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METHODS OF LABORATORY ASSESSMENT OF POTATO CULTIVARS FOR RESISTANCE TO BACTERIAL BLACKLEG AND TUBER SOFT ROT
(review)

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Abstract

Bacterial blackleg and soft rot of potato caused by *Pectobacterium carotovorum* subsp. *carotovorum*, *P. atrosepticum*, and *Dickeya* spp. are among the most harmful diseases of potato. Average annual yield losses of potato caused by these bacteria make 10-15 %, but during epiphytoties they may exceed 50 %. Existing commercial potato cultivars do not possess high resistance to these diseases, since the most of current breeding programs do not consider this trait as a priority one. However, in recent years, global potato losses caused by blackleg and soft rot significantly increased that provided a growing demand for resistant cultivars and also for efficient methods for laboratory assessment of breeding material and new cultivars and hybrids for their resistance to these diseases. Weak correlation between the resistance of potato plants to the blackleg and soft rot results in the need of a parallel assessment for each of these two traits (R. Czajkowski et al., 2011). The choice of a preferable assessment method depends on the purpose of a study and the availability of biological material and required equipment and facilities. In large breeding centers, the assessment of potato resistance to the blackleg may be performed in vitro using potato explants. This approach is characterized by good reproducibility and reliability and provides a possibility for rapid large-scale production of revealed resistant genotypes (I. Hudák et al., 2006). If tested plants are planned to be used in further studies, then the detached leaf assay should be chosen (A. Sima et al., 2015). Results of this assay are usually in agreement with the results of field resistance assessment. The assay is preferable for a large-scale screening of resistance donors among wild Solanum species or transgenic potato lines. Breeders, who work with true potato seeds and mini-, micro-, and usual tubers, can use the method for potato resistance assessment under controlled conditions (V.S. Bisht et al., 1993). Tuber resistance to soft bacterial rot can be assessed using the vacuum infiltration method (M. Koppel, 1993) or the method of tuber or slice inoculation under anaerobic (R.A. Bain et al., 1988) or aerobic (I. Hudák et al., 2009) conditions. For aerobic conditions, the assessment may be carried out using whole tubers or their slices; in the last case, the duration of the experiment is significantly reduced (K.S. Tseng et al., 1990). The assessment criteria include the size of tissue necroses in the point of inoculation, weight or volume of affected tissues, and the ratio between the weights of healthy and affected tissues or between the areas of affected and healthy tuber surface. The choice of an assay and an assessment criterion depends on the purpose of the study and available resources. Comparison of results obtained by different methods may be incorrect. Planning and implementation of experiments on the assessment of tuber resistance to soft rot requires a standardization of some factors influencing on the final results; non-observance of this condition will make a comparison of obtained results impossible. Such crucial factors include the species of the inoculum, temperature of tuber tissues and bacterial suspension during inoculation stage, temperature of incubation after inoculation, and the point of inoculation or the part of tuber from which a slice was obtained. In the case of assessment of potato resistance to blackleg, the study of such factors was not conducted. This paper reviews in details advantages and disadvantages of the described approaches and factors and conditions able to influence on the results of assessment and on the possibility to compare results obtained in different experiments.

Keywords: potato, bacterial diseases, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium atrosepticum*, *Dickeya* spp., resistance assessment, bacterial blackleg, soft rot

Modern cultivars have a high genetic yield potential, which is, however, only realized by 30-50% under production conditions [1, 2]. One of the main causes is thought to be diseases caused by phytopathogenic microorganisms. In this regard, the assessment of persistance of source materials and cultivars and hybrids thereof to infectious diseases is an important place in plant breeding. It objectively speaks for their breeding value and applicability in various regions of Russia. Information on assessment methods is necessary for researchers to select new cultivars or perform registration tests.

The purpose of this article is to review foreign publications on methods for assessing the persistance of potatoes to bacterial diseases caused by *Pectobacterium carotovorum* subsp. *carotovorum*, *P. atrosepticum* and *Dickeya* spp. Its relevance is due to the increased economic importance of these pathogens in recent years, and almost full absence of publications on this subject in the Russian language.

Potatoes are one of the most important crops in the Russian Federation, however, having quite low yield (15-20 t/ha) [3]. Potato diseases associated with phytopathogenic bacteria rank second after late blight in economic terms [4]. Unlike viruses, bacterial infection is able to persist even during in vitro production of pathogen-free plantlets via meristem culture and degrade the quality of seeds and reduce yield [5]. Bacterial lesions cause weakening and death of plantlets during growth, rotting of tubers in the soil and during storage. Annual yield loss from bacteriosis is 10-15% and it can reach up to 50% during high epiphytotic periods [6]. Over the past period, potato losses from bacteriosis have increased significantly in the world. This is due to climatic changes conducive to the reproduction of phytopathogenic bacterial flora and the emergence of new harmful bacteria, the widespread switching to mechanized harvesting of potatoes, which increases the risk of damage to tubers, and the lack of timely and accurate diagnosis of bacteriosis [7].

One of the most harmful bacterial diseases of potatoes is the so-called 'blackleg" accompanied by soft rot of tubers. Damage to the underground parts of stolons at the beginning of the vegetation period leads to slower growth and gradual top necrosis, whereas the development of the infection in the middle of the vegetation period appears in the form of blackening and necrosis of the root part of the stems, accompanied by the decay and yellowing of the leaves [8]. Generally, the affected stems die back until the tubers are fully ripe, which negatively affects crop quality. Tubers harvested from the affected plants are often infected with a causative agent of the disease, which leads to the development of soft rot symptoms during the vegetation or storage period. Tubers can be infected from the affected stolons or lentils, when bacteria from the affected parent tuber enter the root zone [8].

Blackleg pathogens include several distinct, however, closely related species of pectolytic bacteria, originally called *Erwinia carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica* and *E. chrysanthemi*. However, based on the results of phylogenetic analysis using the nucleotide sequences of the 16S ribosomal RNA gene, it was proposed to isolate them and some other *Erwinia* species into a separate *Pectobacterium* genus and re-name them, respectively, to *Pectobacterium carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *atrosepticum* and *P. chrysanthemi* [9]. Subsequently, *P. carotovorum* subsp. *atrosepticum* atrosepticum was identified as a separate *P. atrosepticum* species [10], and *P. chrysanthemi* was transferred into a new genus called *Dikeya* [11].

P. carotovorum subsp. *carotovorum* and *P. atrosepticum* are widespread potato pathogens. *Dikeya* species were first reported as causative agents of potato stem rot in the Netherlands in the early 1970s [8]. At about the same time, a

Dickeya sp. was also identified as the causal agent of a blackleg disease of potato in Japan and tuber soft rot in Australia [12, 13]. Subsequently, the association of *Dickeya* spp. with above mentioned potato disease was reported from France, Israel, and South Africa [14-16]. Since 2004, *Dikeya* spp. has spread throughout Europe, which is associated with an increase in the frequency of hot spring/summer seasons in the northern regions, as well as the emergence of a new *D. solani* biovar [17-19]. In recent years, this phytopathogen has become a serious problem for the whole of Europe [20-23] and countries importing seed potatoes from Europe [24-26]. Currently, the *D. dianthicola* bacteria are included in the EPPO (European and Mediterranean Plant Protection Organization) A2 list of pests recommended for regulation as a quarantine pest with limited distribution in the EPPO member countries [17]. In Russia, *Dikeya* spp. strains were first reported in 2009 [27].

Few potato varieties as well as wild potato species are resistant to the disease and none are immune to pathogens of blackleg and soft rot of tubers [28, 29]. Currently, none of the commercial potato varieties is highly resistant, because this feature has not been prioritized in most existing breeding programs [30]. The resistance of potatoes to the manifestation of blackleg symptoms and the resistance of tubers of the same varieties or hybrids thereof to soft tuber rot do not always correlate with each other. This complicates the breeding for disease resistance, because weak correlation between the resistance of potato plants to the blackleg and soft rot results in the need of a parallel assessment for each of these two traits [31-34; J. Dobránszki, personal communication).

Generally, field tests for resistance are more labor-intensive and complex, therefore considerable attention is paid to the development of fast and easy-to-use laboratory methods. There are currently no standards for such methods, although most of them use the same inoculum concentration — 1×10^8-2×10^8 CFU/ml, which only differs significantly for a few variants. The established methods vary depending on the object, technique, assessment scale and can be divided into several groups.

The assessment of potato resistance to the blackleg may be performed in vitro using potato explants. When using this technique, the cultured explants are artificially contaminated. Plant age and methods of contamination may vary. The use of explants free of viral and fungal pathogens excludes variations in sensitivity to the blackleg pathogen associated with infection.

I. Hudák et al. [35, 36] used potato plants of different varieties grown on a hormone-free Murashige-Skoog (MS) medium under controlled conditions at a temperature of 20-22 °C and a 16-hour photoperiod. In 3 weeks, plant stalks were inoculated with a help of a tweezer moistened with *E. chrysanthemi* bacterial suspension or sterile distilled water (control). After 1 week after inoculation, the development of symptoms of the disease was assessed. The plants were divided into five groups depending on the severity of the disease [35]: 1 — no symptoms; 2 — 1-25% of decayed leaves; 3 — 26-50% of decayed leaves; 4 — 51-75% of decayed leaves; 5 — 76-100% of decayed leaves.

For each variety, the infection index F_i was calculated by formula:

$$F_i = \frac{\sum\left[(N_1 \cdot 1)+(N_2 \cdot 2)+(N_3 \cdot 3)+(N_4 \cdot 4)+(N_5 \cdot 5)\right]}{\sum N},$$

where N_1-N_5 is the number of affected plants in each of the 5 groups, $\sum N$ is the total number of plants used in the test. According to the proposed methodology, a variety was considered stable at $1 \leq F_i \leq 2$, moderately susceptible at $2 < F_i \leq 3$, and very susceptible at $F_i > 3$.

Two other options for using potato explants to assess a variety's resistance to *P. atrosepticum* are presented in I. Hudák's research [37]. For both cases, they took 30-day plantlets cultured in a MS medium under conditions similar to those described above. In the first case, the plant was inoculated with a bacterial suspension of *P. atrosepticum* using a sterile toothpick, which was first immersed into the suspension, and then pressed against the top of the plant. In the second case, the plants were cut at the level of the second node, then they were dipped into a bacterial suspension and finally placed on a MS medium (6%). The degree of infestation was measured after 72 hours on a 6-point scale: 1 point — 0% of decayed leaves (highly resistant), 2 points — 1-25% (resistant), 3 points — 26-50% (moderately resistant), 4 points — 51-75% (moderately susceptible), 5 points — 76-99% of decayed leaves (susceptible), 6 points — 100% (highly susceptible). The results of the two measurements were consistent with each other and with the results of tests performed in the greenhouse. Both resistant (up to 4 points) and susceptible (> 4 points) varieties were identified, among the tested plants.

Measurement of resistance to the blackleg using potato explants is characterized by good reproducibility and reliability and well suited for application in breeding centers, when there are a significant number of explants. Once we have found a resistant clone by using potato explants we are provided a possibility for rapid large-scale production of revealed resistant genotypes.

Assessing the potatoes resistance to blackleg in vitro using separated leaves. If tested plants are planned to be used in further studies, then the detached leaf assay should be chosen, which is also suitable for breeders who are not able to work with explants.

P.S. Bains et al. [33] worked with the plants cultured from explants, true seeds or microtubers on the soilless Ter-ra-Lite 2000 medium under controlled conditions for 5-6 weeks. Sterile silica sand was poured at the bottom of the vessels with lids and moistened with inoculum (10^5 CFU/ml). Five leaves with petiolules 85 mm long and 2-3 mm in diameter were cut from a plant of a tested variety and placed in sand down to a depth of 5 mm. Then, the vessels were left for 72 hours at 20 °C and a 16-hour photoperiod, with lids closed. Pathogen resistance was measured by the length of the infected area of the cutting using a 4-point scale: 1 point — 0-5 mm (highly resistant), 2 points — 6-10 mm (stable), 3 points — 11-50 mm (susceptible), 4 points — > 50 mm (highly susceptible). A similar, but slightly modified approach was applied to measure transgenic potato resistance to blackleg [38].

The advantages of this approach include high reproducibility, suitability for mass screening and technical simplicity. The approach is highly sensitive and provides satisfactory results at an inoculum concentration of 10^4-10^5 CFU/ml. Results of this assay are usually in agreement with the results of field resistance assessment [39]. It should be noted, however, that in the two studies mentioned, all potato varieties were assessed as susceptible and highly susceptible varieties. Resistant and highly resistant samples were found only among wild-growing *Solanum* species and somatic hybrids of potatoes with related species. The assay is preferable for a large-scale screening of resistance donors among wild *Solanum* species or transgenic potato lines.

Potato resistance assessment under controlled conditions. This method involves the inoculation of a bacterial suspension of plants grown in pots under controlled greenhouse conditions.

O.A. Hidalgo et al. [31] grew several clones of *S. tuberosum* subsp. *andigena* from tubers planted in pots with a mixture of peat, sand and soil (1:2:3)

for 4 weeks at 25-30 °C. Then the plantlets of a height of about 35 cm were inoculated by pricking a toothpick dipped in a suspension of bacterial cells of *E. chrysanthemi* for 5 minutes in the axil film of the 3rd leaf. The inoculated plants were placed into closed humidity chambers (28-30 °C, humidity ~ 100%), for 24 hours, and moved transferred to a greenhouse and kept for 3 days at 25-30°C under high humidity conditions. After that, the resistance of the plants was assessed by the length of the affected part of the stem. The degree of difference in the resistance was found to depend on the concentration of the bacterial suspension. When using an inoculum of 5.5×10^7 CFU/ml, the clones with the length of the affected stem area of 5-20 mm were rated as resistant clones, the clones with the length of the affected stem area of 33-36 mm were rated as moderately resistant clones, and the clones with the length of the affected stem area of 70-85 mm were rated as susceptible, and the clones with the length of the affected stem area of 118-128 mm were rated as highly susceptible.

A clearer rating scale was given by A. Sima [37]. Potato seedlings were rooted on a sand substrate for 14 days, then transplanted onto the soil and grown in the greenhouse for another 14 days, after which the stems were inoculated by pricking a sterile toothpick dipped in a suspension of *P. atrosepticum* at a height of 5 cm from the soil. The stem was wrapped with Parafilm laboratory tape to prevent it from drying out immediately after pricking. Infected plants were kept in a greenhouse for 21 days under high humidity conditions, after that, the length of the affected part of the stem was measured. The symptoms were assessed using the following scale: 1 point — 0-19 mm, 2 points — 20-49 mm, 3 points — 50-79 mm, 4 points — 80-109 mm, 5 points — 110 mm, 6 points — death. There is a high degree of compliance with the results obtained in the in vitro assessment of the same varieties.

A variation of the described approach is provided in the work dedicated to the production of transgenic potato plants resistant to bacterial and fungal diseases [40]. Transgenic plants derived from explants were grown on the soil for 3-4 months in climatic chambers (at 20 °C, 16-hour photoperiod), and then inoculated by injection with a bacterial suspension into the root bud. The degree of disease was assessed at regular intervals: 1 point — no symptoms, 2 points — minor manifestations of leaf chlorosis, 3 points — mature leaf chlorosis and necrosis, 4 points — stem necrosis and decay, 5 points — death of the plant (see the original article with photo).

The approach aimed at assessing the resistance of plants under controlled conditions can be convenient when using botanical potatoe seeds and mini-, micro-, and ordinary tubers as planting material. The approach should not be applied if there is a limited amount of seeds.

Approaches for assessing tuberous resistance to soft rot. *Vacuum infiltration.* The most technically complicated tuber inoculation approach. Surface sterilized tubers are immersed in a bacterial suspension and incubated for 1 min at a negative pressure of 80 kPa [41]. After that, the tubers are placed in plastic bags with a small amount of distilled water and incubated for 3 days at 25 °C, then weighed, washed out with a stream of water affected by rotten tissue, and weighed. The degree of damage is assessed by the ratio of the weight of the affected and healthy tissues. This approach helps bacteria penetrate into the tissues of tubers through lentils and aims to simulate the condition of tubers in moist soil moist tubers during storage [42]. In addition to technical complexity, the approach is limited by poor reproducibility [43].

Spot inoculation in an anaerobic environment. After the tubers have been washed and dried, they are provided with holes drilled 10 mm deep along the

longitudinal axis. A standardized concentration suspension of bacterial cells is applied to the bottom of a hole, and the hole is then sealed with a mixture of melted wax and vaseline. Tubers are then placed in plastic boxes, filled with nitrogen, sealed and kept for 6 days at 15°C, after that, the width and depth of tissue damage is measured. The criterion for assessing the resistance of the tuber is the degree of tissue damage (P), calculated by formula: $P = [W/2 + (D - 10)]/2$, where W is the width, D is the depth of tissue damage. It is also possible to assess the damage by the ratio of the weight of the affected and healthy tissue.

To apply a simplified version of this technique one should inoculate the sterilized tubers by 2-3 injections with a microsyringe to a depth of 2 cm [31]. All injection sites are placed on one side of the tuber and covered with a layer of vaseline, after that the tubers are wrapped in wet filter paper and plastic film, placed in plastic boxes and kept for 72 hours at 25 °C. After that, the tubers are cut through the points of inoculation and the width of the affected area is measured. The authors propose the following scale to assess the resistance: 0 points — width of tissue necrosis at the site of inoculation of a tuber 0-5 mm (resistant), 1 point — 5.1-10 mm (moderately resistant), 2 points — 10.1-15 mm (susceptible), 3 points — > 15 mm (highly susceptible).

Another option is to incubate solid tubers previously immersed in a bacterial suspension in chambers with a constantly maintained introductory suspension for 20 minutes, which ensures the continuous presence of a water film on the tubers [45]. As a result, the oxygen content in the tuber tissues decreases rapidly, which grows into a stronger manifestation of infection. The damage area is assessed (as a percentage of the total surface tuber area) after 96 hours of incubation at 20 °C.

This approach involving the incubation of tubers under anaerobic conditions ensures the progressive development of the infection and excludes the formation of wound periderm in potato. Therefore, the resistance of varieties is lower than that when assessed by using other approaches [46]. This approach helps assess how well the tuber tissue can serve as additional food basis for the pathogen, simulate tuber storage and transportation conditions, and select a highly resistant breeding material.

Inoculation of tuber slices and cut-outs in an aerobic environment. The inoculation of tuber slices in an aerobic environment is used to simulate the tuber response to a wound infection under aerobic conditions that are similar to the tuber post-harvest storage conditions, and to assess the tuber wound repair ability [44]. After the tuber surface has been sterilized, three slices of appr. 10 mm thick are cut out from the tuber and placed at right angles to the tuber longitudinal axis. Cavities with a depth and diameter of 5 mm are made in each slice, then left to initiate the formation of wound periderm for 6 hours, and then inoculated with a bacterial suspension. Pallets with slices are wrapped in wet filter paper and kept at 15 °C for 3 days. Tuber resistance is measured by the diameter of the affected tissue.

In later studies, the authors inoculated the slices immediately after cutting, without waiting for the formation of wound periderm [46-48]. There is also an option, where filter paper disks with a diameter ~ of 1 cm soaked in a bacterial suspension are placed on slices or halves of tubers [46, 47, 49]. Here is another approach, which is described by I. Hudák et al. [48]. After the tuber surfaces have been sterilized, cylindrical cut-outs were obtained from the tubers and cut into disks with a height of 1 cm (20-25 disks per option). The weighted discs were put into sterile Petri dishes on wet filter paper, covered with a 0.1 ml bacterial suspension or water and incubated at 26 °C for 24-26 hours. The affected tissues were then washed from the discs, and the disks were dried out and

weighed, and the resistance was assessed using the ratio of the weight of the remaining healthy tissues and the initial weight as a criterion: 1 point — < 5% (highly susceptible), 2 points — 5.1-10% (susceptible), 3 points — 10.1-15% (moderately susceptible), 4 points — 15.1-20% (poorly susceptible), 5 points — 20.1-30% (moderately resistant), 6 points — > 30.1% (resistant).

By inoculating slices or cut-outs we reduce the amount of testing time as compared to the inoculation of solid tubers. This approach shows high variability of results and lack of standardization for experimental conditions [46].

Inoculation of solid tubers in an aerobic environment. In recent years, a variety of techniques to inaculate and incubate solid tubers under aerobic conditions have developed. Tubers can be inoculated by pressing a plastic drop-tube tip containing a small amount of inoculum into a tuber to a depth of 1-1.5 cm [50-51]. Tubers are placed in high-humidity plastic containers with wet filter paper or wet chambers for 3 days [51] or 6 days [50] at 20-22°C and then assessed using the following scale: 0 points — no tissue necrosis at the inoculation area (resistant), 1 point — necrosis width < 2 mm (susceptible), 2 points — 2-10 mm (susceptible), 3 points — > 10 mm (susceptible).

T. Thangavel et al. [52] propose two more inoculation options. Firstly, the tubers were intentionally damaged by a falling weight with a steel tip, and then immersed for a short time into the pathogen suspension. Secondly, a hole with a depth of about 10 mm was made by a corkscrew in the tuber, at the bottom of which a bacterial suspension was then applied. In both cases, the tubers were weighed in 5 minutes after inoculation, wrapped in plastic film, distributed to plastic bags with a damp filter paper inside and tightly closed. After a 14-day, incubation in the dark at a temperature of 20-25 °C, the decayed tissues were washed out with water, the tubers were dried out and weighed, and the ratio of the weight of the decayed tissues and the healthy weight was measured. The damaged tissues can be used to assess tuber resistance. The approach is to determine the amount of damage by measuring the volume of water that is needed to fill the cavity remaining after careful removal of the decayed tissue [40, 53].

Factors influencing the assessment of blackleg and soft tuber resistance. When performing laboratory assessment of the resistance of potato plants to said diseases, it is necessary to take into account that, although the resistance is mainly determined by genetic characteristics [28], the degree of its manifestation depends on a number of external factors. These include seed quality, tuber physiology, the amount and virulence of inoculum, tuber infestation by other pathogenic microorganisms, weather conditions (temperature and humidity) during growing and harvesting, damage to tubers during harvesting, transportation and storage conditions. Therefore, a very strict observance of one and the same experimental conditions is a must. See below the main factors that can influence the outcomes of laboratory testing and are subject to standardization at the planning and testing stages.

Firstly, the degree of resistance depends on the inoculum origin. There were shown differences in the potato clone resistance to the blackleg caused by artificial inoculation with three different pathogens, *P. carotovorum* subsp. *carotovorum*, *P. atrosepticum* and *E. chrysanthemi* [31], and differences in the tuber response to the infestation by *P. carotovorum* subsp. *carotovorum* and *P. atrosepticum* [54]. Secondly, the spread of bacterial infection on tubers depends on the tissue temperature, and the temperature of the suspension, if inoculated by immersion in bacterial suspension. For example, the degree of damage caused by *P. carotovorum* subsp. *carotovorum*, was higher for tubers with the temperature of 23-24 °C at the time of inoculation than for cold tubers (4 °C) or excessively warm

tubers (26 °C) [55]. In the same study, it was shown that the maximum spread of tuber rot occurs when warm tubers are immersed in cold (10 °C) suspension. Thirdly, the extent of the damage can significantly depend on the incubation temperature after inoculation. For example, the increase from 20 to 30 °C resulted in the increase of the diameter of the affected potato slice area when inoculated with *P. carotovorum* subsp. *carotovorum* and *E. chrysanthemi* by about 25%. It should be noted, however, that in the case of *P. atrosepticum* the change was only 2.5% [47]. Finally, when performing tuber-slice tests, the degree of tissue damage strongly depends on the area from which the slice was cut out (or the inoculation point on the slice). Maximum tissue damage was observed for the slices obtained from the middle of a tuber, whereas the slices cut out from the apical parts turned out to be more resistant [46]. This phenomenon wad proved at different varieties of tubers. Further research showed that the diameter of the damage was significantly higher in the inoculation of the core part of the slice, but not the peripheral (cortex) one. For example, in one of the tests, the diameter of the damage for the two inoculations was 23.2 and 3.1 mm, respectively.

Therefore, there is little correlation between potatoes resistance to blackleg and soft tuber rot. For this reason, individual tests are required. Choosing the right assessment approach depends on the goals of the researcher and their access to the biological material, equipment and premises. Assessment of the potato resistance to the blackleg can be performed in vitro on explants or separated leaves, and on whole plants under controlled conditions. When assessing tuber resistance to soft bacterial rot, one can apply various approaches simulating these conditions or any other environment favorable to the spread of infection, by using either whole tubers or slices or cut-outs. These approaches include vacuum infiltration, spot inoculation in an anaerobic and aerobic environment. The size of tissue necrosis at the inoculation site, the weight or volume of affected tissues, and the ratio of the weight of healthy and affected tissues or the area of the affected surface and the surface of the whole tuber can serve as a criterion for assessing tuber resistance. It must be noted that the described approaches to the analysis of resistance to soft rot do not always give similar and comparable results, since they presumably activate different plant defenses. Hence, a comparison of the results obtained by applying various approaches to assessing tuber resistance may turn out to be irrelevant. When planning and practicing the assessment of tubers for resistance to soft rot one should take into account that the lack of standardized benchmarks for the factors that may influence outcomes will hinder any objective comparison among the outcomes. It should yet be noted, that the problem requires further study, because said factors have practically not been studied in the context of the plant resistance to the manifestation of blackleg symptoms.

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