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Original scientific paper

IN VITRO PATHOGENICITY TESTS OF SEVEN *PHYTOPHTHORA* SPECIES ON EUROPEAN CHESTNUT

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In this study, pathogenicity tests were conducted on seven *Phytophthora* species previously recorded on various tree species in North Macedonia. Single representative isolates of *P. cactorum*, *P. colocasiae*, *P. taxon walnut*, *P. cinnamomi*, *P. gonapodyides*, *P. inundata*, and *P. cambivora* were inoculated onto European chestnut twigs, with 40 replicates per isolate, categorized by two twig thicknesses. The results revealed a range of necrotic lesion lengths, from 1.6 to 5.9 cm, highlighting the varying impacts of the pathogen species on chestnut twigs. A two-way ANOVA indicated significant differences in lesion lengths between the two twig thickness categories across all *Phytophthora* species. Overall, *P. inundata* caused the shortest total lesion lengths, while *P. cactorum* caused the longest. Notably, lesion length variability for *P. colocasiae* was consistent across both twig thicknesses, whereas *P. inundata* showed the greatest variability between the two thickness categories.

Key words: *Castanea sativa*; Oomycetes; plant pathogen; invasive alien species

INTRODUCTION

Species of the genus *Phytophthora* are among the most destructive plant pathogens, affecting a wide range of host plant species and causing significant economic losses worldwide [1]. They are responsible for severe diseases in numerous agricultural and horticultural crops, as well as in natural ecosystems and forests, making *Phytophthora* one of the best-studied genera of plant pathogens [2].

As members of the oomycetes, a group of filamentous eukaryotic microorganisms, *Phytophthora* species are characterized by motile zoospores with two distinct flagella and thick-walled oospores, enabling both rapid spread and survival in diverse environments [3]. Highly adaptable, these pathogens thrive in various climates, from tropical to temperate regions, and can persist for extended periods in soil, water, or plant tissue, allowing them to spread over long distances [3-5]. Currently, more than 150 *Phytophthora* species have been described [6], with estimates suggesting a total of 400–600 species [7].

According to Tsao (1990), over 66 % of all fine root diseases and more than 90 % of collar rots in woody plants are caused by *Phytophthora* species. However, misidentification is common, with abiotic factors or secondary pathogens often mistakenly identified as the primary cause of these diseases [8, 9]. An extensive survey in European forest nurseries and forests recorded 68 different *Phytophthora* species, 44 of which were previously unknown to science. These species infected more than 80 % of nursery stands in over 90 % of tested nurseries and affected two-thirds of young plantings [10].

The spread of *Phytophthora* is often facilitated by human activities, such as the international trade of plants and plant materials, which introduces pathogens to non-native regions [11]. Environmental factors like temperature, humidity, and water availability also influence the growth and virulence of these pathogens [3, 10, 12, 13].

One of the most infamous species, *P. infestans*, the causal agent of potato late blight, was the primary driver of the Great Irish Famine (ca. 1845), which led to approximately one million deaths and the migration of over one million people from Ireland within a decade. Ironically, these migrants unknowingly introduced *P. infestans* to new regions through propagative materials [14].

Other *Phytophthora* species continue to cause devastating epidemics in tree species worldwide, with *P. cinnamomi* and *P. ramorum* among the most well-known. *P. cinnamomi* is considered one of the most historically destructive plant pathogens, with over 1,000 host plant species [16], and has been linked to the decline of oak species in central and southern Europe [17, 18]. Meanwhile, the airborne *P. ramorum* causes Sudden Oak Death in western USA and has been recorded in Europe, where it infects a wide range of host species [19–21].

Understanding the biology and ecology of *Phytophthora* species is critical for developing effective management strategies to control their spread and mitigate their impact on plant health. Research efforts focus on unraveling the genetic and molecular mechanisms underlying their pathogenicity, as well as on developing diagnostic tools and management strategies [3].

Pathogenicity tests are essential for evaluating the virulence of *Phytophthora* species on different host plants. These tests involve inoculating plant tissues with *Phytophthora* isolates and assessing disease development over time. By quantifying disease severity, researchers can determine host susceptibility, pathogen aggressiveness, and the effectiveness of control measures [26]. Common methodologies for pathogenicity testing include leaf disc assays, root inoculation, and detached leaf or whole-plant inoculation in controlled environments [27].

Advances in molecular genetics and genomics have greatly enhanced our understanding of *Phytophthora* pathogenicity mechanisms. Genome sequencing projects have identified genes encoding effectors, virulence factors, and components of the oomycete secretome, shedding light on the molecular basis of host-pathogen interactions [29]. Transcriptomic and proteomic analyses have further clarified the dynamics of gene expression and protein secretion during infection, revealing key pathways involved in pathogenicity [30].

Studying *Phytophthora* pathogenicity is crucial for understanding how these pathogens cause disease in plants, a fundamental step in developing targeted strategies for disease control and management.

MATERIALS AND METHODS

A single representative isolate from each of seven *Phytophthora* species (*P. cactorum*, *P. colocasiae*, *P. Taxon walnut*, *P. cinnamomi*, *P. gonapodyides*, *P. inundata*, and *P. cambivora*; Table 1), previously recorded on various tree species in North Macedonia [31], was selected from our collection to assess pathogenicity on *Castanea sativa* (European chestnut) twigs.

Dormant one-year-old shoots, ranging from 10–15 mm in diameter [32], were used as test material. Young shoots were collected from a single tree coppice immediately after bud development in May. Leaves were removed, and the twigs were cut into 10–15 cm lengths and sterilized using 70 % ethanol. Twigs were inoculated with 5 × 5 mm agar plugs, placed with the mycelium side facing the cambium, taken from fresh cultures of the selected *Phytophthora* isolates grown on V8 agar medium. The inoculation site was wrapped with Parafilm.

Table 1. List of representative *Phytophthora* isolates used for the pathogenicity tests

No.	Isolate code	Species	Database	Used DNA sequence for comparison	Match %	Difference in base pairs
1	MKDF-102-1	*P. cactorum*	*Phytophthoradb.org*	PD_00278_ITS	100 %	0
2	MKDF-3	*P. colocasiae*	*Phytophthoradb.org*	PD_01573_ITS	100 %	0
3	MKDF-9	*P. taxon Walnut*	*Phytophthoradb.org*	PD_02830_ITS	100 %	0
4	MKDF-33	*P. cinnamomi*	*Phytophthoradb.org*	PD_01976_ITS	100 %	0
5	MKDF-08	*P. gonapodyides*	*Phytophthoradb.org*	PD_02725_ITS	100 %	0
6	MKDF-46	*P. inundata*	*Phytophthoradb.org*	PD_02731_ITS	100 %	0
7	MKDF-80	*P. cambivora*	*Phytophthoradb.org*	PD_01869_ITS	100 %	0

A total of 40 replicates were set per isolate, with 20 inoculations on twigs in the thickness range of 5–10 mm and 20 inoculations on twigs 10–15 mm in diameter. The inoculations were applied superficially on exposed cambial tissue after bark removal (Figure 1). The inoculated twigs were placed in glass Petri plates (15 cm diameter) lined with moist filter paper (Figure 2), with ten replicates of a single isolate per plate. The plates were incubated at room temperature (24 °C ± 4 °C) in the dark for seven days.

After the incubation period, the twigs were examined for necrosis. The lengths of the recorded necroses were measured precisely after debarking (Figure 3). Re-isolations from the necrotic tissue were performed on selective nutrient media (PARPNH and CARP+) to confirm the causal agent of the necrosis.

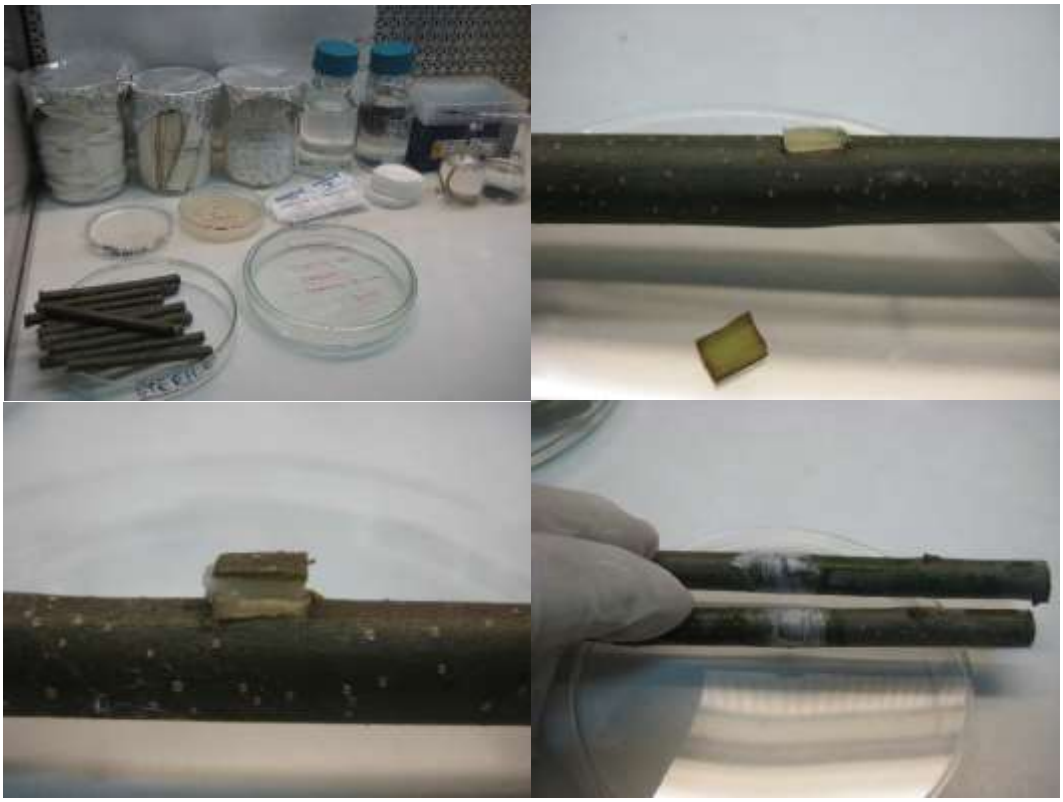

Figure 1. Setting the inoculation assays with *Phytophtora* isolates

Figure 2. Sets of ten replicates of *Phytopthora* isolates on chestnut twigs per dish, for incubation

Figure 3. Incidence of necrosis on chestnut twigs inoculated with a *Phytophthora* isolate

RESULTS AND DISCUSSION

After the incubation period, the examination of the inoculation sets revealed the presence of necrosis on nearly all twigs inoculated with the selected *Phytophthora* species. The measured dimensions of the induced necrosis on the inoculated twigs are presented in Table 2.

Table 2. Lengths of necroses on chestnut twigs inoculated with isolates of the selected *Phytophthora* species

Number of assays	Twig diameter (mm)	Lenghts of the induced necrosis according to species *Phytophthora* (cm)						
		P.colocasiae	*P.cambivora*	*P.taxon Walnut*	*P. cinnamo-mi*	*P.inundata*	*P.gonapodyides*	*P.cactorum*
1	5–10	4.9	3.3	0	1.9	0	1.6	5.7
2		3.2	2.9	4.2	3.5	2.1	2.4	4.9
3		3.4	3.5	2.4	2.4	2.1	2.2	4.1
4		0	2.2	2.2	2.4	2.4	2.4	4.7
5		3.8	2.9	2.1	2.1	1.9	1.8	4
6		5.1	3.1	0	3	2.2	3.4	2.9
7		3.1	3.5	2	2.9	1.8	2.1	3.4
8		4.7	3.1	2.4	3.1	2.3	2.2	4.7
9		4.1	3	2.2	2.2	2.2	1.8	5.1
10		3.2	3.4	2.1	2.2	1.9	1.9	3.9
11		3.4	3.6	2.5	2.4	2.2	2.6	4.2
12		3.9	2.4	2	2.4	2.6	2	4.6
13		3	3	2	3.6	2	2.4	4.9
14		4.7	2.2	2.5	2.6	2	3.6	5
15		5	3.9	2.6	3.3	2.4	2.1	4
16		3.2	3.3	2.5	2.9	2.5	2.6	5.9
17		5.2	3	2.1	3.3	2	2.4	3.6
18		3	3.9	2	2.3	2.6	2.4	3.2
19		4.1	3.6	2.9	2	2.2	2	2.2
20		4.9	3	3.9	2.6	2	2.7	3.1
1	10–15	0	3.3	2.9	2.3	2.4	2	3.9
2		3	2	2.2	2.7	2.3	2.7	3.5
3		2.9	2.2	2.4	3.1	2.1	2	4.4
4		4.1	2.2	1.9	2.9	2.2	2.9	4.9
5		3.8	2.1	4.4	2.8	2.4	2.8	3.3
6		0	2.2	2.1	1.9	2.2	2.9	3.9
7		3	1.9	2.4	2.9	2.1	2.3	3.3
8		3.3	3.1	1.9	2.4	2.7	1.9	4.9

Table 2 Continued

9	10–15	2.9	2.9	1.9	3.1	2.9	2.9	3.8
10		3.5	3.6	3.9	2.7	2	2.5	4.7
11		3.6	3.6	2.2	3.6	2	2	5.3
12		1.9	3.1	2.1	2.6	2.4	2	3
13		3.9	3.5	2.3	2.4	2.4	2.4	3.9
14		5.1	3.2	4.1	2.6	2.2	2.3	5.9
15		5.1	3	4.1	3	2.5	2	5.5
16		3.2	2.2	2.2	2.2	2.2	2.6	4.1
17		3.2	3.4	2.1	3.2	2	2.4	4.1
18		4.7	2.9	2.4	2	2.6	2.4	4
19		3.3	3.1	2.1	2.2	2.1	2.3	3.1
20		4.2	3.4	2.2	2.7	2.7	3.3	3.5

For each *Phytophthora* species, the shortest and longest lengths of the observed necroses were recorded (Table 3).

In Table 3, we present the minimum and maximum lengths of necrotic lesions developed on chestnut twigs caused by the *Phytophthora* cultures under assessment. The lesion lengths ranged from 1.6 cm to 5.9 cm. A descriptive statistical analysis was performed on the pathogenicity test results (Table 4). Additionally, the differences in lesion length were analyzed using two-way ANOVA, with the two thickness categories of chestnut twigs (5–10 mm and 10–15 mm) as factors, and the seven *Phytophthora* species as parameters. The descriptive statistical results for each parameter (*Phytophthora* species) are shown in Table 4.

The descriptive statistical summary highlights significant variation in lesion lengths between the two twig diameter categories. Specifically, *P. colocasiae* showed consistent lesion variability across both twig thickness categories. In contrast, *P. inundata* exhibited the greatest difference in lesion length variability between the two twig thickness categories. *P. cactorum* and *P. cinnamomi* induced lesions with considerable variability in both thickness categories. The total lesion size was smallest for *P. inundata* and largest for *P. cactorum*, which caused lesions twice as large as those induced by *P. inundata*. These findings suggest that *P. inundata* is the least pathogenic, while *P. cactorum* is the most pathogenic toward chestnut twigs.

From the two-way ANOVA, it was concluded that there is no statistically significant difference in lesion length for individual *Phytophthora* species in relation to twig thickness ($p > 0.005$; $F = 0.087$). However, the analysis revealed a statistically significant difference in lesion length between the *Phytophthora* species themselves ($p > 0.005$; $F = 34.97$), indicating that lesion development depends on the inoculated species. The results also indicate that twig thickness is not a crucial factor in determining necrosis length in this case.

Table 3. Values for the shortest and longest observed necroses, for each of the tested *Phytophthora* species

Diameter (mm)		**Used *Phytophthora* species for pathogenicity tests**						
		P.colocasiae	*P.cambivora*	*P.taxon Walnut*	*P.cinnamomi*	*P.inundata*	*P.gonapodyides*	*P.cactorum*
5-10	Shortest necrosis lengths (cm)	3,3	2,2	2,1	1,9	1,8	1,6	2,2
	Longest necrosis lengths (cm)	5,2	3,9	4,2	3,5	2,6	3,6	5,9
10-15	Shortest necrosis lengths (cm)	1,9	1,9	1,9	1,9	2,0	1,9	3,1
	Longest necrosis lengths (cm)	5,1	3,6	4,4	3,6	2,9	3,3	5,9

Table 4. Results from the descriptive statistics of dimensions of necroses developed on chestnut twigs after seven days of inoculation with selected *Phytophthora* isolates

5–10 mm	*P. colocasiae*	*P. cambivora*	*P. taxon Walnut*	*P. cinnamomi*	*P. inundata*	*P. gonapodyides*	*P. cactorum*
Mean	3.795	3.14	2.23	2.655	2.07	2.33	4.205
Standard Error	0.266	0.108	0.216	0.115	0.121	0.111	0.213
Standard Deviation	1.191	0.485	0.966	0.512	0.539	0.497	0.954
Sample Variance	1.417	0.235	0.934	0.263	0.291	0.247	0.911
Range	5.2	1.7	4.2	1.7	2.6	2	3.7
Minimum	0	2.2	0	1.9	0	1.6	2.2
Maximum	5.2	3.9	4.2	3.6	2.6	3.6	5.9
Count	20	20	20	20	20	20	20
10–15 mm							
Mean	3.235	2.845	2.59	2.665	2.32	2.43	4.15
Standard Error	0.303	0.131	0.184	0.096	0.058	0.088	0.183
Standard Deviation	1.353	0.588	0.822	0.431	0.259	0.392	0.819
Sample Variance	1.832	0.346	0.676	0.186	0.067	0.154	0.671
Range	5.1	1.7	2.5	1.7	0.9	1.4	2.9
Minimum	0	1.9	1.9	1.9	2	1.9	3
Maximum	5.1	3.6	4.4	3.6	2.9	3.3	5.9
Count	20	20	20	20	20	20	20
Total							
Mean	3.515	2.993	2.410	2.660	2.195	2.380	4.178
Standard Error	0.204	0.087	0.143	0.074	0.069	0.070	0.139
Standard Deviation	1.290	0.553	0.904	0.467	0.436	0.445	0.878
Sample Variance	1.663	0.305	0.817	0.218	0.190	0.198	0.772
Range	5.2	2	4.4	1.7	2.9	2	3.7
Minimum	0	1.9	0	1.9	0	1.6	2.2
Maximum	5.2	3.9	4.4	3.6	2.9	3.6	5.9
Count	40	40	40	40	40	40	40

Figures 4 and 5 further illustrate the data. The highest lesion dimensions, regardless of twig thickness, were caused by *P. cactorum*. The smallest lesions were caused by *P. inundata*. Table 3 provides the minimum and maximum lesion lengths for each *Phytophthora* species across both twig diameters, excluding cases where no lesions developed (length = 0).

All seven species, previously recorded on various tree species in North Macedonia [31], exhibit characteristics typical of many *Phytophthora* species known for their omnivorous nature and remarkable ability to infect a wide array of hosts, including agricultural crops, forest trees, and ornamental plants [5–7, 11, 16, 22].

P. cactorum infects over 200 plant species across 150 genera in 60 families, including *Fagus* spp., *Juglans regia*, *Malus pumila*, and *Castanea sativa* [5]. It causes necrosis on inoculated stems of *Quercus robur* [18], lesions on apple, strawberry, and rhododendron with host specificity [33], and is part of the complex causing ink disease [34]. *P. cambivora* is associated with cankers and root rot in plants across 30 genera and 15 families, primarily woody plants, often alongside other *Phytophthora* species [5, 34, 37]. *P. gonapodyides* is considered a minor pathogen, affecting a limited number of hosts, and is often isolated from aquatic sludge [34]. *P. inundata* primarily causes root and basal rot in deciduous trees and shrubs, including *Aesculus*, *Olea*, *Salix*, *Prunus*, and *Vitis* [39]. *P. colocasiae* has a relatively narrow host range, primarily infecting *Colocasia esculenta* and *Arecaceae* species [5, 40]. *P. taxon walnut* is pathogenic in natural ecosystems and agricultural crops, causing foliar diseases in walnuts [39, 41]. *P. cinnamomi* infects numerous woody hosts across 266 genera and 90 families [5].

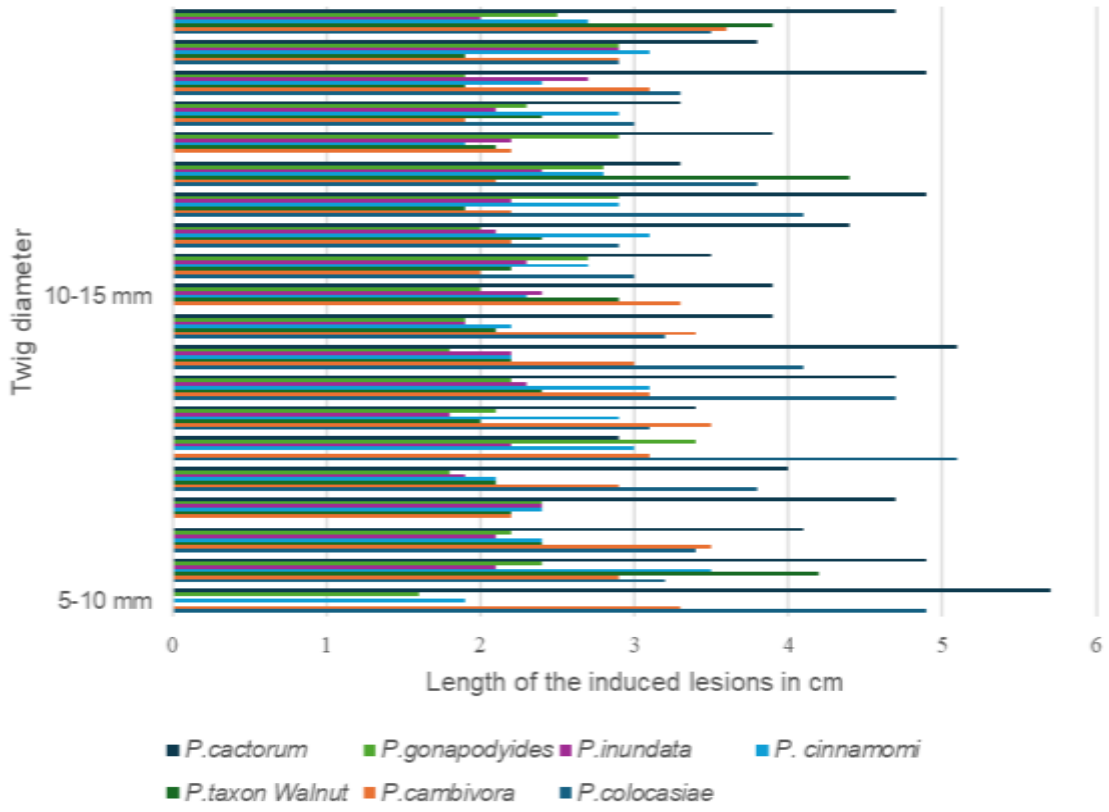

Figure 4. Length of induced lesions on chestnut twigs induced by *Phytophthora* isolates as per category of twig diameter

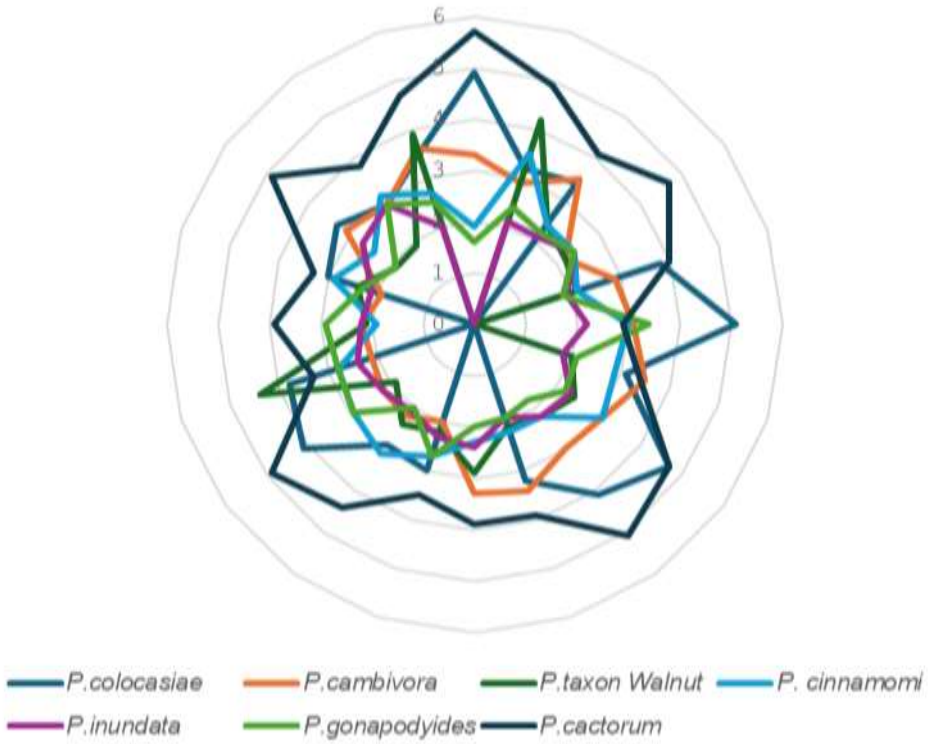

Figure 5. Length of the induced necrotic lesions on chestnut twigs induced by isolates of the selected *Phytophthora* species

The pathogenicity tests on *C. sativa* twigs provided valuable insights into the variability and severity of the lesions caused by the selected *Phytophthora* species. Lesion lengths ranged from 1.6 cm to 5.9 cm, indicating diverse pathogenic potential among the species. Statistical analyses showed significant variation in lesion sizes across the species, with *P. cactorum* being the most pathogenic and *P. inundata* the least pathogenic.

These findings reflect the potential impact of these pathogens on European chestnut trees and underline the importance of continued monitoring and management efforts for *Phytophthora* species in natural stands.

CONCLUSION

Pathogenicity tests were conducted on chestnut twigs using isolates from seven *Phytophthora* species. Among them, *P. cactorum* was identified as the most pathogenic, while *P. inundata* was the least pathogenic, regardless of twig thickness.

In conclusion, these results highlight the varying pathogenic potential of the tested *Phytophthora* species on chestnut twigs and may reflect their potential impact on European chestnut trees in natural stands. This research contributes to disease management strategies aimed at improving plant resistance against these pathogens.

REFERENCES

[1] J. B. Ristaino, M. L. Gumpertz, New frontiers in the study of dispersal and spatial analysis of epidemics caused by species in the genus *Phytophthora*, *Annu. Rev. Phytopathol.*, **38** (2000), pp. 541−576.

[2] P. Scott, M. K. F. Bader, T. Burgess, G. Hardy, N. Williams, Global biogeography and invasion risk of the plant pathogen genus *Phytophthora*, *Environ. Sci. Policy*, **101** (2019), pp. 175−182.

[3] K. H. Lamour, S. Kamoun, *Phytophthora* pathogens: A review of their biology, ecology, and management., *Annu. Rev. Phytopathol.*, **47** (2009), pp. 1−22.

[4] C. M. Brasier, The biosecurity threat to the UK and global environment from international trade in plants, *Plant Pathol.*, **57** (2008), pp. 792−808.

[5] D. Erwin, O. Ribeiro, *Phytophthora diseases worldwide*, APS Press, St. Paul, Minnesota, 1996, p. 592.

[6] T. Jung, L. Orlikowski, B. Henricot, P. Abad-Campos, A. G. Aday, O. Aguín Casal, J. Bakonyi, S. O. Cacciola, T. Cech, D. Chavarriaga, T. Corcobado, A. Cravador, T. Decourcelle, G. Denton, S. Diamandis, H. T. Doğmuş-Lehtijärvi, A. Franceschini, B. Ginetti, S. Green, M. Glavendekić, J. Hantula, G. Hartmann, M. Herrero, D. Ivic, M. Horta Jung, A. Lilja, N. Keca, V. Kramarets, A. Lyubenova, H. Machado, G. Magnano di San Lio, P. J. Mansilla Vázquez, B. Marçais, I. Matsiakh, I. Milenkovic, S. Moricca, Z. Á. Nagy, J. Nechwatal, C. Olsson, T. Oszako, A. Pane, E. J. Paplomatas, C. Pintos Varela, S. Prospero, C. Rial Martínez, D. Rigling, C. Robin, A. Rytkönen, M. E. Sánchez, A. V. Sanz Ros, B. Scanu, A. Schlenzig, J. Schumacher, S. Slavov, A. Solla, E. Sousa, J. Stenlid, V. Talgø, Z. Tomic, P. Tsopelas, A. Vannini, A. M. Vettraino, M. Wenneker, S. Woodward, A. Peréz-Sierra, M. L. Deprez-Loustau, Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of *Phytophthora* diseases, *Forest Pathol.*, **46** (2015), pp. 134−163.

[7] C. M. Brasier, *Phytophthora* biodiversity: how many *Phytophthora* species are there?, in: *Phytophthoras in Forests and Natural Ecosystems*, E. Goheen, S. Frankel (Eds), USDA Forest Service, Albany, USA, pp. 101−115, 2009.

[8] T. Jung, A. Pérez-Sierra, A. Durán, M. H. Jung, Y. Balci, B. Scanu, Canker and decline diseases caused by soil-and airborne *Phytophthora* species in forests and woodlands, *Persoonia-Mol. Phylogeny Evol. Fungi*, **40** (2018), pp. 182−220.

[9] P. H. Tsao, Why many *Phytophthora* root rots and crown rots of tree and horticultural crops remain undetected 1, *EPPO Bull.*, **20** (1990), pp. 11−17.

[10] T. Jung, L. Orlikowski, B. Henricot, P. Abad-Campos, A. Aday, O. Aguín Casal, J. Bako-nyi, S. Cacciola, T. Cech, D. Chavarriaga, Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of *Phytophthora* diseases, *Forest Pathol.*, **46** (2016), pp. 134−163.

[11] S. Woodward, R. C. Beram, H. T. Doğmuş, Drivers of forest pathogen invasions: The roles of global trade and climate change, *South-East Eur. For. Sci. SEEFOR*, **13** (2022), pp. 1−18.

[12] E. M. Hansen, P. W. Reeser, W. Sutton, *Phytophthora* beyond agriculture, *Annu. Rev. Phytopathol.*, **50** (2012), p. 359−378.

[13] N. J. Grünwald, E. M. Goss, C. M. Press, *Phytophthora ramorum*: a pathogen with a remarkably wide host range causing sudden oak death on oaks and ramorum blight on woody ornamentals, *Mol. Plant Pathol.*, **9** (2008), pp. 729−740.

[14] K. Yoshida, V. J. Schuenemann, L. M. Cano, M. Pais, B. Mishra, R. Sharma, C. Lanz, F. N. Martin, S. Kamoun, J. Krause, The rise and fall of the *Phytophthora infestans* lineage that triggered the Irish potato famine, *Elife*, **2** (2013), p. e00731.

[15] E. M. Hansen, *Phytophthora* species emerging as pathogens of forest trees, *Curr. For. Rep.,* **1** (2015), pp. 16−24.

[16] T. I. Burgess, J. K. Scott, K. L. Mcdougall, M. J. Stukely, C. Crane, W. A. Dunstan, F. Brigg, V. Andjic, D. White, T. Rudman, Current and projected global distribution of *Phytophthora cinnamomi*, one of the world's worst plant pathogens, *Global Change Biol.,* **23** (2017), pp. 1661−1674.

[17] C. M. Brasier, F. Robredo, J. Ferraz, Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline, *Plant Pathol.,* **42** (1993), pp. 140−145.

[18] T. Jung, H. Blaschke, P. Neumann, Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands, *Eur. J. For. Pathol.,* **26** (1996), pp. 253−272.

[19] D. M. Rizzo, M. Garbelotto, Sudden oak death: endangering California and Oregon forest ecosystems, *Front. Ecol. Environ.,* **1** (2003), pp. 197−204.

[20] C. M. Brasier, S. Denman, A. Brown, J. Webber, Sudden oak death (*Phytophthora ramorum*) discovered on trees in Europe, *Mycol. Res.,* **108** (2004), pp. 1108−1110.

[21] J. F. Webber; Status of *Phytophthora ramorum* and *P. kernoviae* in Europe, *Third Science Symposium – Sudden Oak Death (Proceedings)*, Albany, USA, 2008, pp. 19−26.

[22] C. M. Brasier, *Phytophthora* species: A review of their biology, ecology and pathogenicity, *Plant Pathol.,* **49** (2000), p. 137−151.

[23] B. M. Tyler, S. Tripathy, X. Zhang, P. Dehal, R. H. Y. Jiang, A. Aerts, F. D. Arredondo, L. Baxter, D. Bensasson, J. L. Beynon, J. Chapman, C. M. B. Damasceno, A. E. Dorrance, D. Dou, A. W. Dickerman, I. L. Dubchak, M. Garbelotto, M. Gijzen, S. G. Gordon, F. Govers, N. J. Grunwald, W. Huang, K. L. Ivors, R. W. Jones, S. Kamoun, K. Krampis, K. H. Lamour, M.-K. Lee, W. H. McDonald, M. n. Medina, H. J. G. Meijer, E. K. Nordberg, D. J. Maclean, M. D. Ospina-Giraldo, P. F. Morris, V. Phuntumart, N. H. Putnam, S. Rash, J. K. C. Rose, Y. Sakihama, A. A. Salamov, A. Savidor, C. F. Scheuring, B. M. Smith, B. W. S. Sobral, A. Terry, T. A. Torto-Alalibo, J. Win, Z. Xu, H. Zhang, I. V. Grigoriev, D. S. Rokhsar, J. L. Boore, *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis, *Science,* **313** (2006), pp. 1261−1266.

[24] S. Kamoun, A catalogue of the effector secretome of plant pathogenic oomycetes, *Annu. Rev. Phytopathol.,* **44** (2006), pp. 41−60.

[25] B. J. Haas, S. Kamoun, M. C. Zody, R. H. Jiang, R. E. Handsaker, L. M. Cano, M. Grabherr, C. D. Kodira, S. Raffaele, T. Torto-Alalibo, Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*, *Nature,* **461** (2009), p. 393−398.

[26] N. J. Grünwald, B. A. McDonald, M. G. Milgroom, Population genomics of fungal and oomycete pathogens, *Annu. Rev. Phytopathol.,* **54** (2016), p. 323−346.

[27] S. Kamoun, Groovy times: filamentous pathogen effectors revealed, *Curr. Opin. Plant Biol.,* **10** (2007), pp. 358−365.

[28] W. Fry, P. Birch, H. Judelson, N. Grünwald, G. Danies, K. Everts, A. Gevens, B. Gugino, D. Johnson, S. Johnson, Five reasons to consider *Phytophthora infestans* a reemerging pathogen, *Phytopathology,* **105** (2015), pp. 966−981.

[29] B. M. Tyler, *Phytophthora sojae*: root rot pathogen of soybean and model oomycete, *Mol. Plant Pathol.,* **8** (2007), pp. 1−8.

[30] S. Dong, G. Kong, D. Qutob, X. Yu, J. Tang, J. Kang, T. Dai, H. Wang, M. Gijzen, Y. Wang, The NLP toxin family in *Phytophthora sojae* includes rapidly evolving groups that lack necrosis-inducing activity, *Mol. Plant Microbe Interact.,* **25** (2012), pp. 896−909.

[31] M. Risteski; Distribution and diversity of pathogenic *Phytophthora* species in the Republic of Macedonia, Doctoral thesis, Ss Cyril and Methodius University in Skopje, Faculty of Forestry, Skopje, p. 143, 2018.

[32] T. Jung, J. Nechwatal, *Phytophthora gallica* sp. nov., a new species from rhizosphere soil of declining oak and reed stands in France and Germany, *Mycol. Res.,* **112** (2008), pp. 1195−1205.

[33] J. Hantula, A. Lilja, H. Nuorteva, P. Parikka, S. Werres, Pathogenicity, morphology and genetic variation of *Phytophthora cactorum* from strawberry, apple, rhododendron, and silver birch, *Mycol. Res.,* **104** (2000), pp. 1062−1068.

[34] A. Vannini, A. M. Vettraino, Ink disease in chestnuts: impact on the European chestnut, *For. Snow Landsc. Res,* **76** (2001), pp. 345−350.

[35] A. Lilja, R. Rikala, A. Hietala, R. Heinonen, Stem lesions on *Betula pendula* seedlings in Finnish forest nurseries and the pathogenicity of *Phytophthora cactorum*, *Eur. J. For. Pathol.,* **26** (1996), pp. 89−96.

[36] A. Lilja, R. Karjalainen, P. Parikka, K. Kammiovirta, H. Nuorteva, Pathogenicity and genetic variation of *Phytophthora cactorum* from silver birch and strawberry, *Eur. J. Plant Pathol.,* **104** (1998), pp. 529−535.

[37] G. Waterhouse, J. Waterston, *Phytophthora cactorum*, *CMI Descriptions of pathogenic fungi and bacteria,* 1966, p. 2.

[38] C. M. Brasier, P. Hamm, E. Hansen, *Phytophthora* diseases: Status of *P. gonapodyides*, *P. drechsleri*, and *P. cryptogea*, *J. For. Res.,* (1989), pp. 45−46.

[39] C. M. Brasier, D. E. L. Cooke, J. M. Duncan, E. M. Hansen, Multiple new phenotypic taxa from trees and riparian ecosystems in *Phytophthora gonapodyides-P. megasperma* ITS Clade 6, which tend to be high-temperature tolerant and either inbreeding or sterile, *Mycol. Res.*, **107** (2003), pp. 277−290.

[40] L. P. Kroon, F. T. Bakker, G. B. M. van den Bosch, P. J. M. Bonants, W. G. Flier, Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences, *Fungal Genet. Biol.*, **41** (2004), pp. 766−782.

[41] B. Ginetti, A. Ragazzi, S. Moricca, First report of *Phytophthora* taxon walnut in Lombardy, North Italy, *Plant Dis.*, **98** (2014), pp. 424−424.

IN VITRO ТЕСТОВИ НА ПАТОГЕНИТЕТ НА СЕДУМ *PHYTOPHTHORA* ВИДОВИ ИЗВЕДЕНИ НА ПИТОМ КОСТЕН

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Во ова истражување беа извршени тестови за патогенитет на седум видови *Phytophthora*, претходно регистрирани на различни дрвни видови во Северна Македонија. Единечни репрезентативни изолати од *P. cactorum*, *P. colocasiae*, *P. taxon walnut*, *P. cinnamomi*, *P. gonapodyides*, *P. inundata* и *P. cambivora* беа инокулирани на отсечени гранчиња од питом костен, со 40 репликати по изолат, поделени во две категории според дебелината на гранчињата. Резултатите покажаа широк опсег на должини на предизвиканите некротични површини, од 1,6 до 5,9 cm, потенцирајќи го различното влијание на секој вид патоген врз костеновите гранчиња. Двонасочната ANOVA статистичка анализа покажа значајна варијабилност во должината на некрозите меѓу двете тестирани категории на дебелина кај сите репрезентативни изолати на *Phytophthora*. Севкупно, најкратка должина на лезиите беше забележана кај *P. inundata*, додека најдолга беше кај *P. cactorum*. Дополнително, кај *P. colocasiae* варијабилноста на должината на лезиите беше конзистентна кај двете категории дебелина на гранчињата, додека *P. inundata* покажа најголема варијабилност меѓу двете категории.

Клучни зборови: *Castanea sativa;* Oomycetes; растителен патоген; инвазивни алохтони видови