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BOOK OF PROCEEDINGS

XV International Scientific Agriculture Symposium

"Agrosym 2024"

Jahorina, October 10-13, 2024

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PREFACE

Dear colleagues,

It is with great pleasure that I introduce the *Book of Proceedings* for the 15th International Scientific Agricultural Symposium "AGROSYM 2024." I trust that you will find it a valuable resource in your work. This year, around 800 contributions have been accepted for inclusion in the *Book of Abstracts*. The themes of AGROSYM 2024 span the full spectrum of agricultural sciences, divided into seven key sessions: 1) Plant Production, 2) Plant Protection and Food Safety, 3) Organic Agriculture, 4) Environmental Protection and Natural Resource Management, 5) Animal Husbandry, 6) Rural Development and Agro-Economy, and 7) Forestry and Agroforestry.

There is growing consensus among scholars and practitioners that technological innovations have the potential to boost agricultural production, enhance supply stability, and reduce the environmental impact of farming. Technology has been especially pivotal in increasing the productivity of annual crops such as maize, rice, soybeans, wheat, and cotton. However, with trees having longer growth cycles, breeding programs for these crops require more time. New plant breeding techniques promise improvements in productivity, but they have sparked an ongoing academic debate regarding their advantages and drawbacks.

While much of the focus in the past has been on the production side of agriculture (e.g., productivity and efficiency), there is an increasing emphasis today on the consumption side and the intermediate stages of the food chain, such as processing and distribution. This shift is driving the transition toward a "farm-to-fork" approach. Globally, consumers are sending clearer signals about their preferences for higher quality, healthier, safer, and more flavorful products. In response, many agri-food companies are exploring innovative ways to gain greater control over production processes and ensure the quality and safety of final products. Furthermore, changes in investment strategies hold the potential to reduce the environmental and social costs of agriculture. It has become increasingly clear to investors that companies that prioritize sustainability and social responsibility tend to yield better long-term returns.

Agri-food systems are at the heart of global discussions surrounding sustainable development and the achievement of the United Nations Sustainable Development Goals (SDGs) by 2030. These systems are deeply connected to numerous global challenges, including climate change, poverty, food insecurity, biodiversity loss, resource depletion, and ecosystem degradation. A key goal of the sustainable agriculture movement is to create farming systems that mitigate or eliminate the environmental harms associated with industrial agriculture. Additionally, it is critical to improve the resilience of food systems to crises, shocks, and pandemics.

I would like to extend my heartfelt thanks to all the authors, reviewers, and colleagues who contributed to the editing of the *Book of Abstracts*. Special thanks are due to our co-organizers and partners for their unwavering collaboration and comprehensive support throughout this endeavor.

Dušan Kovačević

East Sarajevo, 10 October 2024
Prof. Dušan Kovačević, PhD
Editor in Chief, President of the Scientific Committee of AGROSYM 2024

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Abstract

In order to effectively combat wood-degrading factors, particular emphasis is placed on the research of the impact of fungi that cause the most destructive form of decay – brown cubical rot. Among these species, one of the most common in our region is the fungus *Fomitopsis pinicola* (Sw.:Fr.) P. Karst, which attacks both deciduous and coniferous trees and develops as both a parasite and a saprophyte. The aim of the study was to determine whether and to what extent basic parameters of the external environment influenced the successful colonization of nutrient substrates by the fungus *F. pinicola*, which was a probable indicator of infection under natural conditions compared to competing decaying fungi. The research focused on the impact of H-ion concentration on the growth and mass production of mycelium, as well as on changes in substrate pH under the influence of this fungus. Experiments were conducted using dikaryotic mycelium of *F. pinicola* isolated from fruiting bodies taken from fir trees in the Tara National Park. It was found that at constant substrate pH values, the mycelium of *F. pinicola* exhibited maximum growth on a slightly acidic substrate (pH 4.8). Investigations on non-buffered substrates showed that the *F. pinicola* mycelium tended towards a pH value of 2.3, at which the highest dry mass of mycelium was recorded.

Keywords: *brown cubical rot, H-ion, pH, Tara, mycelium.*

Introduction

In protection of our most important tree species, particular emphasis is placed on measures to combat fungi that cause decay of the heartwood, the most valuable part of the tree. Among those species, one of the most common in our region is the fungus Fomitopsis pinicola (Sw.:Fr.) P. Karst, which develops as both a saprophyte and a parasite on coniferous and deciduous trees in temperate regions across Europe and Asia (Bishop, 2020), causing the most destructive form of decay – brown cubical rot. In this type of decay, the wood quickly cracks, becomes brittle, and loses its mechanical properties (Karadžić et al., 2020).
The research was conducted on the basis of samples collected in Tara National Park.
The basis for a rational fight against wood-destruction factors lies in understanding the basic physiological characteristics of the agents of destruction, which involves investigating the most important conditions that determine and enable fungi to initiate infection under natural conditions. In this regard, the influence of hydrogen ion concentration in the substrate was examined, as it is one of the basic parameters of the external environment in which the entire process of infection and decay development occurs over a certain period of time.

Material and Methods

The dikaryotic mycelium of the fungus *Fomitopsis pinicola* (Sw.:Fr.) P. Karst, with which the research was conducted, was isolated using standard methods from the fruiting bodies of fungi taken from fir trees in Tara National Park in Serbia.

The influence of constant substrate pH values on the growth of *F. pinicola* mycelium (on buffered, solid substrates)

To investigate the impact of different constant pH values on the development of *F. pinicola* mycelium isolated from fir, a buffered substrate was prepared. In order to ensure uniform nutrient distribution in certain parts of the buffer, the buffering system was prepared according to the recipe shown and using the Wolpert method, which has been used by several authors (Rypáček, 1957; Mirić, 1993). By mixing different volumes of 0.3 molar phosphate solutions – H_3PO_4, KH_2PO_4, and K_2HPO_4, substrates with different pH values were obtained, but with the same amount of phosphate, so that their different quantities would not affect the results. In that way, 5 series of phosphates of 187.5 ml each were obtained and then diluted in 5 Erlenmeyer flasks with a volume of 300 ml (Scheme 1).

1,000 ml of double-concentrated malt substrate (10 Bé sugar) and agar (4%) were prepared separately and poured into 5 Erlenmeyer flasks with a volume of 300 ml (187.5 ml each). This substrate was autoclaved separately from 0.3 molar phosphate solutions, and following the control of the pH value, they were mixed under sterile conditions. The pH control was performed after the sterilization to determine the stability of the buffer systems. In this way, a substrate of standard concentration (5 Bé sugar and 2% agar) was obtained, with physiologically equal representation of buffers (0.15 M).

Scheme 1. Recipe for preparation of buffered (solid) substrates for *F. pinicola* mycelium cultivation

Series number	Parts of 0.3 M solution (ml)			Parts of solution of the substrate malt (10 Bé and agar 4%) (ml)	pH of buffer	pH of substrate	
	H_3PO_4	KH_2PO_4	K_2HPO_4			after sterilization	at the end of experiment
1	32.5	155	-	187.5	2.9	3.2	2.8
2	11.5	176	-	187.5	3.3	3.8	2.9
3	-	186.7	0.75	187.5	4.5	4.8	2.9
4	-	156.2	31.3	187.5	6.0	6.0	5.0
5	-	42	145.5	187.5	7.2	7.2	6.9

The buffered substrate prepared in this way was poured (20 ml each) into plastic petri dishes (D=90 mm). For each pH value tested, 3 replications were used. Inoculation was performed in a laminar chamber using circular mycelial fragments (D=11 mm), which were placed along the edge of the petri dishes. Cultures were developing in a thermostat at a temperature of 21°C. Mycelial growth was marked at 24 hour increments in three directions - along the diameter and on both sides at an angle of 22.5°. The average daily growth was determined as the mean growth value in these 3 directions, and the number of days of measurement depended on the growth rate of the fungus at certain (constant) pH values of the nutrient substrate. To verify the stability of the buffer system (substrate pH control at the end of the experiment), at the same time a liquid medium was prepared, which was then inoculated and incubated under the same conditions.

The influence of *F. pinicola* mycelium on the change in pH of the nutrient substrate (on non-buffered, liquid media)

To examine the influence of the fungus *F. pinicola* isolated from fir on the change in pH value of the nutrient substrate, a non-buffered (liquid) medium was prepared using the method of Schmidt and Liese (Schmidt, 1994). A total of 2,600 ml of double-concentrated malt substrate (10 Bé sugar) was prepared with distilled water. From this quantity, 408 ml each was poured into 6 Erlenmeyer flasks with a volume of 500 ml (for 6 series), and according to the presented recipe (Scheme 2), the appropriate amount of distilled water and 1M HCl or 1M NaOH solution was

added. This resulted in the required amount of liquid malt nutrient medium of standard concentration (5 Bé sugar).
Before sterilization, the pH values of each series were measured. From each series, 120 ml of substrate was poured into 12 Erlenmeyer flasks with a volume of 300 ml (4 fungi with 3 replications each), so that substrates from 6 series were poured into 72 Erlenmeyer flasks which were sterilized in an autoclave for 20 minutes at a temperature of 120±1°C and a pressure of 1.4 bar. After sterilization, pH values were measured again, and they were treated as initial values. Inoculation with the fungus *F. pinicola* was performed in a laminar chamber, using mycelial fragments of circular shape (D=11 mm). For each series (initial pH value), substrates were inoculated in 3 Erlenmeyer flasks each.

Scheme 2. Recipe for preparation of non-buffered (liquid) substrates

Series number	Parts of solution (ml)		Distilled water (ml)	Parts of solution of double-concentrated malt substrate (10 Bé) (ml)	pH values of substrate	
	MHC1	MNaOH			before sterilization	after sterilization (initial pH)
I	9.60	-	400	408	2.2	2.2
II	0.56	-	408	408	2.9	2.8
III	-	-	408	408	4.2	4.2
IV	-	0.80	408	408	4.8	4.8
V	-	3.24	405.6	408	5.9	5.4
VI	-	25.60	389.6	408	6.4	6.2

The incubation lasted for 21 days at a temperature of 21°C. During the incubation period, pH change was measured every 7 days. For each measurement, 10 ml of substrate was withdrawn under aseptic conditions, using a sterile syringe with a needle, transferred into cuvettes, and pH values were measured with a digital pH meter.

Results and Discussion

The basic prerequisite for understanding the conditions enabling a fungus to colonize wood is knowledge of its fundamental physiological characteristics. It is important to note that different strains of the same fungus show clear differences under optimal environmental conditions (Dresh *et al*., 2015), whereas the fungi isolated from natural habitats and then transferred and cultured in laboratory conditions are under unusual conditions of existence, which causes their somewhat different physiological activity (Vučetić, 1985). This is because it is very difficult to replicate conditions in a laboratory that adequately reflect those of the external environment and vary only one factor without affecting others. Therefore, results obtained through even the most precise laboratory methods cannot directly apply to natural conditions, so they should only be accepted as probable indicators of potential occurrences.
Influence of pH values of the substrate
The pH symbol (the negative logarithm of the hydrogen ion concentration in mol/1 of water) is a measure of the acidity or alkalinity of a medium. The concentration of H ions affects the growth and development of all plant species, including the metabolism of wood-decay fungi (Rypáček, 1957). Substrate acidity can stimulate or inhibit the growth of saprophytic fungi, while changes in pH values have a significant impact on the rate of nutrient consumption and substrate decomposition.
Lilly & Barnett (1951) state that the majority of decay fungi have an optimum for growth in a slightly acidic pH range (pH 5 to 6). The acidity of the environment affects the enzyme system of fungi, which provides the organism's vital needs for food. Many decay fungi produce

organic acids in significant quantities, leading to substrate acidification. By decomposing wood (oxidizing and hydrolyzing wood constituents), epixylous fungi increase its acidity through oxalic acid formed in these processes. Rypáček (1957) states that a neutral substrate reaction suits most decay fungi during the substrate colonization phase, but that during mycelium development, as a result of fungal metabolism, initial pH values change towards acidic conditions. Rayner and Boddy (l988) state that the lower growth threshold of decay fungi is found in the pH range of 2 to 3, with an optimum between 4 and 6, where brown rot agents seek lower pH values than white rot agents. Jačevski (1933) states that epixylous fungi develop in a substrate with a pH between 2 and 8.5, with an optimum between 4 and 6, which represents the natural pH value of the majority of wood species.

Influence of the pH of the substrate on *F. pinicola* mycelial growth

Based on the results presented in Table 1, it can be observed that the mycelium of the tested fungus developed on all substrates with different tested pH values, and that the growth on substrates with certain constant pH values varied.

Table 1. Average daily mycelial growth of *F. pinicola* on buffered (solid) substrates (mm/day)

Series	pH of substrate		Growth of mycelia (mm/day)
	Initial pH	pH at the end of experiment	
1	3.2	2.8	2.14
2	3.8	2.9	2.67
3	4.8	2.9	2.46
4	6.0	5.0	1.86
5	7.2	6.9	0.89

The isolate of *F. pinicola* from fir had maximum growth in slightly acidic conditions with a pH of 3.8. The reduction in growth was more pronounced in slightly acidic substrate, while in a mildly alkaline substrate (pH 7.2), growth was almost completely reduced, amounting to only 0.89 mm/day. On strongly acidic substrate (pH 3.2), the growth of the *F. pinicola* isolate was faster, reaching 2.14 mm/day.

The substrate acidity that nearly halts mycelial growth is a mildly alkaline substrate (pH 7.2), where mycelial growth is extremely low. It should be noted that the range between the tested pH values of 3.2 and 7.2 is relatively large, and there is a possibility that the tested fungus would have higher mycelial growth at pH values between these two. Based on the control series in liquid media, which was set up to verify the stability of the buffer system, it was determined that at the end of the experiment, the fungus altered the initial pH values of the substrate, shifting the pH by 0.3 to 1.9 towards optimal values, which is within the tolerable range, so it can be considered that all buffer systems remained stable throughout the experiment.

The largest change of the pH, amounting to 1.9, was recorded in the 3rd series. Significant pH changes (0.9 and 1.0) were registered in the 2nd and 4th series. In the remaining series, pH changes were 0.3 and 0.4.

Influence of the mycelia of *F. pinicola* on the pH change of the nutrient substrate (non-buffered substrate)

Table 2 presents the change of pH in the standard concentration malt nutrient substrate under the influence of *F. pinicola* isolate from fir.

Table 2. Change of pH values of the substrate under the influence of the mycelia *F. pinicola*

Series	Initial pH	Change of substrate pH				Weight of mycelial
		After 7 days	After 14 days	After 21 days	Total change of pH	
I	2.2	2.4	2.1	2.1	-0.1	0.328
II	2.8	3.0	2.3	2.2	-0.,6	0.387
III	4.2	4.5	2.2	2.2	-2.0	0.571
IV	4.8	4.2	2.5	2.3	-1.5	0.578
V	5.4	5.4	2.7	2.3	-3.1	0.451
VI	6.2	5.5	3.2	2.3	-4.1	0.366

Based on the results shown in Table 2, it can be seen that on the substrate with an initial pH value of 2.2, there was practically no pH change until the end of the experiment (pH was lowered by only 0.1). The greatest pH changes were recorded in substrates with initial pH values of 6.2 and 5.4 (pH decreased by 4.1 and 3.1, respectively, over 21 days). Except for the initial pH value of 2.2, which was almost unaffected by the *F. pinicola* mycelium, at all other initial pH values, the pH decreased to 2.2 and to 2.3 at the end of the experiment. This means that these were the pH values that the *F. pinicola* mycelium gravitated towards during development. For the initial substrate pH value of 2.2, it probably takes longer for the fungus to approach pH values of around 2.3 through its metabolic activities, as was the case in most of the tested series.

Considering that after 21 days of exposure to the fungus *F. pinicola*, the substrate pH at initial values of 2.8 to 6.2 was reduced to a narrow pH range of 2.2 to 2.3, this can be interpreted as a relatively favorable pH value for the development of *F. pinicola*. This is also evident in the dry weight of the mycelium, which was the highest in the 3rd and 4th series, where the substrate pH was reduced to pH 2.2 and 2.3 after 21 days.

Conclusions

The change in pH values of the substrate on which the *F. pinicola* cultures developed gravitated towards a slightly acidic reaction, indicating that it favors a slightly acidic substrate, like most decay fungi. This fact suggests that *F. pinicola* competes with other decay fungi to colonize the substrate with equal chances of success, at least in terms of H-ion concentration. Taking into account the results of the study of the influence of H-ion concentration on the growth and mycelial mass production of the *F. pinicola* fungus, as well as the change in substrate pH under the influence of this fungus, from the perspective of successful colonization of nutrient substrate in natural conditions it can be concluded that the examined species is neither favored nor inhibited by environmental factors compared to the competing decay fungi. The phenomenon of microbial competition on the same substrate, inhibition of growth, or the occurrence of antagonism may be due to the metabolism of the competing species of fungi, secretion of mycotoxins or antibiotics ahead of the growing mycelial front, and the sensitivity or reaction of competing species to them.

This is the phenomenon which the speed, course, and consequences of decomposition of wood will directly depend on – wood as a substrate and nutrition source, but also as a very important raw material for processing, which due to its organic origin, represents food for a large number of organisms and microorganisms. For this reason, it is necessary to investigate the competitive relationships between this and other competing species of decay fungi under controlled conditions in so-called mixed cultures, under conditions of moisture, temperature, and H-ion concentration that are suitable for all types of opposing fungi.

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