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ASPECTS IN PATHOGENETIC FEAUTERS OF *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY TO *FORSYTHIA* SP.

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Abstract. *Sclerotinia sclerotiorum (Lib.) de Bary (1884) is a fungal pathogen that causes substantial losses to a wide range of hosts worldwide. In Romania, Sclerotinia sclerotiorum is known for causing significant damage to plants in field crops and forced crops in greenhouses, warehouses, and silos. Since less are known about the epidemiology of Sclerotinia sclerotiorum in ornamental plants grown in natural landscapes the aim of this paper focuses on the pathogenetic characteristics of the fungus on woody ornamental plants as Forsythia sp. The Forsythia genus is a group of plants in the olive family (Oleaceae) with around 11 species that are primarily native to eastern Asia, with one species from Europe. In Romania, Forsythia spp. Vahl is a perennial plant, appreciated and well known only as an ornamental plant named "golden-bells". This article describes the morphological characteristics and genetic investigation using PCR and Sanger sequencing of Sclerotinia sclerotiorum infection on ornamental plants of Forsythia × intermedia Zab. (F. suspensa × F. viridissima) tissue. Genomic DNA was amplified using ribosomal internal transcribed spacer region-specific primers, and samples were analyzed using sequencing techniques. Constant monitoring and insights record of Sclerotinia sclerotiorum across multiple hosts and time intervals can reduce potential spread and future economic losses in cultivated species.*

Keywords*: Sclerotinia sclerotiorum, morphology , molecular data, new hosts.*

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is a fungal pathogen that can occur in more than 500 plant species worldwide within taxonomic groups as: 64 plant families, 225 genera, 361 species, and 22 other cultivars (BOLAND G.J., HALL R., 1994; FARR D.F., ROSSMAN A.Y., 2017). This soil-borne fungus is an ascomycete, belonging to the *Sclerotiniaceae* family and depending on the location and mechanism of infection can cause different diseases as rot and wilt. *S. sclerotiorum* is characterized by producing white cottony mycelium and melanized sclerotia on infected hosts (PURDY L. H., 1979). *S. sclerotiorum* sclerotia can survive in the soil for up to 10 years and can germinate either carpogenically, producing air-borne ascospores from apothecia, or myceliogenically, producing hyphae (GRABOWSKI M.A., 2017)8. For carpogenic germination, ascospores land on the surface of aerial plant tissues, germinate and then use the dying plant tissue as an exogenous source of nutrients to enable penetration, which represents the most known pathway for infection by *S. sclerotiorum* fungus (SESAN T. E., CRISAN A., 1998). Although extensive research were done on the life cycle of *S. sclerotiorum*, studies focused on understanding the environmental factors in the field that facilitate the emergence and progression of the disease are permanently required (ABAWI G.S., 1979). In carpogenic germination apothecia formation generally involve temperatures among 5 and 25°C, with temperatures above 26°C being detrimental to apothecia production and survival (SMOLIŃSKA U., KOWALSKA B., 2018).

The development of *Sclerotinia sclerotiorum* is strongly controlled by meteorological factors, as air temperature and air humidity, which is why global climate change is likely to influence its spread and impact (BORAH T.R. 2021). Climate change may affect the pathogen

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directly or indirectly via the host plant (MUNOZ M. *et al*, 2023; FIROZ M.J. *et al*, 2016). The *Forsythia* genus is a group of plants in the olive family (*Oleaceae*) with species that are primarily native to eastern Asia and one species from Europe. In Romania *Forsythia* spp. Vahl. is a perennial plant frequently encountered, appreciated and well known only as an ornamental plant named golden -bells, but this plant is also used for a wide range of Chinese medicines and health diets (KIM H. J. *et al,* 2009). In recent years, climatic changes like a cool wet weather in spring and variable winter conditions in the NE region of Romania, have led to the appearance and to an increased incidence of *Sclerotinia sclerotiorum* on *Forsythia* ornamental plants first mention of infection on the *Forsythia* spp. plants being reporten in 2021 (FLOREA A.M. *et al*, 2023).

MATERIAL AND METHODS

Since May 2021 *Sclerotinia sclerotiorum* presence was observed yearly in *Forsythia* spp. plants from the Arboretum Park of the Iasi University of Life Sciences (IULS), located in Iasi, Romania, GPS coordinates: www.google.com/maps 47°11'30.7" N 27°33'25.2" E (FLOREA A. M. *et al*, 2022, FLOREA A. M. *el al*., 2023). In order to indentify, describe the epidemiology and to characterize the pathogenity of *Sclerotinia sclerotiorum* on *Forsythia* sp. plants after a putative micromycete identification and based on visual symptoms infected tissues of the *Forsythia* host plant were collected and investigated in the research laboratory of the phytopathology discipline within the "Ion Ionescu de la Brad" Iasi University of Life Sciences (IULS). According to the fungal morphology, microscopic preparations, and specialized guidebooks, we followed a standard procedure (WANG A. R. *et al*, 2008) for fungal isolation in order to confirm the presence of *Sclerotinia sclerotiorum* in forsythia plants. Therefore infected tissues of *Forsythia* × *intermedia* Zab. were cut into small pieces and then rinsed 3~4 times with diluted water after being treated with 70% (v/v) ethanol for 2~3 s. The treated tissues were transferred to potato dextrose agar (PDA) medium and cultured at 25 °C. Then, whitish, cottony mycelium was transferred into PDA plates for 10 days to obtain pure cultures. White masses of mycelium developed to the edge of the Petri dish, and formed sclerotia on the PDA medium of the selected isolate coded SS_F. As the growth stage of the plants, temperature and humidity have an important role in the onset of infections thus, environmental conditions were also registred with the aid of an iMeteos 1 device within weather station no. 0000198B of Field Climate by Pessl Instruments (https://www.fieldclimate.com).

RESULTS AND DISCUSSIONS

During observation periods the environmental conditions registered in the springs times were extremely favorable to carpogenic germination of *Sclerotinia sclerotioum* (Lib.) de Bary that occured on *Forsythia* spp. plants from arboretum park of Iasi University of Life Sciences (IULS), located in Iasi city (Table 1). Considering cool and wet weather registred in 2021 for Iasi, Romania favored *Sclerotinia sclerotioum* ascospores to initiate infection in *Forsythia* spp. plants. Every spring for the last four years the floral elements, especially the stamens and the pistil, were highly sensitive to infection by ascospores. The ascospores completely colonized the mature and senescent flowers, and after a few days, the mycelium that colonized these flowers caused further infections through direct contact with the leaf and stem. Water-soaked lesions progressed into branch tissue, resulting in the wilting of individual branches (Figure 1a, b). Necrotic tissues from the affected branches were examined, and we observed that they were covered with patches of fluffy white mycelia and sclerotia (Figure 1c).

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Table 1

Average of Air temperature (°C), Dew Point (°C), relative humidity (%) and precipitation (mm) registered during period 2021-2024 for Iasi, Romania (www.fieldclimate.com)

Data	HC Air temperature [°C]			Dew Point [°C]		HC Relative humidity [%]	Precipitation [mm]
	media	max	min	media	min	media	suma
2024-12-01	2,07	13,12	-8,56	0,2	-29,7	13,85	53,6
2024-11-01	3,4	20,88	-4,77	-2	-38,6	42,72	54,2
2024-10-01	10,43	25,74	-2,96	0,2	-0,3	2,39	43,6
2024-09-01	18,33	33,85	4,9	4,5	-0,2	26,14	154,4
2024-08-01	24,18	37,45	10,99	0,6	-18,5	4,5	36,4
2024-07-01	24,76	39,91	11,77	-0,1	-33,5	3,56	36,6
2024-06-01	22,55	34,67	10,94	0,5	-32,3	4,11	58
2024-05-01	16,29	29,98	2	0,1	0	0,67	60,2
2024-04-01	13,88	29,44	-0,12	0,7	-20,2	7,35	35,6
2024-03-01	7,04	27,59	-7,68	0,7	-2	6,67	58,6
2024-02-01	6,66	18,44	-7,49	0,2	-14,9	8,37	14,6
2024-01-01	-0,69	13,67	-19,39	-0,1	-7,9	2,02	21,8
2023-12-01	2,44	17,56	-8,03	-0,4	-31,2	2,67	8,6
2023-11-01	6,78	21,05	-7,14	0	0	0,69	95,6
2023-10-01	14,31	32,7	-2,1	0,5	0	2,6	19,4
2023-09-01	19,55	32,59	7,48	0	0	0	10,6
2023-08-01	24,17	38,8	10,7	0,2	0	0,58	16,4
2023-07-01	23,23	36,88	11,47	0,2	0	1,08	136,8
2023-06-01	20,29	34,25	4,69	7,4	-25,4	41,07	29,6
2023-05-01	15,79	28,28	1,24	4,7	0	31,21	18,4
2023-04-01	8,33	20,68	-1,48	5,3	-1,3	75,53	88,6
2023-03-01	6,71	23,09	-6,04	1,5	-8,7	72,82	5,6
2023-02-01	1,96	18,05	-16,41	-0,9	-16,3	83,41	23
2023-01-01	3,12	17,86	-6,77	2,5	-6,6	96,14	7,4
2022-12-01	1,4	13,68	-9,48	0,9	-9,3	71,44	20,2
2022-11-01	5,67	18,71	-1,27	1,6	0	17,76	69,2
2022-10-01	11,74	27,21	-3,75	4,2	-2,1	45,85	18,8
2022-09-01	15,64	28,31	3,01	5	0	30,98	78
2022-08-01	22,77	34,84	13,63	17,2	0	77,77	53
2022-07-01	23,01	35,79	8,57	14,6	5,6	63,59	20,4
2022-06-01	21,58	37,01	9,17	8,7	0	43,49	24,6
2022-05-01	16,5	31,92	2,08	5,6	0	36,96	24
2022-04-01	10,25	26,31	-3,78	2,2	-6,9	51,07	80,6
2022-03-01	3,09	23,13	-9,56	-4,4	-16,1	63,52	16
2022-02-01	3,63	16,71	-6,85	0,3	-6,4	48,25	16,4
2022-01-01	0,69	13,14	-10,5	-0,5	-11,9	25,83	14,4
2021-12-01	0,79	12,17	-12,12	-0,4	-12	32,38	60,2
2021-11-01	6,69	19,05	-4,6	4,4	-5,2	85,9	14,2
2021-10-01	9,01	22,9	-3,24	3,8	-4,4	73,85	5,6
2021-09-01	14,61	27,52	3,4	9,6	0	73,81	12,4
2021-08-01	20,96	33,73	9,7	14,2	0	69,98	155,6
2021-07-01	23,23	36,65	12,14	16	0	69,81	71,6
2021-06-01	19,79	33,77	8,46	17,2	7,3	87,75	115
2021-05-01	15,19	30,39	0,82	11,1	0	78,62	87
2021-04-01	8,22	23,93	-3,66	3,8	-5	67,42	56,4
2021-03-01	3,37	17,39	-7,24	0,3	-3,3	14,77	65,6
2021-02-01	-0,47	19,42	-15,74	-0,3	-10,6	58,49	22,8
2021-01-01	0,39	11,68	-19,62	-0,3	-19,5	29,39	26,8

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The morphological analysis showed that the fungus *Sclerotinia sclerotiorum* infects the shoots of *Forsythia* only through the withering flowers, especially the stamens and the pistil, and extends up and down the woody branches from those points, causing wilting on the individual branches. Fungal-caused lesions progressed into branch tissue and led to a wilting effect. Taking into account the infection mechanism, effective management practices are necessary to limit the presence of this pathogen in other potential hosts and reduce the possibility of outbreaks.

In order to avoid any confusion regarding the presence and classification of the genus *Sclerotinia*, after a morphological pathogen identification based on described symptoms the fungus was isolated from infected tissues of forsythia and cultured on PDA medium. Thus, the fungus started to produce white masses of mycelium that grew to the edge of the Petri dish. The size of mycelium masses became bigger and their colors became darker as time proceeded then, man black sclerotia formed (Figure 2). Forward Kock's postulates were fulfilled using the isolate coded SS_F to inoculate six branches with a length of 25-30 centimeters of *Forsythia* spp. by placing 1-cm agar plug with actively growing hyphal tips from a colony of *Sclerotinia sclerotiorumon*. Six other branches were inoculated with an agar plug with no hyphae place on the branch. All branches were maintaining in a desinfected area, with conditions for the relative humidity close to 90% and temperature at 28 °C with 10 h of light daily, after which water-soaked lesions similar to arboretum park samples were observed on the six inoculated branches.

Figure 1. *Sclerotinia sclerotioum* on *Forsythia*: (a) wilted flowers; (b) wilted branches; (c) Necrotic tissues of *Forsythia* spp. with patches of white mycelia and sclerotia.

Genomic DNA isolated from plant material infected with *Sclerotinia sclerotiorum* has been subjected to PCR amplification af the 5.8S rRNA region folloded by Sanger sequence. The PCR amplicon sizes obtained from the 4 selected primes are ITS1/ITS2 and LSU D1/D2. Information regarding the similarity of the investigated sequence was obtained by conducting

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BLAST searches (blastn) against the GenBank database (NCBI, 2023) and by using simple neighbor joining clustering based on uncorrected p-distances (ALTSCHUL S.F. *et al,* 1990).

Figure 2. White masses of *Sclerotinia sclerotioum* mycelium and sclerotia developed on PDA medium plates.

The BLAST results indicated that the nucleotide sequence on the selected sample had 99.91% similarity with *Sclerotinia sclerotiorum* chromosome 7 sequence (Sequence ID: CP017820.1) and *Sclerotinia sclerotiorum* isolate KR1121_1 (Sequence ID: KC311494.1). The phylogenetic analysis revealed that isolate SS_R was phylogenetically identical with other *S. sclerotiorum* isolates available in the GenBank database. Phylogenetic analysis of *Forsythia* twig blight the other fungal isolates from NCBI GeneBank indicated high similarity to other *S. sclerotiorum* isolates, with the *Sclerotinia sclerotiorum* chromosome 7 sequence (Sequence ID: CP017820.1) *and Sclerotinia sclerotiorum* isolate KR1121_1 (Sequence ID: KC311494.1). Based on the results of this study, molecular and morphological data suggest that *Forsythia* twig blight was caused by *S. sclerotiorum* (FLOREA A. M . *et al,* 2023) .

CONCLUSIONS

Presented issues can contribute to the knowledges regarding the process of infection and pathogenesis in the *Sclerotinia* disease.

Morphological analysis showed that the fungus *Sclerotinia sclerotiorum* infects the shoots of *Forsythia* only through the withering flowers, especially the stamens and the pistil, and extends up and down the woody branches from those points, causing a wilting on the individual branches.

Awareness of the favorable environmental conditions and knowledge of life cycles of the host species can help to prevent *Sclerotinia sclerotiorum* infections.

For a better understanding of the germination and dormancy requirements of sclerotia caused by *Sclerotinia sclerotiorum* is needed to improve disease-forecasting models.

Even if, currently twig blight symptoms are rare and during this evaluation, we observed a relative small area affected, a constant monitoring of *Sclerotinia sclerotiorum* across multiple hosts and time will reduce the potential spread and future economic losses in cultivated species.

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