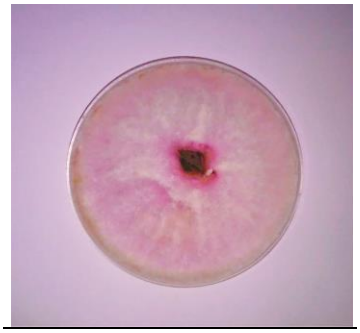

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# The effect of temperature on the *Fusarium sambucinum* growth from the one-year-old *Fraxinus excelsior* seedlings in Montenegro

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## Abstract

Species complex *Fusarium sambucinum* was frequently isolated from ash stands in Montenegro. Previous researches revealed that it is dangerous pathogen for one-year-old seedlings. The aim of this research was to investigate the influence of temeperature on growth and morphological characteristics of morphotype isolated from one-year-old common ash (*Fraxinus excelsior* L.) seedlings in Montenegro. Growth of *Fusarium sambucinum* pure cultures on different temperatures was the fastest on 25°C while the absence of growth occurred on 32.5°C. Cultures on optimum temperature were white reddish, aerial and with a lot hyaline, curved multi septate conidia. This research covers basic ecological and morphological characteristics of *Fusarium sambucinum* strain responsible for high pathogenicity towards one-year-old common ash seedlings in Montenegro.

## Keywords

*Fusarium sambucinum*; *Fraxinus excelsior*; Temperature; Morphology

## Contents



## 1 Introduction

There is increasing threat to ash species in Europe due to ash dieback caused by *Hymenoscyphus fraxineus* (Kowalski) Baral, Queloz and Hosoya (Gross et al. 2014) and occurrence of large number of other fungal species in different stages of dieback (Kowalski et al. 2016). Also, other studies showed a large number of fungal taxa

colonizing common ash tissues (Przybył 2002a; b; Bakys et al. 2009; Vemić and Milenković 2018; Vemić 2020). Due to this, the expansions of researches focused on different fungal taxa recorded on common ash are required in order to better understand ash dieback progress and the role of other fungi in it.

Genus *Fusarium* was established by Link in 1809 (Lesilie and Summerell 2006). First, genus was named *Fusisporium* (Lesilie and Summerell 2013). Also, many authors state this genus cause diseases in plants, humans and animals. Many plants have at least one disease caused by *Fusarium* species (Lesilie and Summerell 2006). Identification of species in this genus is complicated due to number of species in genus was constantly changing in last century according to different taxonomic systems (Moretti 2009). This genus has significant importance in forestry (Karadžić 2010).

During the monitoring of fungal diversity and health condition of common ash (*Fraxinus excelsior* L.) in Montenegro, species complex *Fusarium sambucinum* was frequently isolated from one-year-old common ash trees (Vemić et al. 2019; Vemić 2020). This study was performed to investigate the influence of temperature on growth rate and morphology of strain that caused significant dieback of one-year-old common ash seedlings in Montenegro. Results will contribute to better understanding the conditions contributing infection in field and solving complicated taxonomic situation within this genus.

## 2 Material and method

### 2.1 Tested fungal strain

*Fusarium sambucinum* aff. strain used in this research was taken from the mycological collection in Department of Forest Protection, University of Belgrade. Fungus was isolated from one-year-old common ash (*Fraxinus excelsior*) seedlings using standard procedure (Vemić et al. 2019). Strain was previously identified using molecular methods with ITS1/ITS4 and EF1/EF2 primers (Vemić et al. 2019). Cultures of tested strain were grown in standard petri dishes on 3% MEA (malt extract agar, Scharlau Spain, Torlak, Serbia).

### 2.2 Influence of temperature on mycelium

Cultures of tested strain were grown on next temperatures 10, 15, 20, 25, 27.5, 30, 32.5 and 35 °C. Experiment was finished after three days when all cultures showed clear growth and some of the cultures nearly filled petri dish. Each petri dish was measured at four directions and average growth based on the number of measurements within each temperature was calculated. Measurements were performed in mm using manual steel caliper (Euromaster Imp&Exp Co.Ltd, China) with precision of 0.1mm. Vitality of cultures from 32.5 and 35 °C where growth of mycelium stopped was additionally examined. Vitality was assessed through placing these cultures on optimum temperature after the experiment with different temperatures was finished.

### 2.3 Examination of morphological characteristics

Cultures grown on optimum temperature were chosen for investigation of morphological characteristics including color and texture of colony as well as types

and shapes of spores. Examination was performed on 3% MEA. Characteristics of colony were assessed visually. Characteristics of spores were examined with AmScope B120 C E1 microscope under 400x enlargements. Examined characteristics included: abundance of sporodochia and spores, types of spores and shape of spores.

### 2.4 Statistical methods

Kruskal Wallis test was used to test difference in mycelium growth between tested temperatures. A Mann Whitney U test was used to test difference in mycelium growth between each pair of tested temperatures. Arithmetic mean with standard deviation was used to show average growth of mycelium within different temperatures. All analyses were performed in SPSS 21 and Microsoft Office Excel 2010 software.

## 3 Results

### 3.1 Influence of temperature on mycelium

There was statistically significant difference in mycelium growth between tested temperatures ($H = 300.480$, $p < 0.000001$). The lowest growth was recorded on 10°C while the fastest growth was recorded on 25°C (Figure 1, Table 1). The absence of growth was recorded on 32.5°C (Figure 1, Table 1). Values of growth showed that higher temperatures lead to faster decline of mycelium growth (Figure 1, Table 1). Cultures from 32.5 and 35°C were still alive after the end of experiment. Daily growth of cultures during the experiment is showed on Figures 2-7.

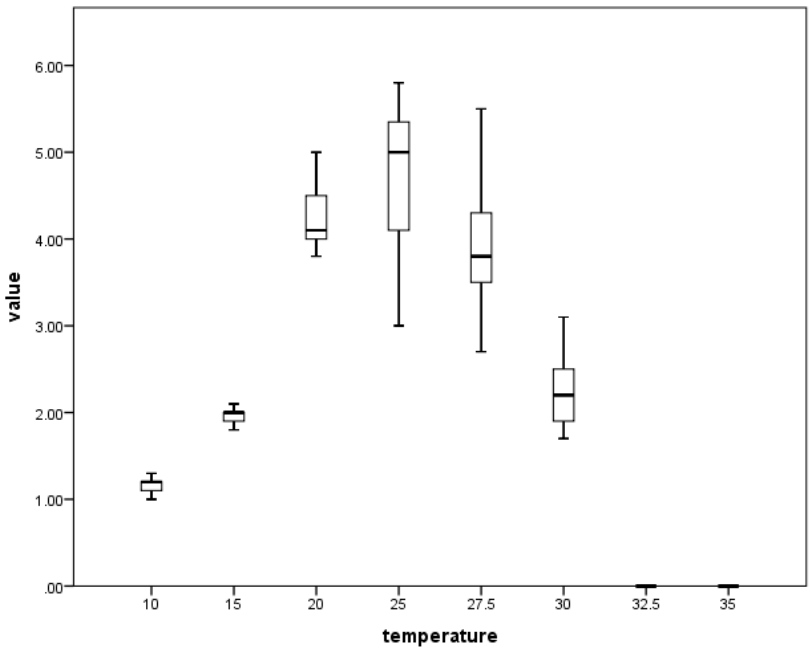


Figure 1. Average growth of cultures according to temperature.

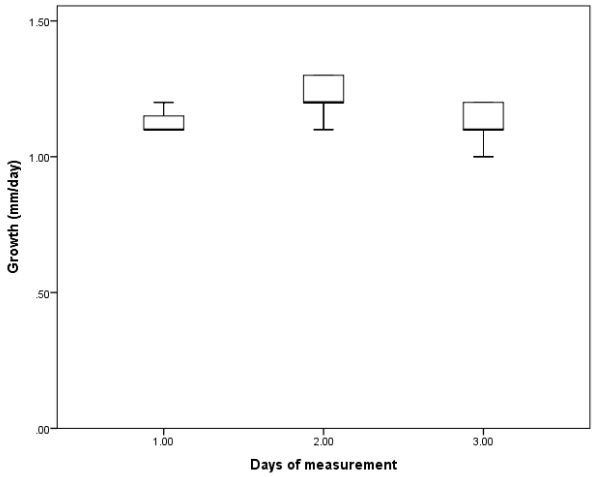


Figure 2. Daily growths of cultures on 10°C.

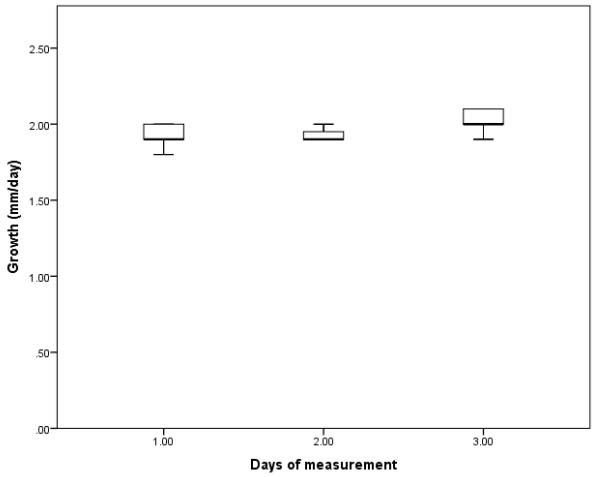


Figure 3. Daily growths of cultures on 15°C.

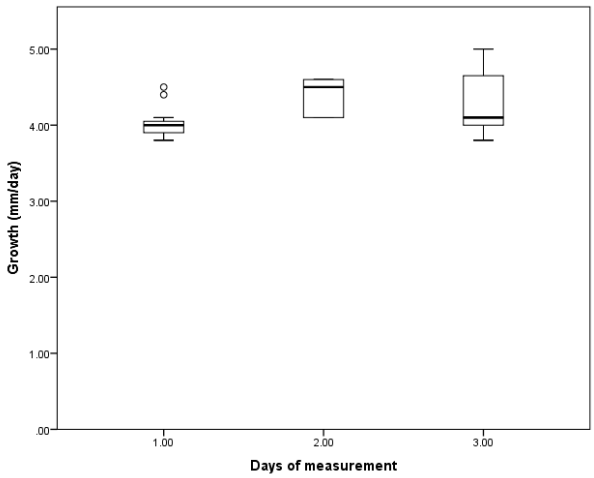


Figure 4. Daily growths of cultures on 20°C.

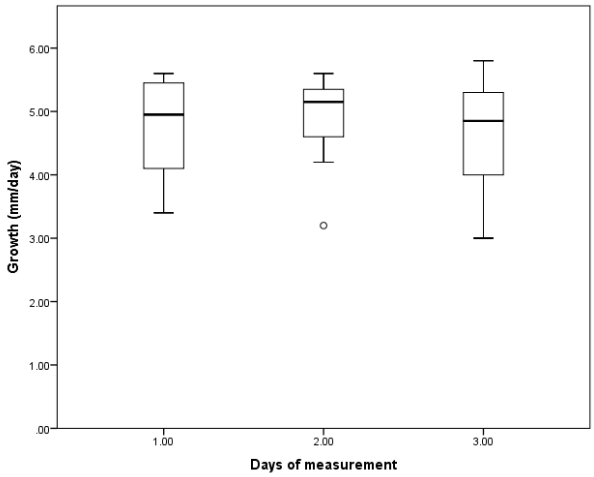


Figure 5. Daily growths of cultures on 25°C.

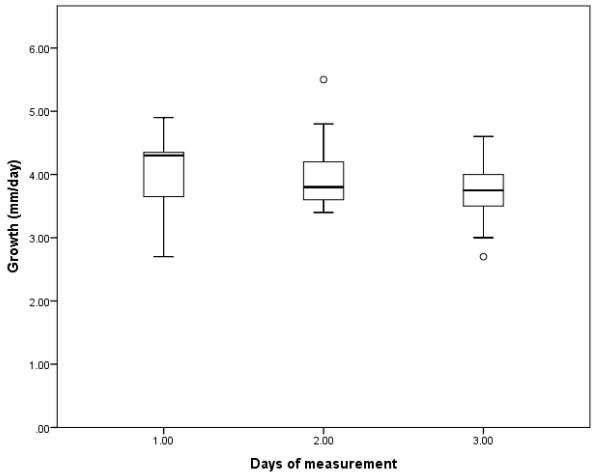


Figure 6. Daily growths of cultures on 27.5°C.

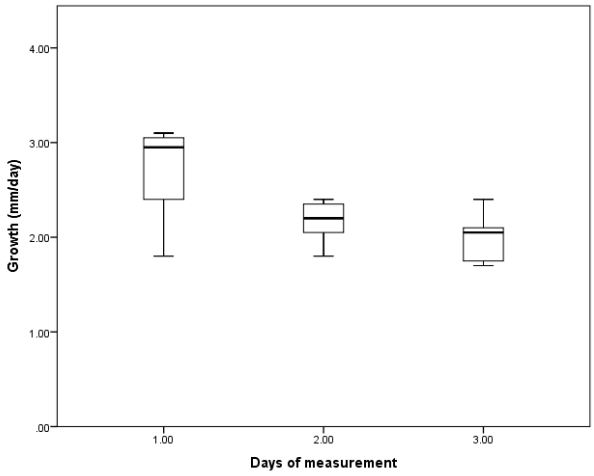


Figure 7. Daily growths of cultures on 30°C.

There was statistically significant difference in mycelium growth between different pairs of temperatures (Table 1).

Table 1. Average growth of cultures according to temperature.

| Temperature °C | Arithmetic mean* (mm/day) | Standard deviation |
|---|---|---|
| 10 | 1.1639a | 0.07617 |
| 15 | 1.9611b | 0.07281 |
| 20 | 4.2333c | 0.32426 |
| 25 | 4.7528d | 0.78065 |
| 27.5 | 3.9167e | 0.60687 |
| 30 | 2.2861f | 0.44411 |
| 32.5 | 0 | 0 |
| 35 | 0 | 0 |

*Statistically significant differences in rows are labeled with different letters

## 3.2 Morphological characteristics

On optimum temperature on MEA culture was aerial, whitish red (Figure 8). It was fast growing, sometimes with small numbers of concentric rings (Figure 8).

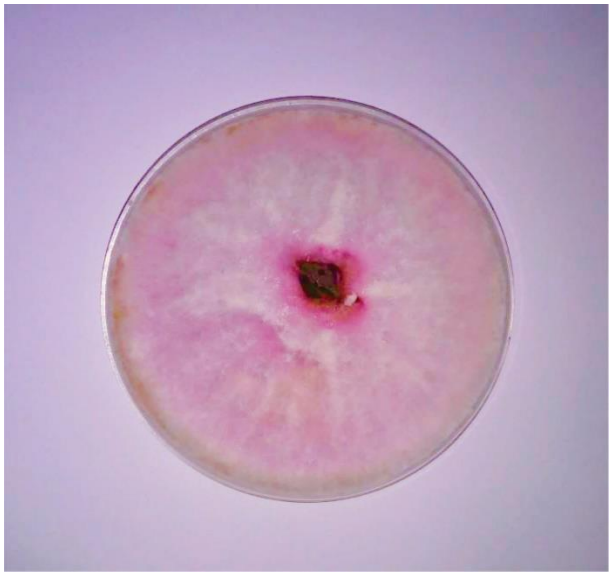

Figure 8. *Fusarium sambucinum* aff. culture.

Sporodochia with spores were abundant. Both macroconidia and microconidia were present and abundant (Figure 9a). Macroconidia were multi septate, slightly curved with pointed apical cells (Figure 9a). Microcinidia were non septate or with one septum (Figure 9a). Chlamydospores were occasionally present (Figure 9b). They were typical in chains and clusters (Figure 9b). Their presence was not always visible and sometimes the age of culture contributed to their occurrence.

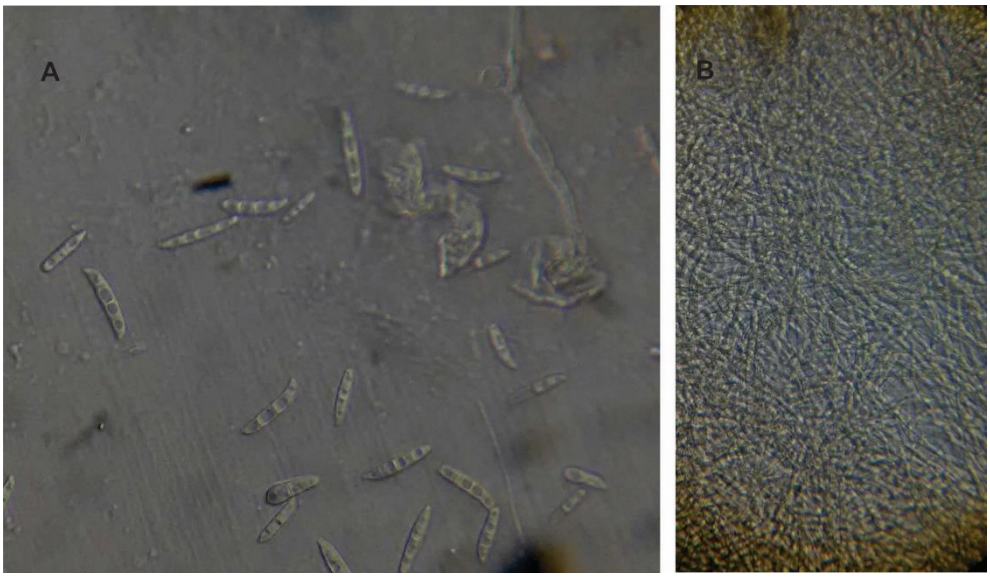


Figure 9. *Fusarium sambucinum* aff. microstructures A-macroconidia and microconidia, B-chlamydospores.

## 4 Discussion

The widespread abundance of ash trees does not make them less vulnerable to dieback (Pautasso et al. 2013). Besides *Hymenoscyphus fraxineus*, there are hundreds of fungal species recorded on common ash (*Fraxinus excelsior*) according to USDA database (Pautasso et al. 2013). Also, other *Fusarium* species occurs on common ash (*Fraxinus excelsior*), (Przybył 2002a; b; Kowalski and Łukomska 2005; Ioos et al. 2009; Bakys et al. 2009; Cleary et al. 2013; Davydenko et al. 2013). All of tested *Fusarium avenaceum*, *Fusarium lateritium* and *Fusarium solani* were slightly pathogenic (Kowalski et al. 2017).

This species complex is found usually in temperate parts of world (Kommedahl et al. 1975, 1988). Also, *Fusarium sambucinum* aff. occurrs on various woody hosts (Booth 1971). Leslie and Summerell (2006) named many records of this species on seed of different plant species. Also, this species complex was frequently found on common ash seed in Montenegro (Vemić 2020). Because isolate from seed belonged to the same morphotype as isolates from one-year-old seedlings it could be concluded that their investigated characteristics are similar. Because tested strain caused massive dieback of one-year-old common ash seedlings, the investigation of its basic ecological and morphological characteristics serve as basis for other researches of this strain. Strains of *Fusarium* species can synthetize hundreds of different toxins whereby not all of them are completely understood (Leslie and Summerell 2013). Due to that, knowledge about basic bio ecological characteristics of this strain will help its faster screening during isolations for further researches about its toxigenicity.

There were many studies about classification of this fungus. According to Gams et al. (1997) *Fusarium sambucinum* is type species in genus *Fusarium*. However, Nirenberg (1995) accepted *Fusarium sambucinum* as type species but also included *Fusarium sulphureum* and *Fusarium trichothecioides* within this species. Also, Nirenberg (1995) separated new species as *Fusarium torulosum* comb. nov and *Fusarium venenatum* sp. nov. while some other species that previously were included

in *Fusarium sambucinum* remained unsolved. Though, this morphological differentiation was not supported with previous molecular analyses by O'Donell (1992). Anyway, *Fusarium sambucinum* can be easily confused with *Fusarium torulosum* and *Fusarium venenatum* due to similar morphology (Leslie and Summerell 2006). Laraba at al. (2021) stated that *Fusarium sambucinum* complex is one of the most taxonomically challenging in genus *Fusarium*. Investigation of population genetics showed that populations in Europe are more diverse than those in United States (Desjardins 1995).

Identification of species in this genus is partly comforted with sequencing single gene tef 1α which is currently widely used for this purpose (Geiser et al. 2004). However, identification based on gene can only serve as guide due to initial identification by person that deposed sequence was performed on the other way (Leslie and Summerell 2013). These results can serve for improving knowledge about designing more accurate identification based on pure cultures.

## 5 Conclusion

Results based on this research pointed to next conclusions:

- Mycelium grew at temperature range 10-30°C. Mycelium growth stopped at 32.5°C. However, temperatures 32.5 and 35°C where growth was absent didn't affect vitality of mycelium during the experiment.
- Optimum temperature for mycelium growth was achieved at 25°C.
- Cultures were aerial, white reddish, with some ambiguously visible concentric rings on upper side and fast growing.
- Strain formed microconidia and macroconidia. However, macroconidia were much more abundant. Macroconidia were multi septate, slightly curved and pointed apical cells. Microconidia were non septate or with one septum. However, variation in visibility of different types of conidia could be due to used technique for their examination as well as that they are formed much rarer than macroconidia.
- Chlamydospores were present, typical for this species complex and formed in chains and clusters. They were middle abundant and their variation in appearance was somewhat dependent on age of culture.

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