage solution at concentrations of 1×10^6, 1×10^7 and 1×10^8 were dropped onto the prepared wounds using a pipette. The tubers were left for 10 min to diffuse the solution into tissues. Then, 10 µL of a bacterial suspension (strain *D. solani* CPPB-050 5×10^5 CFU/mL) was inoculated into the wounds using a pipette. When applied for curative purposes, the bacterial solution was first dropped onto the prepared wounds (mild injury). The tubers were left for another 10 min. Then, a phage solution with a concentration of 1×10^7 PFU/mL was applied into the wounds. The positive control consisted of the variant without phage treatment, where only a bacterial suspension was inoculated. The negative control was the variant where sterile distilled water was applied. Each trial variant used 21 tubers, which were immediately planted into

10 × 10 cm containers with garden substrate after inoculation. The containers were then transferred to the greenhouse and the plants were cultivated at 18–25 °C. Three replications were performed for each trial to ensure accuracy. During the growing season (5–6 weeks after planting), we evaluated the occurrence and severity of blackleg disease. We visually checked the plants for wilting, leaf chlorosis, black stem rot, haulm drying and/or plant dying. The disease severity index (DSI) was calculated using the formula DSI = [Σ (disease scale frequency × score of disease scale)]/[(total number of stems) × (maximal disease severity index)] × 100 [%]. We utilized a disease severity scale ranging from 0 to 5 (refer to Table 2), where 0 indicates healthy plants and 5 indicates deceased plants.

Table 2. Scale of disease severity.

Degree of Infection	Symptoms on Plants
0	uninfected plant
1	stem blackening in the extent of 1–25%
2	stem blackening in the extent of 26–50%
3	stem blackening in the extent of 51–75%
4	stem blackening in the extent of 76–99%
5	the whole plant is affected by stem blackening and dies

2.4. Field Trials on the Efficacy of Lytic Phages on D. solani

A small-plot field trial was carried out on the test site described in [31]. The experimental rows were separated by one row of the untreated variety 'Monika' and, at the end of each row, one plant of the untreated variety 'Monika' was grown.

Planting was conducted on 28 April 2022 using seed tubers of the 'Red Anna' variety with a size of 35–45 mm. The tubers were either severely mechanically wounded with a cross-section or mildly pricked with a needle, as described above. The chance for bacteria to enter the tubers through the wounded area was approximately 1000 times smaller in the case of mild injury. Wounded tubers were immersed in a suspension of *D. solani* CPPB-050 with a density of 5×10^5 CFU/mL and treated with a mixture of phages φDs3CZ and φDs20CZ at a concentration of 10^7 PFU/mL. The phage suspension was applied either preventively, before bacterial inoculation, or curatively, after bacterial inoculation, by immersing the tubers in the phage solution for 5 s. The positive control using a bacterial suspension without phages. The bacterial suspension and phage solution were applied on the day of planting. The treated tubers were kept out of sunlight until planting to prevent interference from UV irradiation.

During the growing season, which was 5–6 weeks after planting, we evaluated the trials. We determined the occurrence and disease severity of bacterial blackleg for individual hills. We visually checked the plants for blackleg symptoms, and DSI was calculated as described in Section 2.4. After 60 days of planting, the trial field was documented using a 20 Mpx UAV camera EVO Lite+ (Autel Robotics, Shenzen, China).

2.5. Statistical Assessment of the Experiments

Statistical analysis of the experiments was performed using variance analysis (ANOVA) and Tukey's HSD test ($p < 0.01$; software STATISTICA 7; Statsoft, Tulsa, OK, USA). Statistical significance was determined between the treatments with and without phage application.

3. Results

3.1. Determination of Potato Varietal Sensitivity to D. solani

In greenhouse experiments performed in 2019, 2020 and 2021, the sensitivity of selected potato varieties from the breeding company Vesa Velhartice a.s. to pectinolytic bacteria that cause potato blackleg was evaluated (Table 3). The sensitivity of potato varieties to the bacteria differed. No resistant (tolerant) varieties to *D. solani* were found among the evaluated range of potato genotypes. 'Bella', 'Vysočina' and 'Katy' were ranked as partially resistant (tolerant) to *D. solani*. None of the varieties showed strong susceptibility to the

evaluated bacteria. Therefore, the highly susceptible variety 'Red Anna' was selected for the greenhouse and field trial [31].

Table 3. Sensitivity of potato genotypes to *D. solani*.

Sensitivity of Genotypes	Mean Degree of Infection	*Dickeya solani*
relatively resistant	8.99–7.50	-
partially resistant (tolerant)	7.49–6.00	'Bella', 'Vysočina', 'Katy'
susceptible	5.99–4.50	'Verne', 'Monika', Suzan', 'Dominika', 'Mariannka', 'David, 'Primarosa', 'Magda'
highly susceptible	4.49–3.00	'Jindra', 'Jasmína', 'Red Anna', 'Bohemia', 'Westamyl', 'Alice'
very highly susceptible	2.99–1.00	-

3.2. Greenhouse Tests of Phage Efficacy on D. solani

In greenhouse tests, plants grown from seed tubers treated only with sterilized water showed no signs of infection, with only rare occurrences of primary plant infection. However, plants grown from tubers of both injury variants inoculated with *D. solani* showed severe infection intensity, including wilting, leaf chlorosis, black stem rot, haulm drying or plant dying (Figure 1). A highly statistically significant difference in the extent of *D. solani* infection was observed between the phage-treated variant and the untreated control for all used phage concentrations (Figure 2). The higher the concentration of the phage solution, the greater the reduction in plant infection intensity was detected. A concentration of 10^8 PFU/mL of phages resulted in a 95.3% and/or 79.7% decrease in infection intensity compared to the untreated control, while a concentration of 10^6 PFU/mL resulted in a 51.5 and/or 46.3% reduction. Further tests were conducted using a concentration of 10^7 PFU/mL due to limited production capacity of highly concentrated phage solutions.

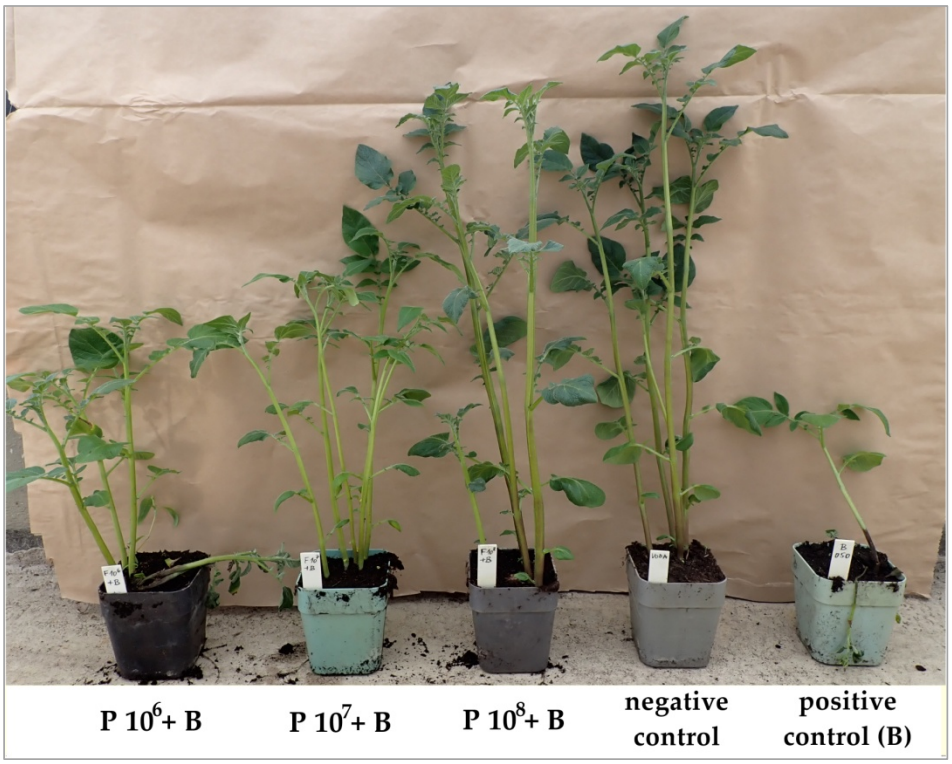

Figure 1. Evaluation of the efficacy of φDs3CZ and φDs20CZ phage solution application at a concentration of 10^6–10^8 PFU/mL using artificial inoculation of tubers (mild injury) in the greenhouse pot trial (P—phage, B—bacteria).

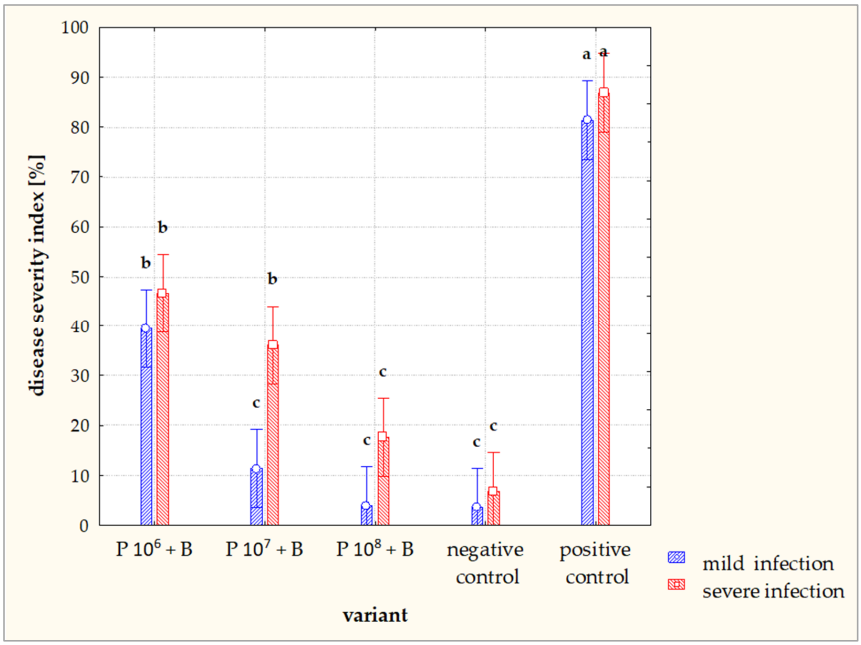

Figure 2. Mean intensity of potato plant infection (% disease severity index) after application of φDs3CZ and φDs20CZ phage solution at a concentration of 1×10^6, 1×10^7 and 1×10^8 PFU/mL to mildly and severely wounded seed tubers; greenhouse test (P—phage, B—bacteria). Statistical assessment of the experiments was performed using variance analysis (two-way ANOVA). All data are means of three replications; each replication is represented by 21 plants; various letters over bars mean significant differences ($p < 0.01$) based on the Tukey's HSD test. Vertical bars indicate 0.99 confidence intervals.

In further experiments, the efficacy of preventive and curative tuber treatment with phages (order of phage and bacteria application) was investigated. Plants grown from tubers treated with sterilized water did not show any infection symptoms. On the contrary, plants grown from tubers inoculated with *D. solani* strain showed severe symptoms of an infection, as wilting, leaf chlorosis, black stem rot, haulm drying and/or plant death. After treating the tubers with the phage solution, a significant reduction in *D. solani* plant infection was observed in both variants of phage application (Figure 3). Plants grown from tubers that were preventively treated with a mixture of phages (10^7 PFU/mL) showed mild disease symptoms, similar to the negative control (Figure 4). The efficacy of the phage solution depended on the order of application. Preventive treatment of tubers with a phage solution prior to inoculation with a bacterial pathogen resulted in a relative decrease in infection intensity of 86.7%. Conversely, curative application of a phage solution after inoculation with the bacterium resulted in a much lower decrease in infection intensity, averaging only 54.6%.

3.3. Field Trials on the Efficacy of Lytic Phages on D. solani

The results of the greenhouse experiments were verified in a small-plot trial under real field conditions. Plants grown from tubers inoculated with *D. solani* strain showed severe infection symptoms. The efficacy of the phage solution was dependent on the order of application, similar to the greenhouse experiments. The effect of the phages was also dependent on the degree of mechanical damage to the tuber. Preventive and curative treatment of mildly wounded seed tubers with the phage solution resulted in a highly significant decrease in *D. solani* infection level compared to the untreated control (Figure 5). Differences were also apparent in a UAV image (Figure 6). Preventive application of phages resulted in a relative decrease in infection of 87.1%. On the contrary, a curative application of the phage solution on mildly damaged seed tubers resulted in a lower reduction of plant infection level compared to the untreated control (by 36.6%). Highly significant differences

were found between the untreated control and the preventive and curative phage application in severely damaged tubers (Figure 5). Differences were also apparent in a UAV image (Figure 7). Preventive application of phages to severely injured tubers resulted in a relative decrease in infection of 26,2%. In contrast, curative application of phage solution to severely injured seed tubers resulted in a relative decrease of 18.9% in infection.

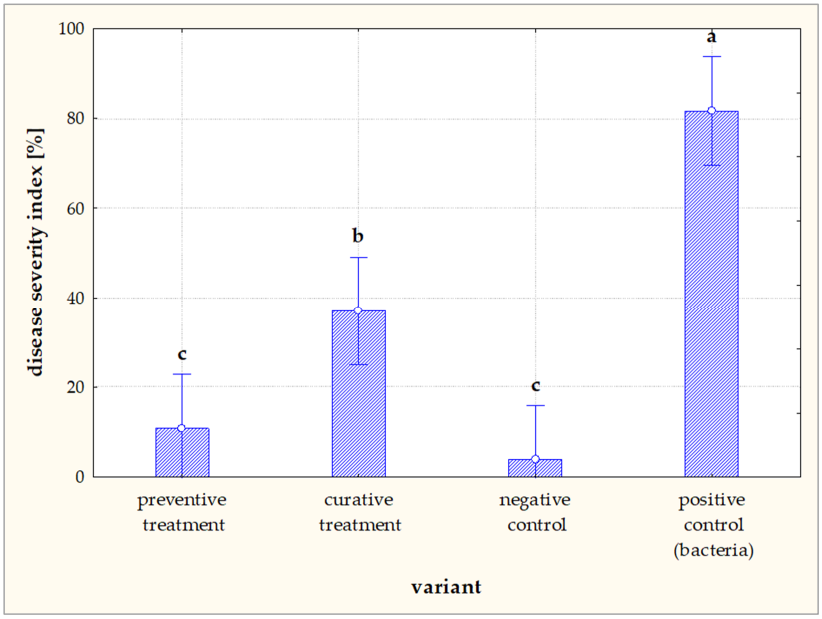

Figure 3. Mean intensity of potato plant infection (% disease severity index) after preventive and curative application of φDs3CZ and φDs20CZ phage solution at a concentration of 1×10^7 PFU/mL; greenhouse test. Statistical assessment of the experiments was performed using variance analysis (one-way ANOVA). All data are means of three replications; each replication is represented by 21 plants; various letters over bars mean significant differences ($p < 0.01$) based on the Tukey's HSD test. Vertical bars indicate 0.99 confidence intervals.

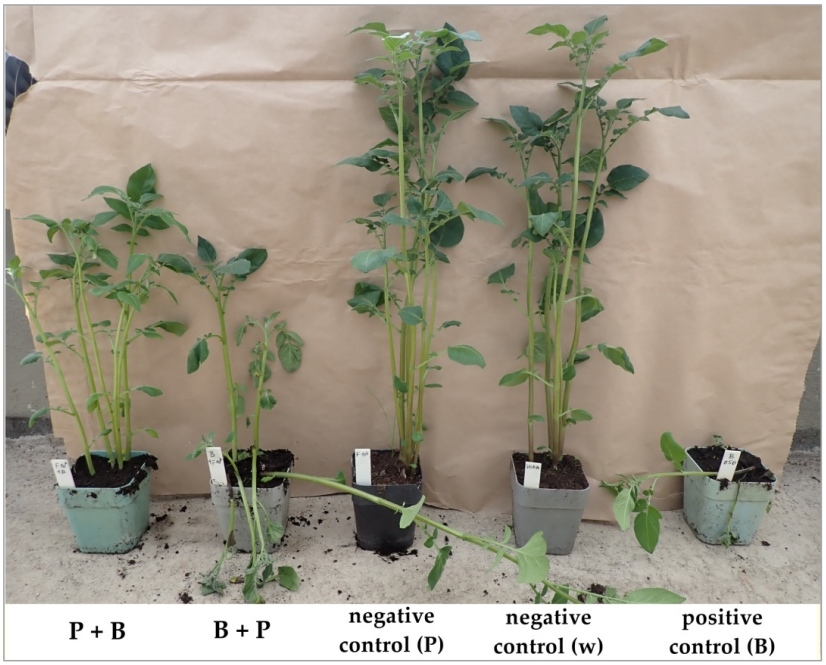

Figure 4. Evaluation of the efficacy of preventive (P + B) and curative application (B + P) of φDs3CZ and φDs20CZ phage solution at a concentration of 10^7 PFU/mL using artificial inoculation of tubers in the greenhouse pot experiment (P—phage, B—bacteria, w—water).

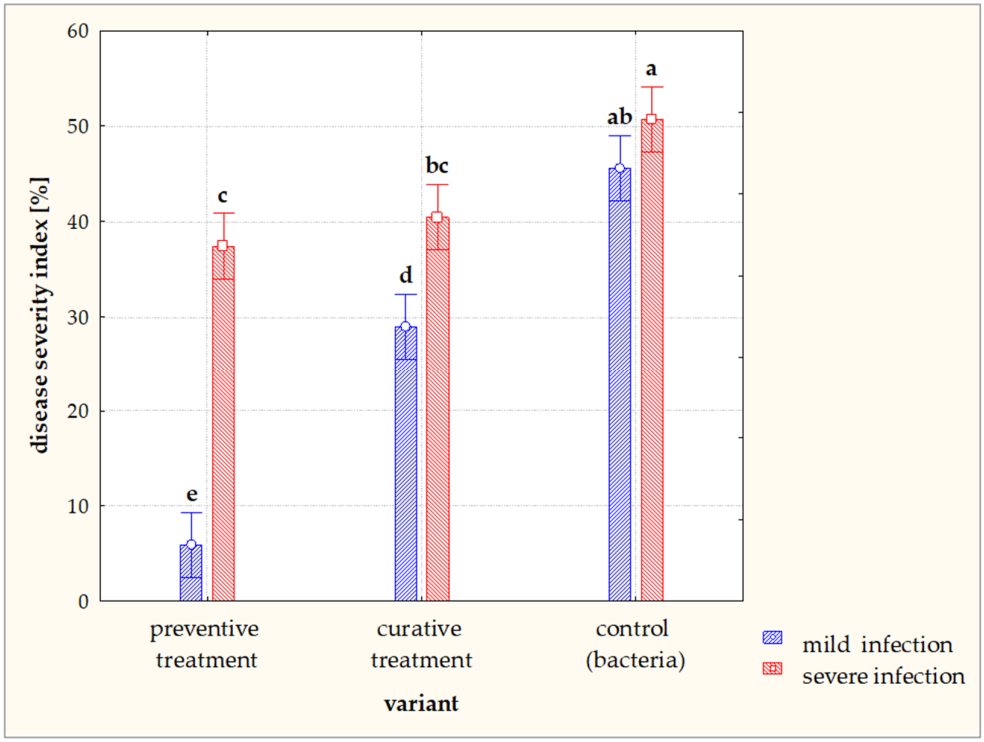

Figure 5. The efficacy of preventive and curative application of the phages φDs3CZ and φDs20CZ on blackleg occurrence in the field trial. Statistical assessment of the experiments was performed using variance analysis (two-way ANOVA). All data are means of three replications; each replication is represented by 90 potato hills; various letters over bars mean significant differences ($p < 0.01$) based on the Tukey's HSD test. Vertical bars indicate 0.99 confidence intervals.

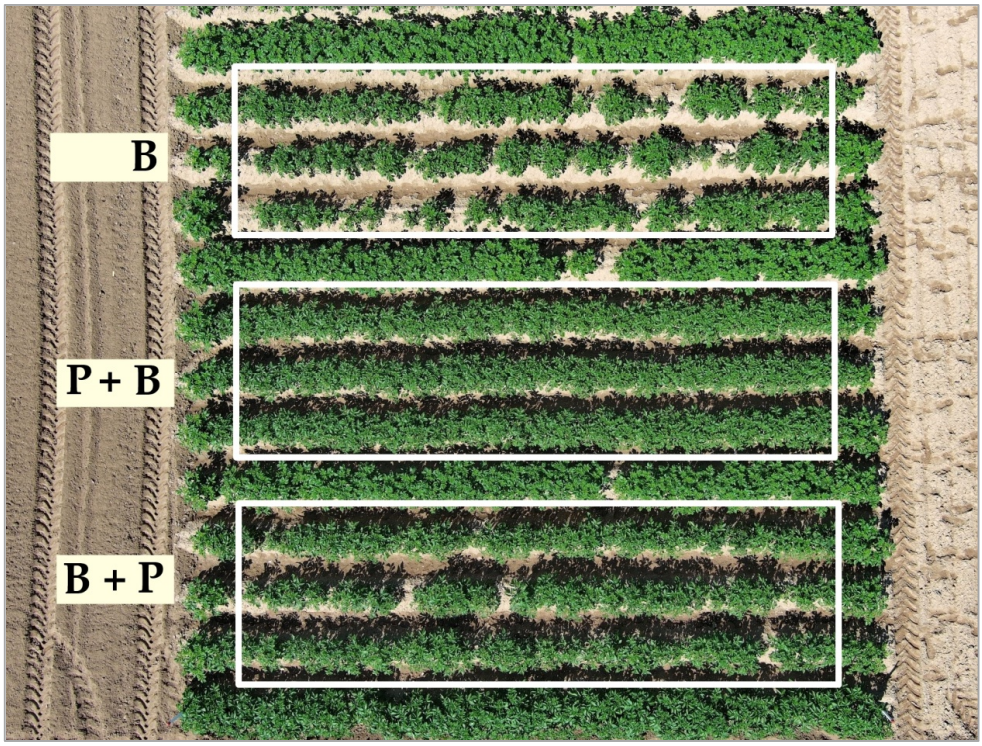

Figure 6. The efficacy of preventive (P + B) and curative (B + P) application of the phages φDs3CZ and φDs20CZ to seed tubers in the field trial (mild tuber injury; UAV view on one replication of the variants) (P—phage, B—bacteria).

Figure 7. The efficacy of preventive (P + B) and curative (B + P) application of the phages φDs3CZ and φDs20CZ to seed tubers in the field trial (severe tuber injury; UAV view on one replication of the variants) (P—phage, B—bacteria).

4. Discussion

The first bacteriophages effective against *Dickeya* spp. and *Pectobacterium* spp. were described in the late 1980s [32]. These phages showed host specificity and were isolated from soil and potato rhizosphere. Czajkowski [23] isolated lytic phages (*Myroviridae*, *Caudovirales*) that were effective against different *Dickeya* species. He also described two phages (φD3 and φD5) that share the genomic organization LIMEstone1. Day et al. [8] described 67 bacteriophages, most of which were species from of the *Myoviridae* family. Andriaenssens et al. [5] published the efficacy of the phages LIMEstone1 and LIMEstone2 against *D. solani*. *D. solani*-infecting phages were isolated from soil samples collected in different regions of Poland and were able to completely stop the growth of *D. solani* under in vitro conditions and protect potato tubers from maceration caused by the bacterium [23]. A unique characteristic of Limestoneviruses is their high number of mobile elements (homing endonuclease), which can be up to 23 in the genome [28].

The high specificity of the bacteriophages is a major drawback for use against bacterial plant pathogens in agriculture [19]. Usually, a specific phage is only able to infect and lyse certain strains of a bacterial species [33]. Studies have shown that *P. carotovorum* subsp. *carotovorum* strains isolated from potato exhibit genetic diversity [34], but *P. wasabiae* and *D. solani* are generally considered to be more genetically homogeneous [7]. While *P. carotovorum* subsp. *carotovorum* is considered to be the dominant cause of blackleg in Europe, *P. wasabiae* and *D. solani* are major causal agents of potato soft rot during storage and potato transport [3]. The use of lytic bacteriophages with a wide host range could be further combined with other methods to control bacterial infections in potatoes [1].

In agricultural practice, the phages could be applied by spraying seed potatoes at planting. Another option is to apply the phages by cold fogging to potatoes stored in bulk or in forced ventilated boxes prior to unloading. Spraying plants with phages is ineffective, due to the rapid degradation of phages by solar radiation. In practice, potato tubers could also be sprayed with a phage suspension prior to packaging. Several discovered and described phages have already been used in agricultural production. There is a commercial product,

Biolyse™ (APS Biocontrol Ltd., Dundee, Scotland), a cocktail of phages capable of targeting *Pectobacterium* and *Dickeya* species. It has been developed as a potato washing solution [8].

In our greenhouse and field trial, the efficacy of the bacteriophages of the genus *Limestonevirus* (family *Ackermannviridae*) isolated from soil in the Czech Republic, against *Dickeya solani*, the causal agent of bacterial blackleg, was determined. The phages were applied prophylactically and curatively to seed potato tubers immediately prior to planting. In both experiments, a highly significant reduction in plant infection levels was observed after tubers were treated with a phage solution prior to planting. The obtained results support the conclusions of previous studies by other authors, which focused on the efficacy of phages of the genus *Limestonevirus* on the pectinolytic bacteria of the family *Enterobacteriaceae.* Czajkowski et al. [23] discovered that using biological tests with the phages φD1, φD2, φD3, φ8D4, φD5, φD7, φD9, φD10, and φD11 resulted in a 30–70% reduction in the incidence of *D. solani* soft rot on tuber slices compared to slices inoculated only with the pathogen. Carstens et al. [22], who isolated bacteriophages from wastewater and organic waste, confirmed a 48.9 and 93.3% reduction in the incidence of *D. solani* tuber infection after the application of bacteriophages. Czajkowski et al. [25] reported a significant reduction of soft rot symptoms by 80 to 95% after application of phages φPD10.3 and φPD23.1, compared to controls inoculated only with a bacterial suspension. Adriaenssens et al. [5] isolated *D. solani*-infecting LIMEstone bacteriophages from a potato field that were effective in both laboratory and field trials, and that resulted in an increase in potato yield. In our study, phage efficacy was dependent on solution concentration and application sequence. The application of phages to tubers before bacterial inoculation resulted in a statistically significant reduction in blackleg symptom development compared to the application of phages after bacterial inoculation. Muturi et al. [26] had similar results in their study. Although the application of the phage solution did not completely eliminate the infection, it resulted in an effective suppression of *D. solani* blackleg development, which is consistent with the studies of Ravensdale et al. [35], Czajkowski et al. [23], Carstens et al. [22] and Muturi et al. [26], among others. The possible emergence of bacterial resistance to the phages in the mixtures is not yet fully understood. To reduce the probability of the development of potential bacterial resistance to all phages, a genetically diverse set of phages could be used. Because the stability of bacteriophages is affected by various environmental factors, including temperature [36], the viability of phages should be maintained for a long time after application by using an appropriate formulation. The use of phages in pest control is limited by their sensitivity to UV radiation and low ambient humidity [20]. According to Czajkowski et al. [37], the phages effective against *D. solani* maintain their viability on the surface of potato tubers for several weeks. The causal agent of blackleg may not be able to survive the winter in central Europe, and seems to be localized in various sites after planting tubers that are latently infected [38]. Fortunately, the soil contains numerous phages that can limit the abundance of bacteria [39]. However, phages require their hosts to exist and be available, without which they quickly disappear from the environment. In the experiment described in [31], the bacteriophages φDs3CZ and φDs20CZ were detected in 6 out of 15 soil samples where they were applied in the middle of the growing season. At the end of the growing season, however, they were only detected in 1 out of 15 samples. Therefore, effective protection against *Dickeya* requires the addition of bacteriophages to the soil. The use of phages as a preventative measure at the time of planting has been shown to be highly effective in the reliable prevention of disease development [31].

5. Conclusions

The study examined the effectiveness of lytic phages φDs3CZ and φDs20 CZ in reducing potato blackleg caused by *D. solani*. Results showed a significant decrease in plant infection levels when tubers were treated with a phage solution before planting in both greenhouse and field trials. Although the application of the phage solution did not completely eliminate the infection, it resulted in effective suppression of blackleg development o caused by *D. solani*. The effect of the phages depended on the concentration

of the solution, the rate of tuber injury, and the sequence of application. Preventive application of phages to tubers prior to bacterial inoculation resulted in a higher statistically significant reduction in disease symptom development compared to the curative application of phages after bacterial inoculation. Phages φDs3CZ and φDs20 have the potential for biological control of potato blackleg caused by *D. solani*, which is important for the development of new biopesticides. Currently, there are no effective and ecologically acceptable systems for direct chemical of potato against pectinolytic bacteria, so the phages can be used for ecological protection.

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