

## Ring Rot Symptom Development in Georgian and Polish Potato Varieties Inoculated with *Clavibacter Sepedonicus* Isolates

Agnieszka Węgierek-Maciejewska\*, Tinatin Sadunishvili\*\*,  
Dali Gaganidze§, Nanuli Amashukeli§, Giorgi Kvesitadze\*\*

\* Plant Breeding and Acclimatization Institute - National Research Institute, Radzików, Poland

\*\* Academy Member, Sergi Durmishidze Institute of Biochemistry and Biotechnology, Agricultural University of Georgia, Tbilisi, Georgia

§ Sergi Durmishidze Institute of Biochemistry and Biotechnology, Agricultural University of Georgia, Tbilisi, Georgia

**Abstract.** *Clavibacter sepedonicus* (Cs), the causal agent of potato bacterial ring rot, is a quarantine disease characterized by latent infections and variable symptom expression. The lack of fully resistant potato cultivars highlights the importance of evaluating varietal susceptibility. This study assessed the responses of five Georgian and three Polish potato cultivars to inoculation with Cs isolates originating from Georgia and Poland. Tubers were inoculated with a bacterial suspension ( $10^8$  CFU/mL) and grown under controlled conditions. Disease development was evaluated over a six-month period using visual assessment, immunofluorescence antibody staining (IFAS), and PCR. Statistical analyses (ANOVA and Tukey's test) revealed significant effects of cultivar, isolate, and their interaction. The Georgian cultivars Meskhuri Adgilobrivi, Meskhuri Tseteli, and Javakheturi showed the lowest susceptibility, whereas Marabeli, Omega, and the Polish cultivar Owacja were the most susceptible. The most virulent isolates were of Polish origin (PL 2150, PL 4553, PL 194). These results demonstrate that susceptibility to Cs depends on both host genotype and bacterial isolate and identify Georgian cultivars as promising sources of reduced susceptibility for breeding programs. © 2026 Bull. Natl. Acad. Sci. Georg.

**Keywords:** *Clavibacter sepedonicus*, potato ring rot, potato varieties, IFAS, analysis of variance

### Introduction

Potato ring rot, caused by the Gram-positive bacterium *Clavibacter sepedonicus*, is a quarantine disease of major economic importance in global potato production. *Clavibacter sepedonicus* (Cs) is the current name of the historical taxon *Clavibacter michiganensis* subsp. *sepedonicus*, which has been elevated to species rank (EFSA Panel on Plant Health, 2019). The pathogen has a restricted

distribution in some regions due to strict phytosanitary control measures, but it has been reported from Europe, North America, and Asia, where potatoes are cultivated (Osdaghi et al., 2022). Cs is regulated under European plant health legislation because of its ability to cause asymptomatic (latent) infections and the difficulty of reliable detection, which together make disease control particularly challenging (Gryń et al., 2021).

The disease primarily affects the vascular system of potato plants and tubers. Latency – where infected plants or tubers remain symptomless – plays a key role in pathogen dissemination, particularly through seed tubers. Symptomless plants may still harbor high bacterial populations (Lelliott & Stead, 1987). When symptoms do develop, they may include leaf chlorosis, wilting, stunting, and internal vascular browning of tubers; however, symptom expression varies widely depending on host genotype, inoculum load, and environmental conditions (Slack, 1987a; Franc, 1999a, 1999b; Brown et al., 2002; Pietraszko et al., 2015).

Accurate detection of Cs remains problematic because low bacterial populations and delayed symptom expression can allow infections to escape visual inspection. Consequently, molecular diagnostic approaches, including CRISPR/Cas-based detection and other highly sensitive amplification methods, are being developed to improve the reliability of latent infection detection (Khmeleva et al., 2024).

Potato cultivars differ markedly in their response to Cs infection. Some cultivars express symptoms rapidly, whereas others sustain latent infections. Recent studies report substantial cultivar-dependent variation in symptom expression and levels of asymptomatic infection under both field and controlled conditions, highlighting the continuing challenges in breeding for resistance or tolerance (Manzer et al., 1987; Baer & Gudmestad, 1995; Gryń et al., 2021).

Although breeding for resistance is considered essential for effective disease control, no potato cultivars with complete resistance to bacterial ring rot are currently available. Some tolerant cultivars may remain symptomless while carrying significant pathogen populations, thereby posing a risk of pathogen spread through seed production systems (Sletten, 1985; Manzer et al., 1988). Understanding varietal responses, pathogen virulence, and the dynamics of latent infection is therefore essential for seed certification, quarantine enforcement, and breeding programs aimed at reducing susceptibility. The present

study assesses ring rot symptom development in plants and tubers of selected potato cultivars, including previously unstudied Georgian varieties, following inoculation with Cs isolates originating from Poland and Georgia.

## Material and Methods

The Cs strains used in this study (##40, 45, 97, 98, 961, 923, 931, and 953) were isolated from potato tubers in 2017 at the Durmishidze Institute of Biochemistry and Biotechnology, Agricultural University of Georgia (DIBB, Georgia) (Sadunishvili et al., 2020). Strains (PL 194, PL 4553, and PL 2150) were isolated at the Plant Breeding and Acclimatization Institute – National Research Institute (IHAR, Poland) between 2005 and 2010. The following potato cultivars were included in the study: Polish cultivars Cyprian, Owacja, and Courage, and Georgian cultivars Meskhuri Adgilobrivi, Javakheturi, Meskhuri Tsiteli, Omega, and Marabeli, kindly provided by the Scientific-Research Center of Agriculture, Ministry of Environmental Protection and Agriculture of Georgia.

Bacteria were grown on yeast glucose mineral (YGM) medium at 22°C for 5-7 days. Cultures were harvested and suspended in deionized water to a concentration of  $10^8$  CFU mL<sup>-1</sup> (OD<sub>600</sub> = 0.1), which was used as the inoculum. Experiments were conducted in a growth chamber under controlled conditions (16 h light at 24°C and 8 h darkness at 22°C). In accordance with EU Directive standards, three tubers per cultivar were inoculated with each Cs isolate. Inoculation was performed using a sterile scalpel; each tuber was punctured ten times around the eyes with a bacterial suspension of  $10^8$  CFU mL<sup>-1</sup>. Intact tubers of each cultivar served as negative controls.

Inoculated and control tubers were planted in 15-L buckets (three tubers per bucket per isolate). Qualitative assessment of plant growth was conducted approximately three months after planting and visual monitoring continued throughout the experiment.

Six months after planting, pathogenicity was quantitatively assessed using immunofluorescence antibody staining (IFAS) with polyclonal and monoclonal antibodies (Loewe) (De Boer et al., 1984) and PCR with the specific primer pair PSA1/PSAR (Patrik & Rainey, 1999).

Heel-end fragments from progeny tubers were excised and macerated in 5 mL of 0.05 M phosphate buffer (pH 7.0) for 24 h. Infection levels were evaluated following IFAS procedures defined in Commission Directive 2006/56/EC of 12 June 2006. All progeny tubers from each plant were assessed individually using a fluorescence microscope (Nikon Eclipse E600) at 100× magnification. Each cultivar was evaluated at three extract dilutions (undiluted,  $10^{-1}$ , and  $10^{-2}$ ), with two repetitions per dilution.

Data were analyzed using linear model ANOVA in SAS Enterprise Guide 4.0 (Fisher, 1925, 1935; Scheffé, 1959). Tukey's test ( $\alpha = 0.05$ ) was used to determine significant differences among extracts, dilutions, cultivars, isolates, and cultivar × isolate interactions and to identify homogeneous groups (Tukey, 1953).

## Results and Discussion

Typical bacterial ring rot symptoms, including leaf chlorosis, wilting, and plant stunting, were obser-

ved after three months. The presence of Cs in all progeny tubers was confirmed by PCR and IFAS using both polyclonal and monoclonal antibodies. The relationship between foliage symptom expression and potato cultivar varied depending on the Cs isolate.

Qualitative assessments conducted during the vegetation period revealed differences in susceptibility among Polish and Georgian cultivars. Among the Polish cultivars, Owacja was the most susceptible, whereas among the Georgian cultivars, Omega and Marabeli were the most affected.

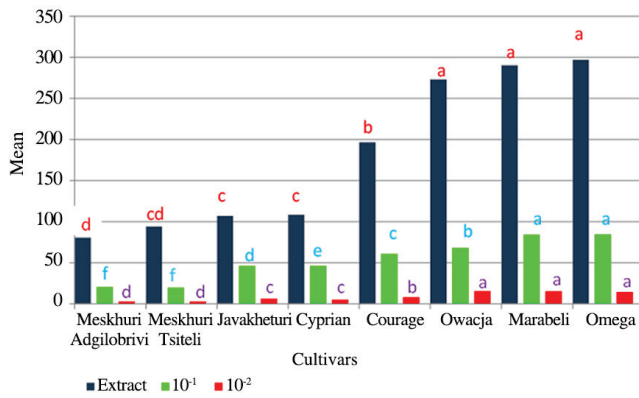
Statistical analyses (ANOVA and Tukey's test) revealed significant differences among cultivars, isolates, and cultivar × isolate interactions across all three serial dilutions (Table 1).

Tukey's test based on IFAS results using both polyclonal and monoclonal antibodies showed that the Georgian cultivars Meskhuri Adgilobrivi, Meskhuri Tsiteli, and Javakheturi were the most resistant to Cs (homogeneous group d), whereas Marabeli, Omega, and the Polish cultivars were the most susceptible (homogeneous group a) across all dilutions (Figs. 1 and 2). These results were consistent with visual observations.

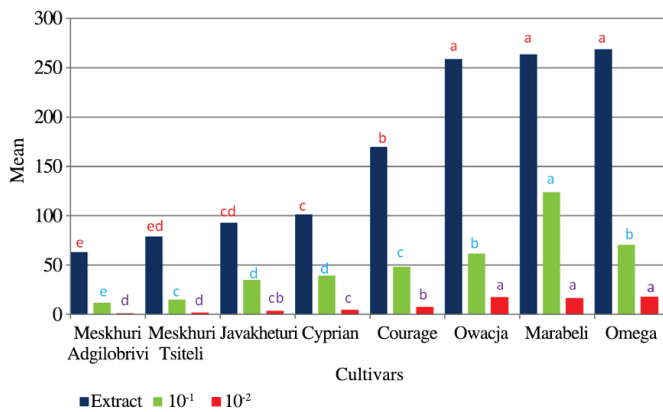
Analysis of Cs isolates using IFAS with polyclonal antibodies indicated that the most virulent

**Table 1. Analysis of variance for IFAS test by using polyclonal and monoclonal antibodies for three serial extract dilutions**

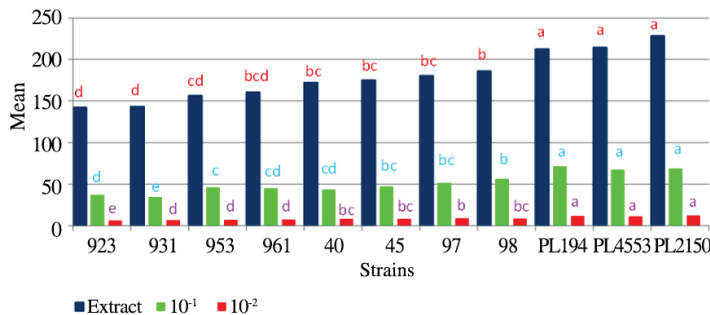
Variant		Extract		Extract dilution $10^{-1}$		Extract dilution $10^{-2}$	
		MS (Mean square)	F statistic (P< 0.01)	MS (Mean square)	F statistic (P< 0.01)	MS (Mean square)	F statistic (P< 0.01)
IFAS test by using polyclonal antibodies	Potato variety	1821318	321.18	108824	150.934	7070.8	232.779
	Cs Isolate	12522	2.21	5335	7.399	72.8	2.397
	Interaction: Variety(*) isolate	10858	1.91	2724	3.778	51.4	1.692
IFAS test by using monoclonal antibodies	Potato variety	1051973	211.593	172190	176.298	14796.8	175.629
	Cs Isolate	18859	3.793	46942	48.062	238.5	2.831
	Interaction: Variety(*) isolate	23885	4.804	35433	36.279	156.6	1.859



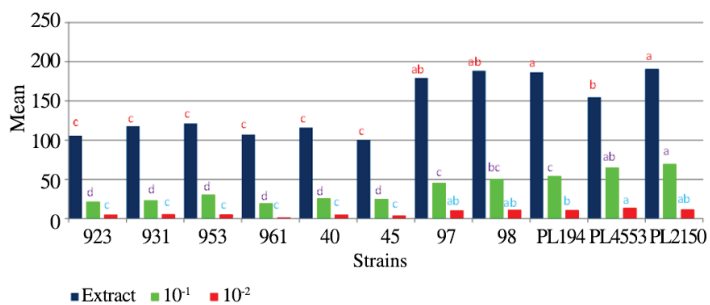
**Fig. 1.** Tukey’s test for data of IFAS test using polyclonal antibodies for different cultivars of potato in three serial extract dilutions. a, b, c, d, e, f – homogeneous groups. The means with the same letter do not differ significantly from each other.



**Fig. 2.** Tukey’s test for data of IFAS test using monoclonal antibodies for different cultivars of potato in three serial extract dilutions. a, b, c, d, e – homogeneous groups. The means with the same letter do not differ significantly from each other.



**Fig. 3.** Tukey’s test for data of IFAS test using polyclonal antibodies for different strains of Cs in three serial extract dilutions. a, b, c, d, e – homogeneous groups. The means with the same letter do not differ significantly from each other.



**Fig. 4.** Tukey’s test for data of IFAS test using monoclonal antibodies for different strains of Cs in three serial extract dilutions. a, b, c, d – homogeneous groups. The means with the same letter do not differ significantly from each other.

(homogeneous group a) were of Polish origin: PL 2150, PL 4553, and PL 194, whereas Georgian isolates did not differ significantly in virulence (Fig. 3). IFAS analysis using monoclonal

antibodies identified PL 2150 as the most virulent isolate across all dilutions (Fig. 4).

Results obtained using competitive PCR were consistent with the IFAS data and correlated well

with observed symptom development. The degree of tuber infection varied depending on the Cs isolate, confirming the statistical analyses. Overall, Marabeli was the most susceptible cultivar, whereas Meskhuri Adgilobrivi, Meskhuri Tsiteli, and Javakheturi were the most resistant.

These findings are consistent with previous reports. Pastuszewska et al. (2010) demonstrated that potato tubers inoculated under identical conditions differed significantly in their responses to Cs. Similar cultivar-dependent variation in symptom expression was reported by Brown et al. (2002).

Breeding efforts have thus far resulted primarily in tolerant rather than fully resistant potato cultivars (Maćkowiak-Sochacka, 2010; Manzer et al., 1987; Manzer & McKenzie, 1988; Yerchyk, 2008). Notably, the present study demonstrates that previously unstudied Georgian potato varieties exhibit substantial resistance, suggesting their potential value for breeding programs.

Further studies under diverse environmental conditions, including field trials, are required to confirm the stability of resistance under practical cultivation scenarios.

## Conclusions

Susceptibility of potato to Cs varies with both cultivar and bacterial isolate. Polish isolates (PL 2150, PL 4553, PL 194) are the most virulent. Georgian potato varieties Meskhuri Adgilobrivi, Meskhuri Tsiteli, and Javakheturi show reduced susceptibility to Cs, highlighting new genetic resources for breeding resistance to bacterial ring rot. This study provides the first evaluation of Georgian potato varieties for resistance to bacterial ring rot, offering new resources for disease management.

## Acknowledgements

This study was supported by the Regional Cooperative Research Grant project #60958, funded by the U.S. Defense Threat Reduction Agency through CRDF Global/GRDF.

We thank Dr hab. Dariusz Mańkowski from IHAR- NRI for making statistical analyzes.

The authors express their sincere gratitude to the late Professor Edward Arseniuk for his support of the project and valuable consultations.

## ბიოქიმია

# რგოლოვანი სიდამპლის სიმპტომების განვითარება კარტოფილის ქართულ და პოლონურ ჯიშებში *Clavibacter sepedonicus*-ის იზოლატებით ინოკულაციის შემდეგ

ა. ვეგერეკ-მაჩიევესკა\*, თ. სადუნიშვილი\*\*, დ. ლაღანიძე‡, ნ. ამაშუკელი‡, გ. კვესიტაძე\*\*

\* მცენარეთა სელექციისა და აკლიმატიზაციის ინსტიტუტი – ეროვნული კვლევითი ინსტიტუტი, რადიკუვი, პოლონეთი

\*\* აკადემიის წევრი, საქართველოს აგრარული უნივერსიტეტი, სერგი დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი, თბილისი, საქართველო

‡ საქართველოს აგრარული უნივერსიტეტი, სერგი დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი, თბილისი, საქართველო

*Clavibacter sepedonicus* (Cs) არის კარტოფილის რგოლოვანი სიდამპლის გამომწვევი აგენტი, რომელსაც ახასიათებს ლატენტური ინფექციები და სიმპტომების ცვალებადი გამოხატულება. ამ დაავადებისადმი რეზისტენტული ჯიშები არ არსებობს, რაც განაპირობებს ჯიშთაშორის მგრძნობელობის შესწავლის მნიშვნელობას. მოცემულ კვლევაში შეფასდა კარტოფილის სრულიად შეუსწავლელი ხუთი ქართული, და, ასევე სამი პოლონური ჯიშის რეაქცია Cs-ის იზოლატებით ინოკულაციაზე, რომლებიც საქართველოსა და პოლონეთში იყო გამოყოფილი. ტუბერები ინოკულირებულ იქნა ბაქტერიული სუსპენზიით ( $10^8$  CFU/მლ) და გაიზარდა კონტროლირებად პირობებში. დაავადების განვითარება ექვსი თვის განმავლობაში შეფასდა ვიზუალური მეთოდით, იმუნოფლუორესცენტული ანტისხეულებით შედგების (IFAS) და პოლიმერაზული ჯაჭვური რეაქციის გამოყენებით. სტატისტიკურმა ანალიზმა (ANOVA და ტუკაის ტესტი) გამოავლინა ჯიშის, იზოლატის და მათი ურთიერთქმედების სარწმუნო გავლენა. ქართულმა ჯიშებმა „მესხური ადგილობრივი“, „მესხური წითელი“ და „ჯავახეთური“ გამოავლინეს ყველაზე დაბალი მგრძნობელობა, ხოლო ჯიშებმა „მარაბელი“, „ომეგა“ და პოლონური ჯიში „ოვაცია“ ყველაზე მგრძნობიარენი იყვნენ. ყველაზე ვირულენტური იზოლატები პოლონური წარმოშობის იყო (PL 2150, PL 4553, PL 194). ეს შედეგები აჩვენებს, რომ Cs-ის მიმართ მგრძნობელობა დამოკიდებულია როგორც მასპინძელი მცენარის გენოტიპზე, ისე ბაქტერიულ იზოლატზე და ქართულ ჯიშებს წარმოაჩენს, როგორც დაბალი მგრძნობელობის პერსპექტიულ წყაროებს სელექციური პროგრამებისთვის.

## REFERENCES

- Baer, S., & Gudmestad, N. C. (1995). Pathogenicity and host range of *Clavibacter michiganensis* subsp. *sepedonicus* isolates. *Plant Disease*, 79(7), 703–707. <https://doi.org/10.1094/PD-79-0703>
- Bonde, R., Nelson, R., & Gudmestad, N. (1942). Early studies on resistance of potato cultivars to bacterial ring rot. *American Potato Journal*, 19(4), 123–134.
- Brown, J. F., Schaad, N. W., & Couch, J. N. (2002). Pathogenicity of *Clavibacter sepedonicus*: Factors influencing symptom expression. *Phytopathology*, 92(10), 1153–1159. <https://doi.org/10.1094/PHYTO.2002.92.10.1153>
- De Boer, S. H., & McCann, H. C. (1990). Variation in pathogenicity of *Clavibacter michiganensis* subsp. *sepedonicus*. *Plant Pathology*, 39(2), 249–257. <https://doi.org/10.1111/j.1365-3059.1990.tb02425.x>
- De Boer, S. H., & Wieczorek, A. (1984). Production of monoclonal antibodies of *Corynebacterium sepedonicum*. *Phytopathology*, 74, 1431–1433.
- EFSA Panel on Plant Health. (2019). Pest categorisation of *Clavibacter sepedonicus*. *EFSA Journal*, 17(4), e05670. <https://doi.org/10.2903/j.efsa.2019.5670>
- European Commission. (2006). *Commission Directive 2006/56/EC of 12 June 2006 amending the Annexes to Council Directive 93/85/EEC on the control of potato ring rot*. Official Journal of the European Union.
- Fisher, R. A. (1925). *Statistical methods for research workers*. Oliver & Boyd.
- Fisher, R. A. (1935). *The design of experiments*. Oliver & Boyd.
- Franc, G. D. (1999a). Persistence and latency of *Clavibacter michiganensis* subsp. *sepedonicus* in field-grown seed potatoes. *Plant Disease*, 83(3), 247–250.
- Franc, G. D. (1999b). Epidemiology of ring rot of potato: Influence of environmental factors. *Plant Disease*, 83(7), 657–662. <https://doi.org/10.1094/PDIS.1999.83.7.657>
- Gryń, J., Maćkowiak-Sochacka, A., & Pastuszewska, M. (2021). Latent infection and symptom development in potato cultivars infected with *Clavibacter sepedonicus*. *Potato Research*, 64(3), 275–290. <https://doi.org/10.1007/s11540-020-09486-6>
- Khmeleva, S. A., Kurbatov, L. K., Ptitsyn, K. G., Timoshenko, O. S., Morozova, D. D., Suprun, E. V., Radko, S. P., & Lisitsa, A. V. (2024). Detection of potato pathogen *Clavibacter sepedonicus* by CRISPR/Cas13a analysis of NASBA amplicons. *International Journal of Molecular Sciences*, 25(22), 12218.
- Lelliott, R. A., & Stead, D. E. (1987). *Bacterial plant pathogens*. Academic Press.
- Maćkowiak-Sochacka, A. (2010). Host range and symptom expression of *Clavibacter sepedonicus*. *Journal of Plant Pathology*, 92(1), 25–32.
- Maćkowiak-Sochacka, A. (2010). *Bakterioza pierścieniowa ziemniaka: Clavibacter michiganensis* subsp. *sepedonicus*. Plant Protection Institute, Poznań.
- Manzer, F. E., Gudmestad, N. C., & Nelson, G. A. (1987). Factors affecting infection, disease development and symptom expression of bacterial ring rot. *American Potato Journal*, 64, 641–676.
- Manzer, F. E., & McKenzie, A. E. (1988). Cultivar response to bacterial ring rot infection in Maine. *American Potato Journal*, 65, 333–339.
- Manzer, F., Nelson, R., & Gudmestad, N. (1988). Tolerance of potato cultivars to bacterial ring rot. *American Potato Journal*, 65(10), 545–554. <https://doi.org/10.1007/BF02852287>
- Nelson, R., Gudmestad, N., & Brown, J. F. (1992). Sources of resistance to *Clavibacter sepedonicus* in potato. *Plant Disease*, 76(8), 754–758. <https://doi.org/10.1094/PD-76-0754>
- Osdaghi, E., van der Wolf, J. M., Abachi, H., Li, X., De Boer, S. H., & Ishimaru, C. A. (2022). Bacterial ring rot of potato caused by *Clavibacter sepedonicus*: A successful example of defeating the enemy under international regulations. *Molecular Plant Pathology*, 23(7), 911–932. <https://doi.org/10.1111/mpp.13191>
- Pastrik, K. H., & Rainey, F. A. (1999). Identification and differentiation of *Clavibacter michiganensis* subspecies by polymerase chain reaction-based techniques. *Journal of Phytopathology*, 147, 687–693.
- Pastuszewska, M., Pietraszko, M., & Maćkowiak-Sochacka, A. (2010). Mechanisms of symptom development in *Clavibacter sepedonicus* infections. *European Journal of Plant Pathology*, 127(3), 357–369. <https://doi.org/10.1007/s10658-010-9645-1>
- Pastuszewska, T., Gryń, G., & Franke, K. (2010). Susceptibility of potato cultivars to *Clavibacter michiganensis* subsp. *sepedonicus* infection. *Progress in Plant Protection*, 50(1), 1297–1300.
- Pietraszko, M., Gryń, G., Pastuszewska, T., Przewodowski, W., & Przewodowska, A. (2015). Susceptibility of potato cultivars to infection by *Clavibacter michiganensis* subsp. *sepedonicus* under different soil conditions. *Biuletyn IHAR*, 277, 99–108.

- Sadunishvili, T., Węgierek-Maciejewska, A., Arseniuk, E., Gaganidze, D., Amashukeli, N., Sturua, N., Amiranashvili, L., Kharadze, S., & Kvesitadze, G. (2020). Molecular, morphological and pathogenic characterization of *Clavibacter michiganensis* subsp. *sepedonicus* strains of different geographic origins in Georgia. *European Journal of Plant Pathology*, 158, 195–209. <https://doi.org/10.1007/s10658-020-02066-x>
- Scheffé, H. (1959). *The analysis of variance*. John Wiley & Sons.
- Slack, S. A. (1987a). Biology and ecology of *Corynebacterium sepedonicum*. *American Potato Journal*, 64, 665–669.
- Slack, S. A. (1987b). Potato ring rot: Biology and control. *Annual Review of Phytopathology*, 25, 253–273. <https://doi.org/10.1146/annurev.py.25.090187.001345>
- Sletten, A. (1985). Symptomless carriers of *Clavibacter sepedonicus* in potato cultivars. *European Journal of Plant Pathology*, 91(3), 217–222. <https://doi.org/10.1007/BF01972813>
- Tukey, J. W. (1953). *The problem of multiple comparisons*. Princeton University.
- Węgierek-Maciejewska, A., Masny, A., Wołkowicz, T., Jagiełło, A., & Arseniuk, E. (2019). PCR MP method for differentiation of *Clavibacter michiganensis* subsp. *sepedonicus* isolates. *American Journal of Potato Research*, 96, 1–10. <https://doi.org/10.1007/s12230-019-09752-5>
- Westra, A. A. G., Arnesin, C. P., & Slack, S. A. (1994). Effect of interaction of inoculum dose, cultivar and geographic location on the magnitude of bacterial ring rot symptom expression in potato. *Phytopathology*, 84, 228–235.
- Yerchyk, O. (2008). Breeding potatoes for resistance to bacterial ring rot. *Journal of Plant Breeding and Seed Science*, 60(1), 31–39.
- Zhao, X., Jordan, S., & Castro, C. (2023). Complete genome sequence resource for a recently isolated potato ring rot pathogen, *Clavibacter sepedonicus* K496. *Microbiology Resource Announcements*, 12(6), e36265158. <https://doi.org/10.1128/mra.36265158>

Received December, 2025