Anadolu Orman Araştırmaları Dergisi
Anatolian Journal of Forest Research
http://dergipark.org.tr/ajfr

Pathogenicity of the *Sydowia polyspora* originating from different hosts on *Pinus brutia* in Türkiye

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ARTICLE INFO
Received: 05/07/2024
Accepted : 05/09/2024
https://doi.org/10.53516/ajfr.1511176
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ABSTRACT **Research Article**

Background and aims Sydowia polyspora is a significant fungus associated with seasonal needle necrosis, particularly on *Abies* spp. and *Pinus* spp. in Europe and North America. *Pinus brutia* is a species widespread in the Mediterranean basin with high economic and ecological value. This study aimed to determine the susceptibility of *P. brutia* seedlings in Türkiye to local *S. polyspora* isolates and to investigate the virulence differences among isolates obtained from different hosts.
Methods S. polyspora isolates from various hosts were inoculated into the shoots of two-year-old *P. brutia* seedlings. The seedlings were monitored in comparison with a control group, and pathogenicity indicators such as lesion length were recorded.
Results The results showed that 7 out of 10 tested isolates were pathogenic on *P. brutia* seedlings and successfully colonized the living heartwood tissue. Isolates from *Pinus pinea* were found to be pathogenic on *P. brutia*, and isolates obtained from shoots caused longer lesions in hosts. Additionally, isolates from symptomatic trees exhibited higher virulence compared to those from asymptomatic trees. Isolates from *P. brutia* exhibited higher virulence compared to isolates from *Pinus nigra* and *P. pinea*.
Conclusions P. brutia seedlings are highly susceptible to local *S. polyspora* isolates, posing a potential threat to the region's forest ecosystems. The fungal pathogen was successfully re-isolated from symptomatic tissues, and no symptoms were observed in the control plants.

Key Words: Conifer, current-season needle necrosis, *Sydowia polyspora*, tip dieback, virulence

Türkiye'de farklı konukçulardan elde edilen *Sydowia polyspora* izolatlarının *Pinus brutia* üzerindeki patojenitesi

ÖZ

Giriş ve Hedefler Sydowia polyspora, Avrupa ve Kuzey Amerika'da özellikle *Abies* spp. ve *Pinus* spp. üzerinde mevsimsel ibre nekrozu ile ilişkili önemli bir fungustur. *Pinus brutia*, Akdeniz havzasında yaygın olarak bulunan ve yüksek ekonomik ve ekolojik değere sahip bir türdür. Bu çalışmada, Türkiye'deki *P. brutia* fidanlarının yerel *S. polyspora* izolatlarına duyarlılığını belirlemek ve farklı konukçulardan elde edilen izolatlar arasındaki virülans farklılıklarını araştırmak amaçlanmıştır.
Yöntemler Farklı konukçulardan izole edilen *S. polyspora* izolatları, iki yaşındaki *P. brutia* fidanlarının sürgünlerine inokule edilmiştir. Fidanlar, kontrol grubuyla karşılaştırmalı olarak izlenmiş ve lezyon uzunluğu gibi patojenisite göstergeleri kaydedilmiştir.
Bulgular Çalışma sonuçları, test edilen 10 izolatın 7'sinin *P. brutia* fidanlarında patojenik olduğunu ve canlı öz odun dokusunda başarılı bir şekilde kolonize olabildiğini göstermiştir. *Pinus pinea*'dan elde edilen izolatların *P. brutia* üzerinde patojenik olduğu, sürgünlerden elde edilen izolatların ise konukçularda daha uzun lezyonlar oluşturduğu tespit edilmiştir. Ayrıca, semptomatik ağaçlardan elde edilen izolatların, semptomatik olmayan ağaçlardan elde edilenlere göre daha yüksek virülans gösterdiği belirlenmiştir. *P. brutia*'dan elde edilen izolatlar, *Pinus nigra* ve *P. pinea*'dan elde edilen izolatlara göre daha yüksek virülans göstermiştir.
Sonuçlar P. brutia fidanları, yerel *S. polyspora* izolatlarına karşı oldukça duyarlıdır ve bu durum, bölgedeki orman ekosistemleri için potansiyel bir tehdit oluşturmaktadır. Fungal etmen, semptomatik dokulardan başarılı bir şekilde yeniden izole edilmiştir. Kontrol bitkilerinde herhangi bir semptom gözlenmemiştir.

Anahtar Kelimeler: Konifer, mevcut sezon ibre nekrozu, *Sydowia polyspora*, geriye doğru ölüm, virülans

Citing this article:
Beram, R.C., 2024. Pathogenicity of the *Sydowia polyspora* originating from different hosts on *Pinus brutia* in Türkiye. Anatolian Journal of Forest Research, 10(2), 36-40.

1. Introduction

Sydowia polyspora (Bref. and Tavel) E. Müller (anamorph*: Hormonema dematioides* Lagerb. and Melin) is a prevalent fungal species commonly found in association with conifers, often as an epiphyte (Kowalski and Pozdzik, 1993; Kowalski and Kehr, 1996) or an endophyte (Sieber-Canavesi and Sieber, 1993) on asymptomatic plants and within seeds. This fungus has the potential to turn pathogenic in colonized hosts, particularly under conditions of abiotic or biotic stress (Ridout and Newcombe, 2018; Talgø et al., 2010). *Sydowia polyspora* is frequently linked with current-season needle necrosis and tip dieback on conifer species, notably *Abies* and *Pinus* spp., across Europe and North America (Talgø et al., 2010; Tinivella et al., 2014; Pan et al., 2018; Silva et al., 2020; Çakar et al., 2022; 2023, Beram and Demiröz, 2024). It is considered a significant fungal species in various regions, especially in the Mediterranean, due to its significant impact on timber yields and economic values (Silva et al., 2020). Primary symptoms include the development of tan-to-yellow-colored bands or spots on needles, which turn reddish-brown during summer, along with tip dieback observed in *Pinus* spp. (Tinivella et al., 2014; Pan et al., 2018; Silva et al., 2020;). These chlorotic lesions on needles are randomly distributed throughout healthy foliage (Pan et al., 2018).

Pinus brutia Ten., commonly known as Turkish red pine, is the predominant tree species in the Aegean and Mediterranean regions of Türkiye (Arbez, 1974). It covers the largest area in Turkish forests, extending up to 5.2 million hectares (GDF, 2021). Renowned for its success in reforestation efforts, particularly in southern and western Türkiye, Turkish red pine is a highly favoured species. As such, it is imperative that all forestry activities within Turkish red pine stands adhere to the principles of science and technology to ensure the sustainability of this vital resource. Decades of intensive forestry management practices in Türkiye have been influenced by the species' robust regeneration capabilities, positioning it as one of the most preferred species for afforestation initiatives, including industrial plantations, in Turkish forestry (Koski and Antola, 1993). Recognizing the pivotal role of Turkish red pine, understanding its susceptibility to pathogens is crucial for its continued success, necessitating the development of effective disease management and control strategies.

The aim of the work reported here was to (i) determine the susceptibility of the *P. brutia* in Türkiye to local *S. polyspora* (ii) to investigate whether differences in virulence occurred between isolates originating from different hosts (iii) to determine whether virulence differs between isolates obtained from symptomatic and non-symptomatic trees.

2. Materials and Methods

2.1 Plant material and fungal isolates

To test the pathogenicity of *S. polyspora* isolates on *P. brutia*, inoculations were performed on healthy 2-year-old *P. brutia* seedlings obtained from Denizli Karahasanlı Forest Nursery. Ten isolates of *S. polyspora*, obtained from needles and shoots of various hosts in different locations in Türkiye, were randomly selected from the isolates stored in the fungal biotechnology laboratory at Pamukkale University (Table 1). Inocula were prepared by subculturing *S. polyspora* isolates on 2% Potato Dextrose Agar (PDA; Merck, Germany) for 10 days at 25°C.

Table 1. Isolates included in this study

No.	Isolate code	Host	Location	Symptom	Tissue
1	KA1CZ1	*Pinus brutia*	Denizli/Kale	+	shoot
2	E16CK	*Pinus nigra*	Çankırı/Eldivan	-	shoot
3	FE1CZ5	*Pinus brutia*	Muğla/Fethiye	-	needle
4	US1CZ2	*Pinus brutia*	Uşak	+	shoot
5	US1CZ1	*Pinus brutia*	Uşak	+	needle
6	IZ1CF2	*Pinus pinea*	İzmir	+	shoot
7	IZ1CF1	*Pinus pinea*	İzmir	+	shoot
8	ESN2CZ6	*Pinus brutia*	Denizli/Beyağaç/Eşenler	+	needle
9	ES2CZ4	*Pinus brutia*	Denizli/ Beyağaç/Eskere	+	shoot
10	ES1CZ4	*Pinus brutia*	Denizli/Beyağaç/Eskere	+	shoot

2.2 Inoculation, incubation and sampling

In order to test the pathogenicity of *S. polyspora* isolates on *P. brutia*, inoculation was performed in April 2023. Five replicate *P. brutia* plant shoots were inoculated per isolate; the control treatment included treating five plants with fresh, sterile PDA. The shoots were wiped with 70% ethanol before being wounded. A 5 mm wound was made on the surface of each shoot using a cork borer, and an agar plug was inoculated between the phloem and the bark. After inoculation, the wounds were wrapped with sterilized cotton and sealed with Parafilm M (American Can. Co., Greenwich, CT, USA) (Pan et al., 2018; Silva et al., 2020). Plants were incubated in the growth chamber at 18–20°C, with natural light and watering at least twice a week. After 8 weeks, the bark tissues were removed, lesion length was assessed, and re-isolation performed. For each shoot, the necrotic area was cultured on PDA and incubated at 25°C for 7 days in the dark.

2.3 Statistical analyses

All data were analysed using the SPSS GLM procedure (IBM SPSS Statistics for Windows, Version 20; IBM Corp., Armonk, NY, USA). Differences between host and isolate mean values were analysed using Duncan's multiple range tests. Isolates were compared with two ways ANOVA and host–isolate interaction was determined using multivariate test in SPSS package program.

3. Results

A total of 55 *P. brutia* seedlings used in the study were an avarage 6.5 mm in diameter, and 29.3 cm tall. At the end of the 8 weeks incubation period, necrotic areas extending beyond the inoculated points were observed on the phloem of all shoots inoculated with 7 isolates (US1CZ2, US1CZ1, IZ1CF2, IZ1CF1, ESN2CZ6, ES2CZ4, ES1CZ4).

The fungus was re-isolated successfully from the shoots inoculated with these isolates, thus confirming that *S. polyspora* was able to cause necrosis on red pine shoots. In controls and with the other 3 isolates tested (KA1CZ1, E16CK, FE1CZ5), necrosis was observed only in the inoculation point, without further extension. No seedling mortality occurred during the incubation period.

Table 2. Differences between lesion size caused by isolates of *Sydowia polyspora* on seedlings of *Pinus brutia*

Sample code	Lesion length (mm)	Lesion width (mm)
Control	-	-
KA1CZ1	-	-
E16CK	-	-
FE1CZ5	-	-
US1CZ2	6 (0.3)[1] b[2]	3 (0.2) b
US1CZ1	4 (0.4) a	2 (0.2) a
IZ1CF2	5 (0.5) ab	2 (0.2) a
IZ1CF1	7 (0.4) c	3 (0.2) b
ESN2CZ6	6 (0.5) b	-
ES2CZ4	10 (1.1) d	3 (0.2) b
ES1CZ4	11 (1.3) d	12 (1.1) c

[1]: Standard deviation, [2]: The same letters in a column of lesion length and lesion width are not significantly different ($p \leq 0.05$). The post hoc tests (Duncan) for the lesion length and lesion width were analyzed separately because the interaction factor was significantly different.

Symptom development varied among *P. brutia* shoots inoculated with different *S. polyspora* isolates. Following the ANOVA analysis, the Duncan test was employed to assess differences in arithmetic means. Out of 10 isolates tested, 7 were found to be pathogenic on the hosts and capable of growing in living sapwood. The results revealed homogeneous grouping between isolates US1CZ2 and ESN2CZ6, as well as ES2CZ4 and ES1CZ4 for lesion length. Isolate IZ1CF1, US1CZ1 and IZ1CF2 formed their own homogeneous group. Lesion lengths in the phloem following inoculation with isolates ES2CZ4 and ES1CZ4 were significantly longer than those induced by other isolates ($p < 0.05$; Table 2 and Fig 1).

The results revealed homogeneous grouping between isolates ES2CZ4, IZ1CF1, US1CZ2, as well as US1CZ1 and IZ1CF2 for lesion width. Isolate ES1CZ4 formed its own homogeneous group. Isolate ES1CZ4 showed significantly wider lesions than other isolates ($p<0.05$; Table 2) and completely surrounded the shoot, showing obvious symptoms. Following ES1CZ4, the widest lesions were observed in isolates ES2CZ4, IZ1CF1 and US1CZ2.

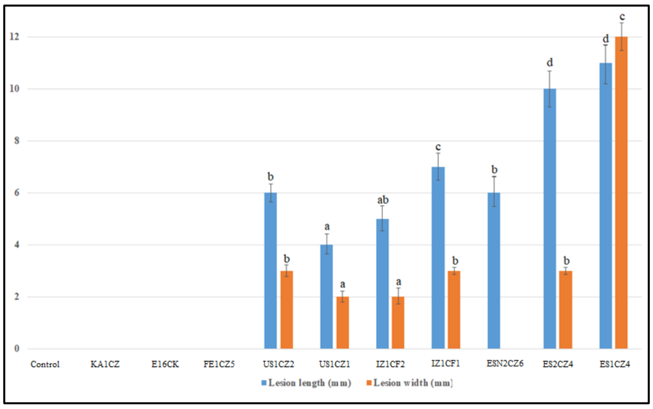

Figure 1. Differences between isolates of lesion length and lesion width on *Pinus brutia*

Isolates originating from shoots (ES2CZ4 and ES1CZ4) caused longer lesions on the hosts (Table 1), while those from symptomatic trees were more virulent than those from non-symptomatic trees. Additionally, isolate ES2CZ4 caused varying degrees of chlorosis or discoloration of the needles 27 days after inoculation.

While the lesion length was determined to a certain extent in isolate ESN2CZ6 obtained from the needle, this isolate did not develop a lesion width. The IZ1CF2 and IZ1CF1 isolates obtained from symptomatic shoots of *P. pinea* caused lesions on *P. brutia* seedlings. Additionally, the isolate obtained from symptomatic shoots of *P. brutia* did not surprisingly show any lesions. Isolate E16CK obtained from healthy shoots of *P. nigra* and isolate FE1CZ5 obtained from healthy needle of *P. brutia* did not cause any lesions. Also, no symptoms were observed the control plants.

4. Discussion

Inoculation tests showed that seven of the ten isolates caused lesions on *P. brutia* shoots. This suggests that *S. polyspora* is able to colonize shoot phloem of *P. brutia*. Lesions were consistent in all replicates of the isolates. Three isolates used in the inoculation tests did not cause necrosis on the shoots. Beram and Demiröz (2024) observed that each of the five isolates used in their study caused lesions in red pine shoots in at least three out of five replicates. They suggested that the lack of lesion induction in some replicates might be attributed to procedural errors during inoculation or variations in host genotype susceptibility. Silva et al. (2019) found that two out of three isolates used in their inoculation tests did not result in necrosis on the shoots. Similarly, Pan et al. (2018) observed varying degrees of symptom development on needles of *P. yunannensis* inoculated with different *S. polyspora* strains, with four out of ten strains showing no symptoms. Talgø et al. (2010) observed no symptom development in one out of three tests conducted with the same strains, suggesting that a range of abiotic and biotic factors may influence disease development by affecting pathogen ecology or host susceptibility.

In this study, the maximum average lesion length on living sapwood of pine shoots was 11 mm after 8 weeks. In a previous 2-month pathogenicity study, the maximum average lesion length of *S. polyspora* isolates obtained from Denizli was recorded as 16 mm (Beram and Demiröz 2024). The lesion lengths in the phloem following inoculation with Denizli isolates (ES2CZ4 and ES1CZ4) were significantly greater than those induced by the other isolates ($p<0.05$; Table 2). Importantly, these isolates were derived from shoots displaying CSNN symptoms, underscoring their potential virulence. This observation suggests that the specific conditions of the sample area may influence isolate virulence. Given that environmental factors and local pathogen pressures can vary considerably, it is likely that the unique conditions of the sample area contribute to the observed increase in pathogenicity of these isolates. Further investigation into the environmental and genetic factors prevalent in these regions is warranted to elucidate the mechanisms underlying this heightened virulence.

Additionally, after 27 days, typical symptoms of CSNN were observed on two seedlings inoculated with the isolate ES2CZ4. The isolate ES1CZ4, obtained from *P. brutia* shoots, completely encased the pine shoots horizontally following pathogenicity, exhibiting highly noticeable symptoms. No such horizontal encasement was observed in the other isolates. Although isolate ES1CZ4 showed significantly larger lesions than other isolates ($p < 0.05$; Table 2), isolate ES2CZ4, another isolate showing the longest lesion, showed reduced lesion width. There may be differences in virulence between isolates, or this may be due to any situation in the tree structure.

Isolates obtained from *P. pinea* were found to be pathogenic on *P. brutia*. This finding suggests a potential cross-species threat, highlighting the need for further research into the mechanisms of infection and potential measures for disease management in forest ecosystems.

Our inoculation experiments indicate that *S. polyspora* has the potential to induce necrosis on *P. brutia* shoots. The observed symptoms could significantly compromise the health of *P. brutia* trees. This emphasizes the need for comprehensive investigations and assessments to better understand the disease's impact in the future. However, it's worth noting that not all pine shoots inoculated with *S. polyspora* exhibited typical symptoms, suggesting the possibility of the fungus existing on shoots with less obvious signs. Factors such as host resistance, pathogen genotype, inoculum efficiency, and environmental conditions can influence the development of necrosis (Talgø et al., 2010).

Beram and Demiröz (2024) have reported the involvement of *S. polyspora* in the occurrence of needle necrosis and shoot dieback across five distinct *P. brutia* sampling sites in Denizli, Türkiye. Additionally, this fungus has the potential to cause serious diseases in other pine species under stress conditions. Silva et al. (2020) have reported its potential to affect peanut production, particularly in *P. pinea*. They reported that the necrotic shoots in the upper part of the canopy of *P. pinea* are particularly concerning due to their potential to compromise the development of pine cones and, consequently, the production of pine nuts. These effects can lead to economic losses for this species. In addition, the lesions caused by isolates obtained from *P. pinea* (IZ1CF2 and IZ1CF1) in our study raise concern. Therefore, future studies should focus on detailed investigation of the relationship between *P. pinea* and *S. polyspora* on a national scale in Türkiye. In a other study conducted in Türkiye, *S. polyspora* was isolated from 25% of the *Abies equi-trojani* needles exhibiting CSNN symptoms. Noticeably, the percentage of *S. polyspora* isolates obtained from asymptomatic needles was reported as 1% (Çakar et al., 2023).

Furthermore, *S. polyspora* was recently detected in asymptomatic seeds of various *Pinus* spp. collected from Europe and North America, including *Pinus pinaster*, *Pinus radiata*, *Pinus strobus*, *Pinus sylvestris*, and *Pinus mugo* from Portugal, as well as *P. pinea* from Türkiye (Cleary et al. 2019). These findings underscore the necessity for a deeper understanding of the dissemination pathways and interactions between the fungus and its host plants. Further research endeavors are currently ongoing to comprehensively understand the pathways, as well as the biotic and abiotic factors, that contribute to the manifestation of this disease within the forests of Türkiye. Investigating CSNN comprehensively in pine forests is crucial for determining suitable silvicultural and other control methods.

Acknowledgements

The author is thankful to the Forestry Ministry for providing the seedlings for this study. Special thanks are also due to Dr. Funda Oskay and MSc. Fatma Demiröz for their efforts in obtaining some isolates, and to MSc. Sultan Akyol for her assistance in conducting the inoculation studies.

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