

**SUSTAINABLE PRACTICES IN
HORTICULTURE AND
LANDSCAPE ARCHITECTURE**



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CONTENTS

PREFACE

Res. Assoc. Dr. Jovana OSTOJIĆ	
Assoc. Prof. Dr. Arzu ÇIĞ.....	1

❖ CHAPTER I SUSTAINABILITY VERSUS AESTHETICS IN LANDSCAPE ARCHITECTURE

Assist. Prof. Dr. Ivana SENTIĆ	
Dr. Ivana ŽIVOJINOVIĆ	
Prof. Dr. Jelena TOMIĆEVIĆ-DUBLJEVIĆ.....	3

❖ CHAPTER II REALIZATION OF THE MAP OF SOIL SUITABILITY FOR AGRICULTURE VIA GEOGRAPHIC INFORMATION SYSTEMS GIS: CASE OF THE SAIDA PROVINCE ALGERIA

Dr. Zohra ARABI.....	35
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❖ CHAPTER III SELECTION OF HORSE CHESNUT GENOTYPES (*Aesculus hippocastanum* L.) FROM SECONDARY POPULATIONS

Assoc. Prof. Dr. Jelena ČUKANOVIĆ	
MSc. Research Assistant Tijana NARANDŽIĆ.....	53

❖ CHAPTER IV REVIEW ON ORNAMENTAL ROSE OF SHARON (*Hibiscus syriacus* L.): ASSESSMENT OF DECORATIVENESS, INVASIVENESS AND ECOSYSTEM SERVICES IN PUBLIC GREEN AREAS

Assoc. Prof. Dr. Mirjana LJUBOJEVIĆ	
Junior Researcher MSc. Magdalena PUŠIĆ.....	71

❖ CHAPTER V
TRUE BUGS (HETEROPTERA) AS PESTS IN ORNAMENTALS

Assoc. Prof. Dr. Aleksandra KONJEVIC.....123

❖ CHAPTER VI
INVASIVE PATHOGENS AND PESTS ON WOODY ORNAMENTALS

Dr. Senior Researcher Jelena LAZAREVIĆ
Dr. Senior Researcher Kateryna DAVYDENKO.....145

❖ CHAPTER VII
ROOFTOP FRUIT GROWING AS A NATURE BASED SOLUTION TO MITIGATE THE CLIMATE CHANGE

Assoc. Prof. Dr. Mirjana LJUBOJEVIĆ
Junior Researcher MSc. Landscape Architecture Milica GRUBAČ..213

❖ CHAPTER VIII
DROUGHT STRESS IN FRUIT TREES – A REVIEW (MORFOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL BASIS OF TOLERANCE)

Dr. Sc.Ines MIHALJEVIĆ.....245

❖ CHAPTER IX
POLYPHENOL PROFILE AND ANTIOXIDANT CAPACITY OF RED FRUITS FROM THE CONTINENTAL PART OF MONTENEGRO – A PROMISING SOURCE OF FUNCTIONAL FOOD

Junior Researcher MSc. Radenka KOLAROV
Assoc. Prof. Dr. JOVANA ŠUĆUR
Prof. Dr. Đina BOŽOVIĆ
Prof. Dr. Biljana LAZOVIĆ
Prof. Dr. Đorđe MALENČIĆ.....277

PREFACE

Sustainable practices in horticulture and landscape architecture are necessary from several aspects. First, intense globalization is expanding market boundaries, resulting in increased and more diverse demand for horticultural products. The increased demand for new plant species and varieties depends, among other things, on the introduction and selection of new plant species from natural populations, but also on the intensification of existing production, which can have a negative impact on ecosystem sustainability. In recent years, this issue is also increasingly being researched in urban and peri-urban areas from a landscape architecture point of view, including social programs, all with the aim of improving the health and quality of life of local communities. In order to conserve natural resources, improve our environment, create economic opportunities, and meet our nutritional needs, it is necessary to develop best practices for sustainable horticulture and landscape architecture

This book presents topics that cover the above areas from different aspects. The chapters deal with the selection of some ornamental and fruit plants, their physiology and biochemical content, their pathogens and pests, urban cultivation and health benefits, ornamental value and invasiveness.

We would like to thank all the authors who have contributed to make this monograph of high scientific value, and we hope that this edition will bring answers and new knowledge to all those who are looking for it.

Sincerely Yours,

Jovana OSTOJIĆ & Arzu ÇIĞ

CHAPTER I

SUSTAINABILITY VERSUS AESTHETICS IN LANDSCAPE ARCHITECTURE

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1. INTRODUCTION

The most famous garden of all is invisible to the eyes of mortals, and it is not on the Earth - it is the Garden of Eden, also known as Terrestrial Paradise, a symbol of eternal life and the pursuit of perfection, found in religions and mythologies of many people (Kovačić, 2009). In the Middle East, 10,000 years ago, in the valleys of the rivers Euphrates and Tigris, the first gardens appeared and the most famous of which is certainly the Gardens of Babylon (Gotlin Čuljak et al., 2019). In these gardens (later in Roman gardens as well), the landscape design was far ahead of its time, since it is clearly visible that the great power of man was over nature (Currie, 2005). As the author Currie (2005) states during the Middle Ages, gardens had a utilitarian purpose and, according to their design they were closed to nature. The Renaissance and the Baroque separated gardens from nature once again with a tendency to express the power of man in society. In these kinds of gardens the great accent in design was on the aesthetics that deviated gardens from their natural surroundings. A formal geometric design was at the heart of the landscape composition, ornamentation in the parterres was common and the introduction of exotic species was noticeable. Later on, the wave of informal landscaping arrived; gardens became a part of the landscape (and their natural surroundings) with prominent view points. During the 19th and 20th centuries, the public park stood out as part of the social reform. The working class had a space for enjoyment and recreation. These kinds of parks fitted into the surrounding landscape.

A new modern age and new design challenges came with the 21st century. The design of green areas has strived for high functionality and aesthetics (Vujković, 2018). The growth of the world's population and more intensive urbanization that suppresses green areas have required from professionals to make a deeper analysis of the environment and natural resources as a starting point in the process of landscape design (Birnbaum, 2004). Designing and planning sustainable landscapes has become one of the great challenges and imperatives of the 21st century and landscape architects have been handed this important role. (Chen & Wu, 2009; Blagojević & Gačić, 2012; Byoung-Wook, 2012; Blagojević et al., 2014).

The popular concept of landscape design is limited and it is believed that the art of design is reduced only to the spaces of private gardens and parks (Jellicoe & Jellicoe, 1998). The mission of a landscape architect is to show equal care for every scale of each landscape (Chen & Wu, 2009).

When taking human and professional care of the landscapes, the landscape architect needs to establish the sustainability of the great chain of being (the human-plant-animal natural chain). Aesthetics have to find a place in the process of sustainability as well (Sentić, 2019).

When the landscape is being shaped and designed, its functionality should be satisfied (Vujković, 2018) and its *genius loci* identified (Stender, 2015). This means that no matter what idea the designer was guided by in the composition of the landscape, the function of the landscape content must be considered. They must be in a harmonious

relationship (Vujković, 2018). The landscape that has no history has no identity. Therefore, nurturing the *genius loci* is one of the first steps for the sustainable survival of the landscape (Stender, 2015) and a potential method of integrating the landscape contents (Bell, 2004).

This implies the responsibility of the landscape architect in the design process – the ecology, the art (the aesthetics), and the functionality must be provided in harmony through the story of the *genius loci*. In order to achieve these requirements, the landscape architect has to integrate the function and, ecology, and to emphasize the state of art in the landscape (Chen & Wu, 2009). What is more, in light of urban sprawl and major environmental changes, the unity of man and nature needs to be achieved (McHarg, 1995; Chen & Wu, 2009).

Despite all this, it is not uncommon for a landscape architect to place design over sustainability (primacy is given to the aesthetics of the landscape). In this way the issue of continuity and issues of the sustainability of the landscape itself are neglected (Vujković, 2018). In the 21st century, when climate change is becoming more intense, and environmental challenges more and more demanding, the question that arises is - can we afford the luxury of degrading nature, its character and values, or hampering its permanence? Does pre-designing landscapes and dealing exclusively with the aesthetic field of working not directly degrade the landscape? Can we talk about sustainability through landscape aesthetics? Should aesthetics oppose sustainability, or could the landscape be seen as a synergy of these two elements?

These are some of the questions that we will try to answer in this chapter. The chapter theoretically and through prominent examples discusses the role of aesthetics in the process of landscape design and the relationship between landscape and aesthetics in order to emphasise the importance of ecological aesthetics, more precisely landscape sustainability.

1.1. Principles of Landscape Architecture

Landscape architecture involves the planning, designing, management, and nurturing of urban and rural environments. The main task is to deal with outdoor spaces (ASLA, 2017). Landscape architecture has an important and unique role to play in developing and maintaining sustainability on local, regional, and global scales. It deals directly with the relationships between man and nature, and its theory and practice are influenced significantly by the philosophies and ideals about how humans should relate themselves to the nature (Chen & Wu., 2009). Some objects of landscape architecture are well known such as private gardens, parks, squares, green roundabouts, etc. Others are less well known - meadows, river banks, transportation corridors, national parks, protected natural sites, landfills etc. (Vujković, 2018).

No matter what kind of site or area is the subject, landscape architecture is always dedicated to the design of a healthy environment and the well-being of people, as well as for communities of plants and animals, their protection and harmony (Cervera Alonso de Medina, 2020). As the authors Chen & Wu (2009) underline, landscape architects intentionally modify and create landscapes of different kinds and various sizes, but

the accent of the design process should be on the ancient philosophy of “harmony between man and nature”. In the context of landscape architecture, landscape design and planning should follow and take advantage of the natural rhythms and ecological principles of a particular location. At the same time, nature must be modified, and artificial elements must be incorporated to meet the social, economic, and cultural needs of the humans who reside in the landscape. Contemporary landscape architecture should be based on applied ecology and a multidisciplinary approach to intervening in the space. If ecology is applied in a creative way, it establishes harmony in the biocenosis, a harmony of living and non-living nature and logical relationships in the structure and image of the landscape (Milinković, 1985). According to the source (ASLA, 2017), landscape architecture is based on **creating a space for people**, a space where people live or spend their free time.

The author Cervera Alonso de Medina (2020) emphasized that a landscape architect designs an open space in such way that activities of different types can take place there. These open spaces should be designed with the principle of “**design for all**”, meaning that content for various types of users is integrated. As stated in the source (ASLA, 2017), in the process of design, the needs of people should be an imperative, but also though should be given to creating a comfortable zone for living the various forms of flora and fauna. Many species are extinct, and there are even more that are endangered. Therefore, the responsibility of a landscape architect is significant. When designing a

landscape, a landscape architect promotes nature by creating a friendly environment between humans, plants and animals, which strengthens **the biodiversity**, making it self-sustainable (Cervera Alonso de Medina, 2020). This does not mean that there is no space for aesthetics. On the contrary, a landscape architect, **with the vegetation as the main tool of working**, emphasizes the beauty in the landscape by harmonizing colour, light, shape and form, as an inspiration for different types of content (Vujković, 2018). The way in which a landscape architect develops, maintains and manages landscapes is in direct connection with global climate change (Wittneben et al., 2012; Alizadeh & Hitchmough, 2019). Many degradations of the landscape have encouraged the faster onset of climate change, especially in urban areas, where the human-plant-animal natural chain has been greatly disrupted (Alizadeh & Hitchmough, 2019). The role of a landscape architect is **to re-establish the natural balance** and raise the landscape to a higher level than the current one by using natural resources such as water (rainwater collection), vegetation (root system, leaf mass), bio-filters (phytoremediation) and biomass (recycling) (ASLA, 2017). Finally, **healthy landscapes also have a positive impact on people's health**, providing places of recreation and relaxation and security, places accessible to people of all ages (Vujković, 2018; Alizadeh & Hitchmough, 2019). Adequately designed landscapes meet **the different needs of social communities**, in light of major climate change and the fight against natural hazards (ASLA, 2017).

1.2. Aesthetics in Landscape Design

In “The Great Dictionary of Foreign Words and Expressions” (Klein & Šipka, 2010), aesthetics is defined as the study of beauty, as a developed sense of beauty, a beautiful external impression, or harmony. In landscape architecture, aesthetics can be approached in two ways - exclusively through a high degree of decorativeness and through the prism of ecological harmony with the environment. In the first case, it often contradicts the sustainability of the landscape, because the designer's tendency to please the eye of the observer is primary. In such landscapes, the tendency with vegetation is to highlight its decorativeness, not to achieve a biological compatibility with the environment; the focus of the content is on visual attractiveness, not on environmental acceptability. In the second case, aesthetics is a part of sustainability over time and space. The design is reduced and emphasizes the qualities of the landscape through its biological and ecological values (Vujković, 2018).

Recent research has emphasized that these two ways of looking at landscape architecture can be joined together and that an aesthetic experience has both biological and aesthetics constituents (Chenoweth & Gobster, 1990; Muir, 1999). However, a landscape is more than simply an object (Chenoweth & Gobster, 1990). It represents a combination of an art and nature. The connections between these categories are complex; above all they must be sustainable (Muir, 1999). As Olin (2014) underlines, the landscape designer is an agent of changes, having an urge to save and protect aspects of the environment

that have become important, precious and meaningful for people. To achieve this, the landscape designer must emphasize aesthetics in the design; again, the aesthetics must be in harmony with nature (Othman et al., 2015). This is also very important since in the last few decades, the need for preservation and conservation of endangered natural resources has become a global goal (Halprin, 2004). The same author (Halprin, 2004) stated that this need is reflected in the preservation and protection of air, water, soil and biological species. These changes are directly reflected in the preservation of the planet and biological way of life.

This puts emphasis on the fact that aesthetics must in some way unite itself with design (Othman et al., 2015). Nohl (2001) states that in order to meet the needs of users of the 21st century, the landscape designer gives preference to the aesthetic values over the quality of the landscape and thus does not predetermine the future as sustainable. This applies to both small and large scale landscapes. Prominent authors (Kaplan & Kaplan, 1989; Nohl, 2001) state that a landscape can have a high aesthetic quality and be sustainable if all its elements are balanced and harmoniously placed and where the traditional cultural landscape is nurtured (*genius loci*).

An interesting explanation has been given by the authors Othman et al. (2015). These authors remind us that landscape aesthetic values have a significant role on the choice of visiting some a place. In a certain studies on recreation and nature (Hansmann et al., 2007; Wolf & Wohlfart, 2014), people gave high marks and reacted positively to

scenery they like to see such as mountains, streams and <https://www.shutterstock.com/search/autochthonous+vegetation> autochthonous, native vegetation. As these authors (Hansmann et al., 2007; Wolf & Wohlfart, 2014) underline, it is believed that the natural landscape environment has an aesthetically pleasing, flexible and functional quality for people to visit and explore it. What is more, visitors are motivated to explore a natural environment that will offer them the opportunity for mental relaxation. Landscapes with a low aesthetic value, with lack of maintenance and with abandoned buildings reduce the motivation for visiting, and if something is not done by the management, these places do not have a bright future. This opens the mind to see the fact that visitors are very sensitive to their environment and always want a quality outdoor recreational landscape (Othman et al., 2015), which again leads us to unite aesthetics and sustainability.

1.3. Landscape Sustainability

Sustainability, a widely recognized common goal for humanity, has become an increasingly dominant theme in design and planning. This is important since landscape architects should, in their design process, meet the needs of the present without compromising the ability of future generations to meet their own needs (McLennan, 2004). As is mentioned above, the role of a landscape architect is to plan, design and manage natural, rural and urban environments, by applying scientific and aesthetic principles to address the sustainability, the health of the landscape quality and the impression of the *genius loci* – the landscape character (IFLA Europe, 2021). A sustainable landscape is achieved

with a comprehensive, sustainable design that protects sites and ecosystems (soil, water and wildlife), reduces water usage, uses native plant species, has minimum mowing requirements and ensures user health (URL-1). The sustainable landscape design recognizes the landscape character by respecting the natural landscape values and social norms. In this way, every landscape management is facilitated and, to a great extent, without negative outcomes (Blagojević & Gačić, 2012).

The landscape architect does not only deal individually with the urban environment and its aesthetics, but also has an inclusive vision to develop landscapes on behalf of the residents (IFLA Europe, 2021). Regional landscapes are a key domain for studying and applying the principles of sustainability, as they integrate human-environmental interactions, connect local, regional and further global processes, whilst also providing a common platform for scientists, designers/planners, policy makers and stakeholders to collaborate on sustainability issues that are of interest to all (Wu, 2019).

All this is done through the process of designing and managing with different environmental approaches in accordance with scientific and social principles and with the aim of helping to set a vision for a city or region (IFLA Europe, 2021). Thus, it can be said without doubt that the role of the landscape architect in defining and designing landscapes must be directed towards sustainability. The decorative quality of the landscape in terms of plant material can be achieved, but with respect to ecological standards (Gačić & Blagojević, 2012).

Considering all this, it can be said that landscape sustainability fits into the latest paradigm of a circular and bio-economy, which is presented as the leading path of the European Union development (European Commission, 2018). Circular economy is a new approach in the process of creating sustainable visions for a city or region, by putting the economic system of landscape development in balance with nature. In general, it is based on several principles: the less change in the landscape, the greater the potential for saving natural resources and labour; sustainable design in order to minimize maintenance; the use of more durable and high-quality materials, which can be recycled and used for some other projects (IFLA Europe, 2021). A sustainable and circular economy presents a way of achieving the goals of sustainable development of the United Nations (European Commission, 2018).

In addition, a sustainable landscape is reflected in its ability to provide long-term specific ecosystem services, necessary to maintain and improve human well-being in the area. The sustainable landscape as a science is based on improving the dynamic relationship between ecosystem services and human well-being in landscape changes in the face of uncertainties resulting from internal qualities and external influences (Wu, 2013).

Sustainable landscape design is generally recognized through the relationship of three principles - environmental health, social justice and economic prosperity. Aesthetics rarely influence the discourse on sustainability, except in negative aspects that combine the visible with the aesthetic and make both superfluous. Moreover, the aesthetic aspect

of a landscape helps to increase knowledge of environmental sustainability by affecting human consciousness and individual emotions (Hemmati, 2016). As Gobster et al. (2007) emphasize, considering aesthetics helps to anticipate landscape change and its environmental impacts. As humans, our sensory system is tied closely to our emotions, and of our emotions, pleasure has a fundamental influence on how we respond to the stimulation of our world. The attention to ecological quality can be influenced by the perceived aesthetic value of landscapes.

1.4. Sustainable Policy

Landscape is not a matter for individual states alone. It also needs to be considered in international policies and programs (Gulinck et al., 2000). Legal and planning documents define the vision of long-term development and the framework of spatial development of the landscape (Scott, 2011).

In the case of sustainable aesthetics in design, planning documents can be used as a guideline for developing a vision towards a sustainable planet. One of the most important planning documents in landscape architecture, which is closely related to the landscape, is the European Landscape Convention (ELC, 2000). According to this convention (ELC, 2000), the landscape is considered to be one of the basic elements of Europe's natural and cultural heritage that contributes to the well-being of humanity and the strengthening of the European identity. In addition, the landscape is considered to be an important factor in the quality of people's lives, wherever they may be - in urban and rural

areas, in degraded areas or in areas of high quality of life, in areas with exceptional characteristics or in everyday environments. In this regard, the landscape should be sustainably nurtured and preserved as a legacy for generations to come. A directly related policy is the Green Infrastructure Strategy, which has been developed by the European Commission. This strategy aims to ensure that the protection, restoration, creation and enhancement of green infrastructure becomes an integral part of spatial planning and territorial development whenever it offers a better alternative, or is complementary, to standard grey choices (Jadžić, 2011).

Climate change affects the stability of the climate in different temporal and geographical dimensions, often causing natural hazards that change people's daily lives (Franch-Pardo et al., 2017). Therefore, the scientific community has a responsibility to improve the overall knowledge and understanding of climate change and its impact on daily human activities. Consequently, sustainable landscape policy must be covered by declarations of the types previously mentioned. In light of climate change, landscapes are becoming extremely vulnerable places, and their design and arrangement must have clear limitations (Linsler & Lier, 2020). Existing climate-related policies differ in many countries, but it should be assured that they are taken up in landscape planning and design, as well as in the management of green areas (URL-2).

Further activities of the EU that are aimed at strengthening sustainability and resilience aspects of societies include fostering the concept of nature-based solutions in its research and innovation policy.

This is equally applicable when thinking of the sustainability of landscape, from their planning to their maintenance. The EU defines nature-based solutions as “Solutions that are inspired and supported by nature, which are cost-effective, simultaneously provide environmental, social and economic benefits and help build resilience. Such solutions bring more, and more diverse, nature and natural features and processes into cities, landscapes and seascapes, through locally adapted, resource-efficient and systemic interventions” (URL-2).

These are just some of examples that show how much these aspects also become prominent and visible in the policy arena and how these are featured in policy documents.

2. LESS IS MORE. EXAMPLES OF THE PRACTICE

Ian McHarg in his book *Design With Nature* (McHarg, 1995), emphasizes nature as an arena of life, saying that a small amount of knowledge about its processes is necessary for survival, and even more for existence, health and pleasure. Thus, it is amazing how seemingly difficult problems seek almost simple solutions. The same author (McHarg, 1995) states that the problem of man and nature is not in providing a decorative background for human play or life, but to maintain nature as a source of life, as an environment that teaches and environment as a holy place. The author Simon Bell (Bell, 2004) agrees with this, and states that instead of looking at landscapes only as beautiful images, it is vital for sustainability to understand *genius loci*.

On the other hand, the authors Jellicoe & Jellicoe (1998) emphasize that the landscape is moving in a direction whereby its design can be recognized as the most comprehensive art. The survival of the environment is disrupted by human activities and only by human efforts can the balance be re-established. Kongjian & Padua (2006) state that the 21st century is a challenge for landscape architecture to return its role to the art of survival. The leading task is to build models for designing different types of landscapes; landscapes that will sustain humanity, that will restore people's identity and make them happy.

In order to justify the 21st century, design is sometimes overemphasized outside the context of the environment. Therefore, it may be better to follow the philosophy less is also more beautiful (Malan & Bredemeyer, 2002). Maybe this should be transferred to the landscape in order to achieve harmony with nature.

2.1. Kamenički Park, Novi Sad, Serbia

Kamenički Park is a natural monument, with an area of 33 ha. It is the oldest landscaped area in the city of Novi Sad and includes the area along the Danube embankment (Aradski et al., 2004). It was built in the 19th century, around the castle of the Marcibanji and Karačonji families. The special value of the park is the preservation of the landscape concept and style in which it was designed - the natural lake stands out, as do the remains of garden-architectural elements. The dendroflora of Kamenički Park represents the most diverse and richest of Novi Sad. Deciduous tree species dominate within forest communities, which are arranged into three levels: along the banks of the river Danube willow

and poplar trees dominate; in the central part of the park oaks rule (the oldest dates from 1805), and in the highest parts of the terrain there are silver linden trees, maple, milkweed, hornbeam, etc. (URL-3).

Kamenički Park was revitalized in 2019. During this process, the principle of “less is more” was used (Figure 1). Namely, the park hasn’t undergone major changes to the existing forms of design, but the new forms clearly highlight and give stability to the spatial composition. The pathways in the park’s landscape are paved and well defined; they follow the lines of spontaneously formed paths by users in the existing design. The outdoor furniture is in harmony with the ambience of the landscape. The lake has been renovated and deepened, and the sculptures are more visually noticeable.

The dendroflora has retained its compositional value with the necessary thinning and removal of diseased items. The aesthetic of space is present, but modest - highly decorative specimens have not been introduced; there is not a high degree of colour nor is there present any modern design elements. The richness of the fauna has been preserved.



Figure 1: Kamenički Park in Its Original and Newly Formed Design (Original by Sentić)

This simple and functional design supports the nature of the landscape and reduces the aesthetics to a minimal but sustainable level. Namely, the emphasis on the aesthetics and sustainability of the natural community of the Kamenički Park area is, without a doubt, of a more modest scope, but their harmony has not been overlooked. The power of *genius loci* hasn't been felt, the connection between nature and aesthetics in the landscape is less noticeable or it can be seen from a

different view, whereby if it is not noticeable, the harmony between the two is satisfied. Projects such as the Kamenički Park are an encouraging step towards sustainable landscape aesthetics. The management measures must not be underestimated. The ecological power of the space is reflected in almost every segment. This is evidenced by the protected dendroflora and vegetation profile that has been preserved compared to the first horticultural landscaping of the park in the 1950s. Kamenički Park is one of the most visited parks in Novi Sad, recognized as a space suitable for children to play, a picnic area, and space for recreation and sports.

2.2. The Floating Gardens — Yongning River Park, China

In 2002, TURENSCAPE “transformed” an area of the concrete embankment of the Yongning River on the east coast of China into a 21-hectare park (Figure 2). The designers had the task of creating a concept that would provide a solution to flood control and atmospheric precipitation management (a model that would be applicable to the entire river valley). On the other hand, it was also necessary to design the whole area as a park, in order to be accessible to the local population and to be a natural oasis for local birds, but also attractive to tourists. Since the space often flooded during the year, the idea was to explore the possibility of using it throughout the year, hence the idea of the so-called “floating gardens” arose (Kongjian & Padua, 2006).



Figure 2: The Appearance of the Concrete Embankment and After Its Levelling and Transformation into A More Natural State (Kongjian & Padua, 2006)

What makes this newly designed landscape unique and special is the vegetation, which is the heart of the whole concept. The total concept of “living with floods” has conditioned the coast to be green, naturally suitable for the area, located in the first line of the flood defence. The concept has grown into the idea of “living in harmony with nature”. Native tree species form massifs along the coast and their permeation through the entire space emphasizes the continuity of the design itself with the surrounding ecosystems, but also the ecological aspect of the restored riverbank, where floodplain species dominate. An attractive and environmentally friendly environment has been created (Figure 3).



Figure 3: Newly Designed Riverbank with Its Contents (Kongjian & Padua, 2006)

During the monsoon season, the entire embankment area is under water. Despite this, thanks to its design, the embankment is accessible to

visitors throughout the year. Namely, on the very slope of the embankment, platforms have been set up that float during the flood period (Figure 4). These platforms have openings through which vegetation can grow, hence the name “floating garden” (Kongjian & Padua, 2006).

Unlike Kamenički Park, where the aesthetic of the space is more modest, the design of the Yongning River Coastal Park is aesthetically very strong. This project represents a harmonious relationship between aesthetics and sustainability of nature. Neither of these two elements predominate, but complement each other. The design of the park itself supported the ecological postulates of the natural environment and, through the power of nature emphasized the potential of sustainable ecological aesthetics. The choice of vegetation is directed towards the natural values and characteristics of the habitat itself, and that is the basis of the sustainability of the space.



Figure 4: One of the Segments of the Yongning River Park- A Platform under Which Water Flows During the Flood Season, Provides an Interesting Ambience and a Pleasant Stay (Kongjian & Padua, 2006)

Above all, the space is nurtured by *genius loci*, which provides a good condition for recognizing and nurturing the character of the landscape. The historical background of the place has been preserved, and the landscape is permanently close to the local population, and an innovative solution to allow visitors to explore. In this way, the landscape provides permanent plant and animal survival. What should be pointed out is that landscapes of this type cannot exist on their own. The intelligent management of the landscape emphasizes the character, history and potential of the place, at the same time they understand the needs of the users, and the planned steps for future survival are carefully focused in that direction.

2.3. Enzo Enea Tree Museum, Near Zurich, Switzerland

The idea of creating a museum of trees arose from the work of Enzo Aeneas as a landscape architect. The park is designed on an area of 75,000 m². The museum exhibits individual trees from the personal collection of this landscape architect (17 years of collecting). The collection consists exclusively of varieties that belong to the climate zone of Switzerland (URL-4).

The goal of building a museum of this type is to emphasize the exceptional beauty and rarity of trees, whilst also looking at the primary attributes of life, such as time and space. Standing in this museum gives the impression that time stands still, because very old specimens are mostly represented. The landscape architect carefully adapted the background to each type, so that the specimen can be seen from several different angles (Figure 5). As it is stated in the source (URL-4), the

museum consists of about 50 trees, more than 25 varieties. There are several examples that are more than 100 years old, and which are there to bring peace to the space and disconnect visitors from the everyday rush of life. The art of bonsai design was applied to the trees, in order to preserve the older trees.

This park is an aesthetically extremely strong space, but not of an over-emphasized design. Simplicity is primary in this project, which is symbolic of nature, and its hidden beauty, which is very easy to look at, but extremely complex in function. The park highlights all that is beautiful in form, light, material, colour, and even its biological profile and the harmony of their unity. As the trees are autochthonous varieties of Switzerland, the park has another message, and that is that aesthetics in design can be successfully achieved without the introduction of exotic species.

Additionally, when considering the sustainability of the park landscape, this is an example of a park that is self-sustainable, and one that thrives. The level of maintenance and applied care measures in this park, as a prominent object of landscape architecture, is very high, primarily because of the bonsai technique that is employed here.



Figure 5: View of Details from the Museum of Trees (URL-4)

All this shows that objects of this type can survive, but with wisely organized management, i.e. landscape management. Also, the “Museum of Trees” is a park that combines the aesthetics and sustainability of the landscape in a very special and very personal way, with a theme and *genius loci* that leaves a strong and lasting mark on the space.

CONCLUSION

Fundamentally, landscape architecture has ecological thinking at the core of its heritage, but the significance and importance of ecology in design weakens, and very often separates the profession. On one side of the continuum are those who see the primacy of landscape design in arranging the ecological process, and on the other side it is perceived in aesthetic explication. However, the truth is in the connection of these two views. Speaking of any scale in landscape architecture, an

ecological aesthetic is achievable, and with the right choice of management measures, it can be highlighted as sustainable.

The aesthetics of the landscape should not be narrowly viewed as a high degree of decorativeness, but as the beauty of the landscape emphasized with vegetation, light, culture, people etc.

We live in the era of major climate change, when vegetation is in deficit, and any preservation of it, is also an extension of the survival of the planet, and thus human life. Therefore, the natural postulates, i.e. the preservation of the natural chain of human-plant-animal, must be very sensitive in the design of any object of landscape architecture. On the other hand, aesthetics is certainly something that leads us to green areas, keeps us in them and makes us mentally stronger, and it is wrong to deny its importance in green surface design.

Consequently, the right and only way must be to create a harmonious design as an accord between the aesthetics and sustainability of the landscape. Legislation can be a very important step in creating the pillar of a sustainable environment of the future.

In a time of great urban change, the issue of environmental sustainability has become the most important concern for the well-being of society. Respect for the history and ecology of the landscape is an imperative from which the landscape architects must not stray. The monitoring of natural laws is in accordance with the permanence of space, i.e. with its sustainability and survival. In order to have

landscapes that will be recognizable and long-lasting, there must be historical recognition and cultural value.

Man is a part of nature and his bond with nature should not be broken; this relationship should be seen as a single entity. Landscape aesthetics and ecology are united around the idea that visual diversity in the landscape is stimulated by natural patterns and linked to biological productivity.

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CHAPTER II

REALIZATION OF THE MAP OF SOIL SUITABILITY FOR AGRICULTURE VIA GEOGRAPHIC INFORMATION SYSTEMS GIS: CASE OF THE SAIDA PROVINCE ALGERIA

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1. INTRODUCTION

The World actuality is dominated by the concept of sustainable soil management to ensure food security, according to the FAO (2015), 95% of food comes directly or indirectly from the soil, whose quality and quantity of these foods are closely correlated with soil quality.

Algeria, like the various countries of North Africa, is lagging considerably behind in terms of sustainable soil management, which prevents it from achieving food self-sufficiency. Agriculture results in great precariousness and is mainly limited to cereal growing. The useful agricultural area represents only 3.6% of the total area of the country (O.N.S., 2022).

More broadly, knowledge of the soils at the scale of the study area and their suitability for agriculture is a crucial ally for the sustainable eco-development of this ecosystem already weakened by severe climatic conditions. Moreover, desertification is the most serious problem on the economy of the region and the inhabitants (Arabi, 2016).

Faced with this situation, it appeared necessary to resort to adequate strategies based on developed tools such as Geographic Information Systems in order to assess the suitability of the soil for agriculture in a prosperous and sustainable way. These tools are particularly useful for decision-making in terms of agricultural development (Paegelow, 2007; Kédowidé, 2010; Olatondji Salomon Chabi et al., 2018; Jaffrain et al., 2021).

The main objective of this work is the integration of multi-source data in a GIS in the implementation of specific development. It will be used to produce the map of the potential suitability of the soil for agriculture and to propose a development model.

2. MATERIALS AND METHOD

The study area is located in the north-west of Algeria (Figure 1). It covers the territory of the three communes: Hassasna, Maamora and Ain Skhouna with an area of 2200.4 km². It fits in the space delimited in longitude by 0.2 °W to 0.9 °E and in latitude by 34.3 °N to 34.9 °N (Arabi, 2020). It has several types of course: agricultural course, agro-pastoral course and steppe course.

The macro-zoning of the territory into large homogeneous or homoeological zones is a mandatory step before any development action (Dobremez, 1976; Benabdeli, 1983). The macro-zoning of the study area reveals three major natural complexes which are:

- The high plateaus in the northwest represented by agriculture
- Djbel Sidi Youssef in the North constitutes a forest massif with arable voids exploited by local residents
- The High Steppe Plains which has significant potential for agricultural and livestock development.

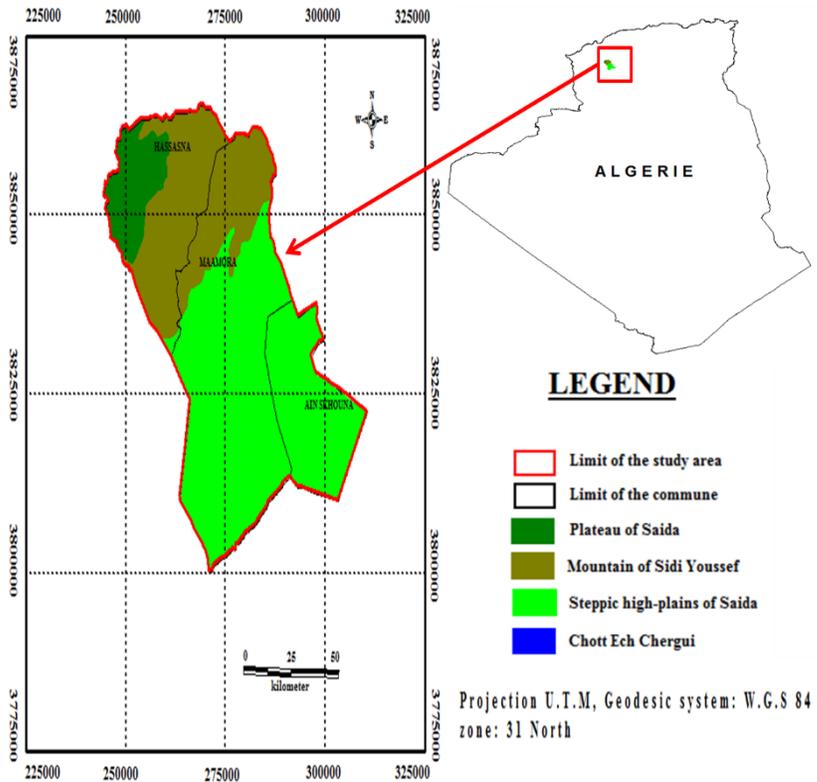


Figure 1: Location of the Study Area

We have tried to examine the real constraints that hinder agricultural success in the study area considered as a semi-arid steppic zone. The study concerns the evaluation of the suitability of the soil for the practice of crops, according to the FAO (1997) the characteristics which must be taken into account are natural (soil, climate, topography, water resources, vegetation, etc.), socio-economic and also infrastructure.

In our case, the map of potential suitability for cultivation expresses the distribution of land units according to purely natural factors. Several criteria were taken into consideration to determine this suitability,

namely: slope, soil and vegetation cover. A classification of land into five categories proved necessary by grouping them by ecological affinity. The resulting map will give us a first estimate of the agricultural potential in the study area, which is characterized by severe climatic conditions and poor structural stability of the soil (Arabi, 2016).

The ability map was produced using Mapinfo software and its own database management system (SGBD). Each parameter studied was presented in a separate thematic map that illustrates the degree of its potential suitability for cultivation. The thematic maps were established by digitizing existing maps, while relying on several sources of information:

- Cartographic documents: Map of land cover (INSID, 2013), Map of soil types (C.N.T.S., 2014), Map of slopes and hydrographic networks, Map of homogeneous areas established as part of the National Forest Inventory.
- Bibliographical information from the Consultation of internal documents of the Conservation of Forests and also of the Directorate of Agriculture of the wilaya of Saida.

Each mapped unit was classified in an evolutionary framework comprising five categories ranging from "very poor ability" to "very good ability" according to its potential suitability for cultivation (Table 1).

The crossing and the combination of the different layers were carried out according to a decision matrix (Table 2). It took place according to the principle of two by two which consists in superimposing two thematic maps to establish a new map, on this same map new polygons are created as well as a new attribute table is installed.

Table 1: Classification of Evaluation Criteria According To Their Potential Suitability for Cultivation

Land Suitability	Soil	Slope	Vegetation
Very poor ability	Raw mineral soils of eolien input, Complex units	>15%	Maquis, sandy veil : alfa grass steppe, <i>Artemisia</i> steppe and
Poor ability	Halomorphic soils	9 – 15%	Mixed steppe (alfa grass and <i>Artemisia</i>), Psammophytes and
Average ability	Little evolved soils of erosion	6 – 9%	Degraded sagebrush steppe
Good ability	Carbonated calcimagnesian soils	3 – 6%	Cereal farming
Very good ability	Little evolved soils of alluvial input	1 – 3%	Agriculture (perimeter of zraguet)

Table 2: Decision Matrix

Soil \ Vegetaion	Very Poor Ability	Poor Ability	Average Ability	Good Ability	Very Good Ability
Very Poor Ability (VPA)	VPA	VPA	PA	AA	AA
Poor Ability (PA)	VPA	PA	AA	AA	GA
Average Ability (AA)	PA	AA	AA	GA	GA
Good Ability (GA)	AA	AA	GA	GA	VGA
Very Good Ability (VGA)	AA	GA	GA	VGA	VGA

3. RESULTS AND DISCUSSION

The great advantage of the georeferenced database lies in its capacity to store a huge amount of graphic and attribute data. The processing and spatial analysis of these data have made it possible to establish summary maps called criteria maps (Figure 2), these three maps obtained will make it possible to better characterize the effect of environmental factors acting on the suitability of the soil for value.

The soil suitability map for agriculture (Figure 3) provides relevant information for planning the use of agricultural land. It will serve as a support for decision-making for the choice of means of intervention according to the particularity of each zone and according to the factors acting on agriculture, as confirmed by Paegelow, (2004); Paegelow & Camacho Olmedo, (2005); Jaffrain et al., (2021).

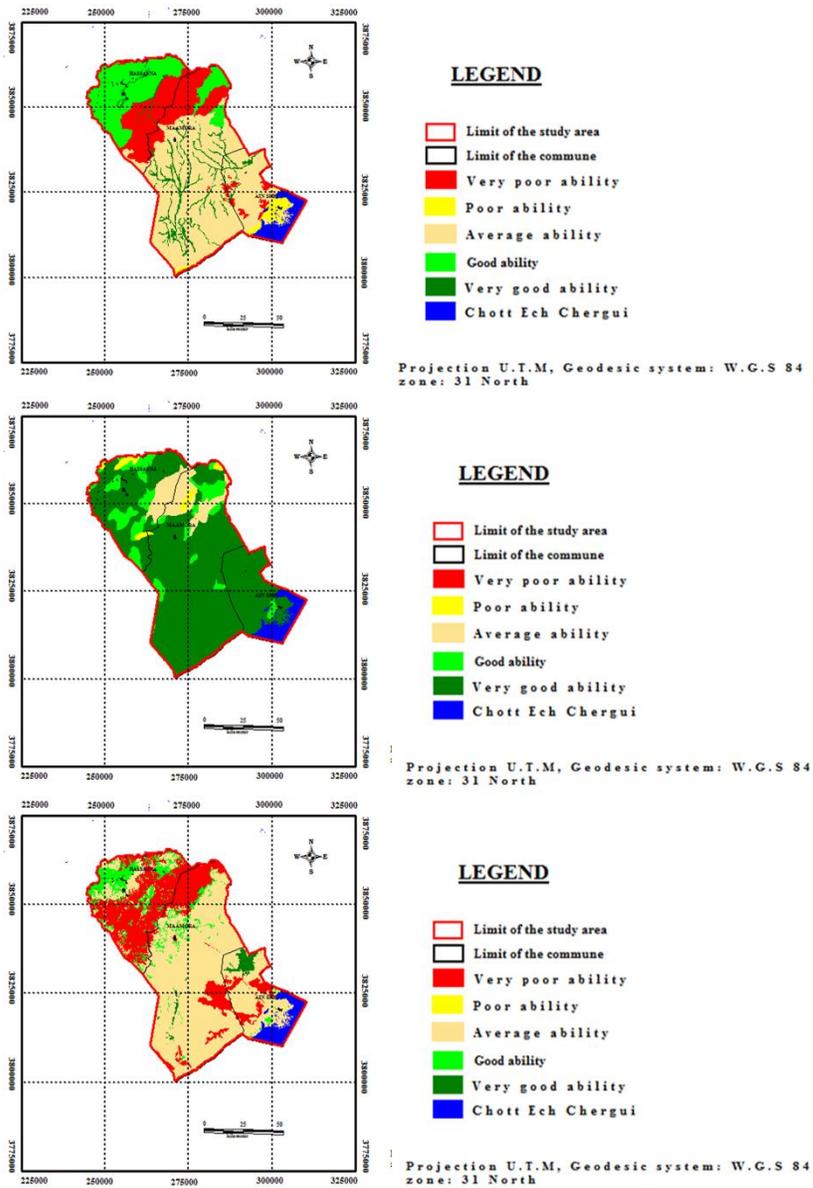


Figure 2: Criteria Maps of Land Suitability (a) Soil; (b) Slope (c) Vegetation

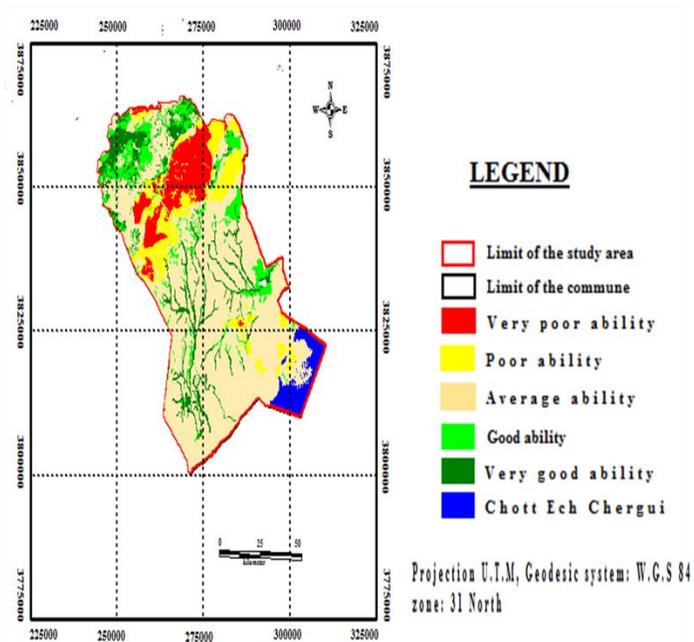


Figure 3: Map of Land Suitability

The map obtained provides a spatialization of land use according to the degree of agro-pastoral potential, which allowed the division of the study area into five distinct classes according to their degree of suitability for agriculture, in addition to the Chott Ech Chergui wetland (Table 3).

Table 3: Estimation of Classes

Class Of Land Suitability	Area (ha)	%
Very Poor Ability	19040	8,654
Poor Ability	23660	10,754
Average Ability	128600	58,454
Good Ability	19740	8,972
Very Good Ability	20470	9,304
Chott Ech Chergui Wetland	8490	3,862

3.1. Distribution of Soil Suitability Classes for Agriculture: Very Poor Ability

This class includes land covered with degraded forests in clear maquis to the south of the municipality of El Hassasna and to the north of the municipality of Maamora. The beautiful Alfa steppes are also part of this class. Its area is estimated at 19,040 ha, or 8.654% of the total area of the study area.

3.2. Poor Ability Class

This class occupies an area of about 23,660 ha, which represents 10.754% of the total area of the study area. It concerns the land occupied by the mixed steppes of Alfa and Armoise in the North-East and North-West of the study area and also near the wetland of Chott Ech Chergui whose vegetation is dominated by Halophytes. The phenomenon of salinity is very advanced.

3.3. Average Ability Class

It is located in almost the entire territory of the study area, of which it occupies the largest area estimated at 128,600 ha, or 58.454% of the total area. The land in this area is either occupied by cereal growing or by low-coverage steppe vegetation, they are characterized by a low slope that does not exceed 3%.

3.4. Good Ability Class

It encompasses almost all the zones characterized by little evolved soils of alluvial contribution which are near wadis and water bodies and also

all the zones of carbonated calcimagnesian soils. It occupies an area of about 19,740 ha, or 8.972% of the total area.

3.5. Very Good Ability Class

It is represented by the entire territory of the irrigated perimeter of Zraguet, it is close to all areas with good aptitude for cultivation. It occupies an area of about 20,470 ha, or 9.304% of the total area.

3.6. Development Action Plan in The Study Area

It is certain that an increase in agricultural production never justifies an increase in area but rather an increase in the production of potential units for agriculture. These areas represent only a small area of the study area. On the other hand, the areas with an average aptitude occupy more than half of the total area with the predominance of dry farming. These areas deserve special attention from the State to strengthen land development actions. The rest presents more than a third of the area, distributed between areas with very poor to poor suitability for cultivation, which testifies that agricultural activity is insignificant and far from expectations, these areas must be used either for pastoralism or for reforestation.

The establishment of a development plan is an action of extreme urgency, according to Smail (1994), the steppe development consists in finding harmonization between the constraints of the natural environment and the requirements of social progress, or between the ecological management of space and development policy.

3.7. For Dry Cultivation

The use of the earth's resources must be done in a reasonable way while respecting the carrying capacity of the soil so as not to cause a drop in its productivity, this is only possible with the practice of fallowing and avoidance of prolonged monoculture on the same land through crop diversification and the application of a crop rotation system that allows the restoration of soil fertility (Arabi, 2010).

The diversification of the types of production by the introduction of plant species, such as the prickly pear, the *Casuarina*, the olive tree, the pistachio tree, and this stage can guarantee the stability of the incomes which leads to a local development of the area of study.

Barley cultivation can also be interesting, especially in the Dayates, but on condition that it is done by prohibiting plowing, which will be replaced by semi-direct (Arabi, 2010).

3.8. For Irrigated Cultivation

The irrigated areas extending along the watercourses and also the irrigated perimeter of Zraguet, these areas can be of great agricultural interest but they have not benefited from development projects to ensure good drainage and a good system irrigation (Arabi, 2016). The use of natural fertilizers based on plant debris instead of chemical fertilizers which increases the salinity of the soil and therefore the water of irrigation.

The development of food crops based on wheat, barley, sorghum, lentils and vegetables that can contribute to food and sustainable security. The

intensification of annual fodder crops is also a possible solution. The return in areas with a slight slope to fruit tree growing such as apple and pear trees and, in irrigated areas, banana trees and market gardening such as potatoes. These areas also have good market gardening and horticultural aptitude

3.9. Lessons Learned

The map of potential suitability of the soil for agriculture makes it possible to propose a division into zones making it possible to define the agricultural and livestock speculations of each zone (Table 4). But it does not fully define the current state of the agricultural sector.

Table 4: Summary of Actions to Be Implemented For the Development

Agro-Ecological Zone	Area (ha) 2020	Precipitation (mm)	Actions
Plateau of Saida	19460	>300	Extension and enhancement of agricultural land
			Cereal and market gardening
			Controlled grazing
Mountain of SIDI YOUSSEF	66290	300-250	Reforestation and repopulation based on fruit trees Controlled grazing torrential correction Reconstruction of illicit extensions of tillable voids Silvicultural work Preservation of Holm Oak seed stands
Steppic high plains of Saida	133300	>250	Forage plantations Installation of green belt and windbreak Olive plantations Fencing
			Land reclamation in zone of Chott Ech Chergui Dune fixing in zone of Chott Ech Chergui

The climate factor must also be taken into consideration, according to Bedrani et al. (2018), climatic disturbances accompanied by unsuitable farming practices are at the origin of the amplification of the wind erosion phenomenon, of which 1.2 billion hectares of land are eroded.

Other factors come into play, such as the modern mechanization of cultivation methods and also the intensification of chemicals such as fertilizers at uncontrolled concentrations. This has made agricultural land unsuitable for cultivation (Arabi, 2016).

4. CONCLUSION

The results obtained only confirm the state of degradation of the steppe ecosystem due to a set of natural, socio-economic and even institutional problems that hinder the economic and ecological sector of the study area.

At the end of this study, it is necessary to point out that the use of remote sensing and GIS techniques has made it possible to achieve a significant advance in the characterization of the agro-pastoral potentialities which are well traced in the map of suitability for agriculture of the study area. This map is a valuable tool for decision-makers to effectively guide towards sustainable eco-development of this ecosystem. This can only bear fruit with a gradual approach while taking into account socio-economic constraints.

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CHAPTER III

SELECTION OF HORSE CHESNUT GENOTYPES (*Aesculus hippocastanum* L.) FROM SECONDARY POPULATIONS

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INTRODUCTION

Horse chestnut (*Aesculus hippocastanum* L.) is endemic to the southern part of the Balkan Peninsula. It is a very interesting deciduous species for the study of secondary populations thriving on green spaces in Serbia, as well as a valuable source for the production of planting material for horticulture and landscape architecture.

As an Arctotertiary endemic, horse chestnut is widespread in the mountain region of Albania, Greece, Bulgaria and Macedonia. In Byzantine empire age it was transferred from its natural habitat to Constantinople, but it was introduced to Europe much later, in XVII. century (Vukićević, 1997). Since then, it is a widely cultivated species in urban areas in small-, medium- and large-sized cities with temperate climate across the Northern hemisphere (North America, Europe and Asia) (Brune, 2016).

Cenosis and trees communities in urban ecosystems are under the influence of many environmental stress factors that lead to major changes in plant species. Cultivated trees exhibited lower growth, compared to the trees of the same species in their natural habitats. The majority of the cultivated trees are characterised by slow growth, weak resistance to environmental factors, shortened juvenile phase, reduced precocity, occurrence of complete sterility and reduced overall life expectancy by at least 30% (Oćokoljić, 2006). The longevity of trees in urban cenosis is determined by their genetic predisposition, which may be reduced several times under the influence of large sidewalks areas, great number of tall buildings, underground and overground

installations, climate (especially temperature), anthropogenised soil and large amounts of pollutants. Among the selected horse chestnut trees (*Aesculus hippocastanum* L.) there are old genotypes that have shown the possibility of existence in terms of urban environment. Hence, the need to examine variability and reproductive capability on such rare individuals, arose. These genotypes represent precious selection material that confirmed adaptivity on urban conditions. Their ability to grow and develop in the polluted air and soil conditions, proved that those genotypes have a great role in environmental quality improving.

The seed of the horse chestnut contains a series of biologically active substances in different quantities which affect the time duration of seed preservation, its germination, and therefore the production of good quality seedlings (Čukanović et al., 2011). Regarding the high level of water and its irretrievable loss from ripening to sowing, the horse chestnut seed is considered to be a recalcitrant. Starch, as organic matter, has the highest incidence in the seed while fats, which play a significant role in the pharmaceutical industry, come second. Proteins, as organic matter, have the lowest incidence in the horse chestnut seed, which, besides its structure, plays a role in the prevention of excessive drying up of the seeds. Tucakov (Čukanović, 2015), a well-known pharmacognosist from the Balkan Peninsula, has thoroughly investigated the healing properties of vegetative and generative organs of the horse chestnut. In the past, the seed was used for different purposes, such as fever and hemorrhoid treatment (Sirtori, 2001). Horse chestnut seeds have long been used in European phytotherapy to treat

inflammatory and vascular problems (Kucukkurt et al., 2010). Today, the horse chestnut seed extract is widely used in Europe and the USA in treatment of rheumatism, rectal complains, bladder and gastrointestinal disorders, chronic venous insufficiency and post-operative edema. The major active substance in horse chestnut seed is aescin, which is a natural mixture of triterpene saponins (Constantini, 1999; Sirtori, 2001). Besides, horse chestnut seed is rich in various other components, such as bioflavonoids (e.g. quercetin, kaempferol), anti-oxidants (proanthocyanidin A2) and coumarins (esculin and fraxin) (Bombardelli et al., 1996; Wilkinson & Brown, 1999; Deli et al., 2000; Sirtori, 2001; Chen et al., 2007). For that reason, horse chestnut secondary populations are recognized as potentially valuable seed sources for farmaceutical industry (Čukanović et al., 2020).

1. SELECTION PERIOD

In the breeding period 50 genotypes were recorded and rated at three sites: Bačka Palanka, Novi Sad and Sremski Karlovci (Vojvodina, Serbia). Seeds were collected, and the analysis of laboratory and field germination was conducted, along with morphological characterization of seedling growth indicators. The chemical composition of seeds was determined concurrently.

During the three-year period based on the extant research findings, 17 genotypes with the best biological, production and medicinal seed properties were identified.

The selected individuals were monitored across different phenological phases—leafing, flowering and fruiting, with intermediate phases. Seeds were collected for determining the morphological and chemical characteristics. Mother trees are characterized by differences in physiological maturity, phenotype character, and seed utility value for nursery production. Generative reproduction of genotypes was monitored and the growth indicators of *half-sib* progeny analyzed.

2. BIOMETRIC CHARACTERISTICS OF SELECTED TREES

At two investigated localities (Bačka Palanka and Sremski Karlovci), experimental horse chestnut trees are in the form of a tree-lined avenue. At the Novi Sad site, they grow freely in green areas. All investigated trees are physically mature, aged between 25 and 60 years.

After quantitative measurement, it was established that the average height of the examined trees is 13.64 m, trunk diameter 50.84 cm and crown width 6.24 m. The genotypes BP4, BP5, P1, P3, P4, P6 stand out with significant values for height from 15.00 m to 15.50 m.

Genotypes from the Sremski Karlovci site were isolated on the basis of high values for trunk diameter (61.14 cm) and crown width (7.50 m). They are located in an alley at a distance of 5 m in relation to the building, which enabled the proper development of the canopy.

The observed deviations of biometric parameters in relation to the literature data are not significant, and it can be stated that the conditions of the urban environment did not negatively affect the growth and

development of the investigated horse chestnut trees in selected localities.

As part of the evaluation of experimental wild chestnut trees, the presence of leaf miner was recorded (*Cameraria ohridella* Desch. & Dimić). Weak intensity of leaf miner occurrence was observed at all three examined localities, which did not significantly endanger the normal growth, flowering and fruiting of trees. Experimental trees, as separate seed sources for the use in horticulture and landscape architecture, produced regular and abundant yield.

Based on high average ratings for vitality (4.20) and decorativeness (4.60), genotypes BP4, BDS1, BDS2, P4, P5 and SK4 stand out, which is a reflection of their adaptation to urban conditions and the possibility of separation for different needs.

3. PHENOLOGICAL DATA OF SELECTED TREES

Phenological observations were performed on 17 selected horse chestnut trees. Phenological phenomena of leafing, blooming, fruit formation and leaf fall with intermediate stages were monitored, according to the BBCH scale (Meier et al., 1994).

The blooming period for all three years of observation at selected localities lasted from April 17, when the flower buds appeared, to May 17, when the end of blooming period was recorded. The peak of full blooming was recorded on April 29.

A significantly longer blooming period was observed in relation to the available literature data, which can be considered a positive feature of

the studied trees. The absolute longest duration of the blooming phenophase was observed in genotypes BP1, BP2 and BP27 (22 days), and the shortest in genotypes P5 and SK9 (13 days). The genotypes BP4, BP5, BDS1, BDS2 and SK4 also stand out for their blooming length (Figure 1 and 2). The long duration of the blooming phenophase increases the aesthetic value of horse chestnut trees, which is one of the reasons for its frequent use in secondary populations in horticulture and landscape architecture (Figure 3).

The dependence of the occurrence and duration of the phenophases of the observed horsechestnut trees on the air temperature was observed. Higher average air temperatures during March and April led to a much earlier start of the growing season.

During the three-year period, significant statistical differences were observed in the occurrence of leafing, fruit formation and seed maturation at the level of genotypes, localities and years of investigation, which can be used in the selection and breeding of horse chestnut.

The onset of seed loss during the three examined years was fairly uniform in terms of observation (from 18 September of the first year of observation to 23 September of the second year). Significant differences occurred between genotypes. The earliest seed decline was observed in genotypes BDS1, BDS2 and P5 (September 15-18).

High temperatures in the autumn months, which is the case in the last few years, significantly delay the beginning of leaf fall, prolong the

vegetation period and negatively affect the preparation of plants for winter. As a consequence of the warm autumn period, appears the vitality of the plant weakens and freezing the young branches.

Climate change may also be the reason for the deviation. The average duration of the growing season in Europe is 10.8 days longer than in the 1960s (Menzel & Fabian, 1999; Menzel, 2000). Walther et al. (2002) state that in the last 30 years in Europe, the warmer period at the beginning of spring led to an earlier beginning of the vegetation period in about 8 days. The same authors state that unusually high air temperatures in autumn cause an average of 4.5 days later change in leaf color.

According to Chmielewski & Rötzer (2002), the dynamics of phenological phenomena of plants within medium latitudes is very dependent on air temperature.



Figure 1: Horse Chestnut Blossom in the Phase of Full Flowering, Genotype BDS1 (Original by Čukanović)



Figure 2: Crown in Full Blooming Phase, Genotype BDS1 (Original by Čukanović)



Figure 3: Tree Line Of Horse Chestnut in the Phase Of Full Blooming, Locality Bačka Palanka (Original by Čukanović)



Figure 4: Occurrence of Repeated Leafing and Blooming, Bačka Palanka Site (Original by Čukanović)

Although air temperature is considered to be the most important factor influencing the development of phenological phenomena in plants, numerous studies indicate a large impact of economically important insect attacks, air and soil pollution and anthropogenic factors as well.

Repeated leafing and blooming of horse chestnut genotypes (BP2 and BP27) was observed at the Bačka Palanka site (Figure 4). The occurrence was recorded in August during all three years of observation. It is assumed that the reason for repeated leafing and blooming is the correlation of radiation temperature, drought, increased salt and heavy metal content in the soil and the attack of horse chestnut leaf miner (*Cameraria ohridella* Desch. & Dimić).

Long-term monitoring of horse chestnut phenophases and their dependence on air temperature in urban areas singled out genotypes that have successfully adapted to temperature extremes, regularly and abundantly bloom and bear fruit (BP4, BP5, BDS1, BDS2, P3, P6 and SK4). The inclusion of these genotypes in the process of reproduction would contribute to the production of high quality planting material for the needs of horticulture and landscape architecture.

4. SEED COLLECTION, STORAGE AND NURSERY PRODUCTION

Depending on the stage of maturity, horse chestnut seeds were collected at all three sites in the period from September 20 to October 15.

Seeds for the needs of nursery production were stored in a cold chamber in controlled conditions, at a temperature of 4 to 6°C. Sowing was carried out in the spring.

The seed material of 17 genotypes, in the three-year period, exhibited very high field germination rate, averaging from 80.94% to 85.64%. Seedling percentage survival, determined at the end of the vegetation

period and the beginning of a successive vegetation stage, ranged from 78.96% to 82.98%.

By measuring the growth indicators of one-year-old seedlings (Figure 5), average values for tree height (12.26 cm), root collar diameter (10.04 mm), root length (12.87 cm), tree weight (3.62 g), root mass (5.34 g), and number of I order roots (26.49) were determined. BDS₁ genotype could be distinguished due to the high values of measured indicators (19.20 cm for height, 17.51 cm for root length, 4.40 g for tree weight and 6.80 g for root mass).



Figure 5: One-Year-Old Seedlings of Horse Chestnut (Original by Čukanović)

According to the growth indicators of two-year-old seedlings (Figure 6a), genotypes BDS₁, BDS₂ and SK₄ could be singled out based on height (30.20 cm, 35.80 cm and 35.68 cm). The highest tree base diameter values were measured for genotypes BDS₁ (19.20 mm) and BDS₂ (19.33 mm).

The average value of the above-ground height and tree base diameter for the three-year-old plant genotypes were 81.45 cm and 26.81 mm, respectively (Figure 6b). In the third year of the horse chestnut seedling *in situ* development in the Rimski Šančevi nursery, maximum values for height and tree base diameter were found for genotypes BDS₁ (92.00 cm and 29.55 mm), BDS₂ (90.80 cm and 31.27 mm) and SK₄ (91.30 cm and 28.45 mm).

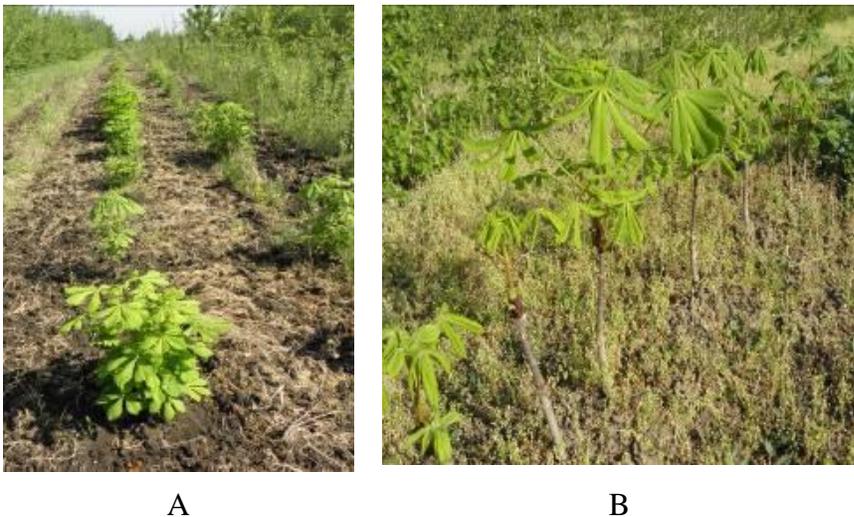


Figure 6: Seedlings of Horse Chestnut in Nursery A) Two-Year-Old; B) Three-Year-Old (Original by Čukanović)

On two- and three-year-old seedlings of genotypes BP₄, BP₅, BDS₁, BDS₂, P₅, P₆, SK₁ and SK₄, grafting of red chestnut hybrid (*Aesculus x carnea* Hayne) was performed (Figure 7a and 7b). Scion-rootstock compatibility was evident, and the average graft uptake was 82.36%. The applied English grafting technique produced good results, as a

vegetative technique for red chestnut hybrid planting material production.

The highest percentage of graft acceptance was recorded on BDS1, BDS2 and P6 rootstocks (84.32%, 82.95% and 82.72%). At the same time, the seedlings of these genotypes had high values of seed germination and survival, formed a favorable set of plants, which led to positive values of growth indicators of *half-sib* offspring that affect the favorable graft acceptance (Figure 8a, 8b, 8c and 8d).

No data on the success of grafting red chestnut hybrids on wild chestnut root have been found in the literature, and the results represent the starting point for further research. In correlation with the observed phenological phenomena and morphological characteristics of seeds, the most favorable results of field germination were shown by horse chestnut trees isolated in Novi Sad.

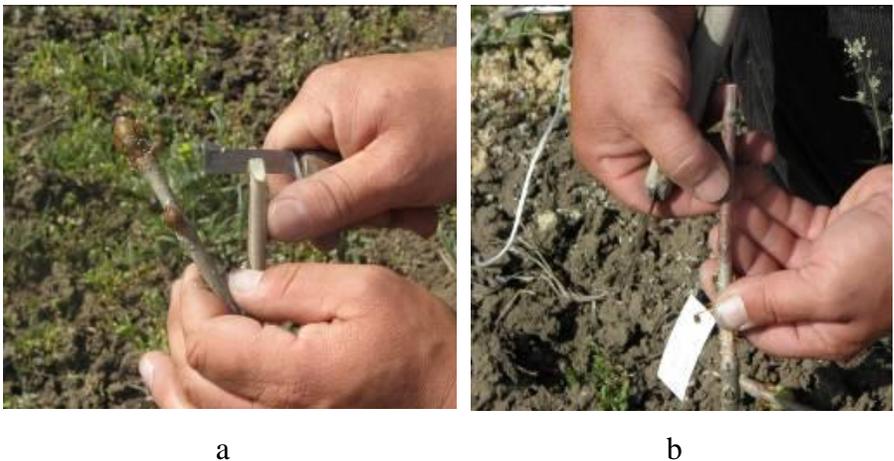


Figure 7: Grafting of Red Chestnut Hybrids on Wild Chestnut Rootstock
(Original by Čukanović)



a



b



c



d

Figure 8: a) Propagation of Red Chestnut by Grafting; b, c and d) Graft Acceptance on Two-Year and Three-Year Rootstocks of Horse Chestnut (Original by Čukanović)

CONCLUSION

The study aim is identification of horse chestnut genotypes from secondary populations developed on the green spaces of Vojvodina in a form of alleys, as individual trees or in group formations. Biological characteristics of genotypes should provide focus for the production criteria of quality horse chestnut planting material, adaptability to urban environment conditions and resistance to the stress caused by abiotic factors.

Horse chestnut does not have a natural population on the territory of Serbia, but there are significant areas occupied by secondary populations. Selection from secondary populations contributes to the conservation of valuable genotypes by applying different breeding methods. In that way, a unique gene pool is created, with offspring that have positive characteristics. Preselection and selection were focused at isolating individuals based on phenotypes adapted to unfavorable urban conditions, while regular and abundant fruiting, high seed germination, favorable composition and ratio of biochemical composition in the seed improve production of horse chestnut planting material in different ways.

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CHAPTER IV

REVIEW ON ORNAMENTAL ROSE OF SHARON (*Hibiscus syriacus* L.): ASSESSMENT OF DECORATIVENESS, INVASIVENESS AND ECOSYSTEM SERVICES IN PUBLIC GREEN AREAS

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INTRODUCTION

Urban dendroflora on public green areas plays a key role in urban development by providing different ecosystem functions (Daniels et al., 2018). Some cities are working to expand and preserve the sustainability of urban greenery, while others are degrading and destroying them to build residential and commercial buildings (Jim, 2004; M'Ikiugu et al., 2012). Urban cenosis forms the lungs of a city, they protect the city from negative climate change, purify the air and improve its quality, reduce the temperature and protect it from other climate disasters. Public green areas appear as semi-natural areas in the form of parks, urban gardens, urban pockets, squares, and as smaller or larger green areas that are enriched with a variety of decorative dendroflora. The design of urban green spaces according to ecological, climatic, and social criteria requires appropriate indicators and assessment strategies that maintain the multiple functions that urban green spaces fulfill (Mörtberg et al., 2017). Assessment of green space, decision-making, and maintenance of urban greenery in cities is increasingly based on the approach of assessing the function of the ecosystem itself (Niemela et al., 2010; Hansen et al., 2015). The biggest deficiency of urban dendroflora in public green areas is that design intentions are usually focused on aesthetic function and visual experience, rather than on the functional values that ornamental plants provide to the urban ecosystem (Kevrešan & Stevanov, 2017), which leads to increasing invasive alien species in public green areas. Invasive alien species were intentionally or unintentionally introduced as ornamental horticultural species, due to the decorative characteristics

they quickly became popular and very often planted on urban green areas (Van Kleunen et al., 2018; Ljubojević et al., 2021). Since they are characterized by a high degree of adaptability to different habitats, they are also considered fast-growing species with an abundant crown and a large number of ornamental flowers and seeds. Also, they represent one of the biggest global threats to biodiversity, they have the power to suppress autochthonous dendroflora, damage and occupy natural areas and change ecosystem processes, which affects its conservation (Gavrilović, 2016; Pyšek et al., 2020). Invasive alien species also provide a large number of ecosystem services whose recognized potential has a positive impact on ecosystem conservation and reduces ecosystem disservices.

Urban dendroflora on public green areas provides direct or indirect ecosystem services. Based on the Millennium Ecosystem Assessment (2005), the term ecosystem represents services that include the provisioning, regulating, supporting and cultural services provided by ornamental species (TEEB, 2011). Urban green areas have a basic role in preserving biodiversity in cities, whose presence has a great impact on the inhabitants of that city from a decorative, health, cultural and psychological aspect. Urban dendroflora in public green areas provides residents with an ideal place to rest, escape from city noise and heat, recreation and other activities, also play a very important role in improving human health and their quality of life in these areas (Wolch et al., 2014; Krellenberg et al., 2014; Iojă et al., 2014; Kabisch, 2015). It is also a source of habitat and food for animals that feed on fruits and

seeds of woody and shrubby species, and form the basis for maintaining and preserving basic ecological functions and processes created by nature itself (Farinha-Marques et al., 2011; Mace et al., 2012; Geslin et al., 2013; Nielsen et al., 2014). The concept of green infrastructure emphasizes the quality and quantity of urban and suburban green spaces, their multifunctional role and the importance they provide to residents, and the preservation of this concept contributes to the protection of biodiversity (Sandström, 2002; Walmsley, 2006; Tzoulas et al., 2007). A crucial role in planning, managing and preserving urban cenoses is played by people who play a key role in shaping, forming and maintaining public green spaces, as inadequate maintenance and management as well as shaping leads to urban decay and loss of ecosystems (Bennett et al., 2017; Christie et al., 2017; Shackleton et al., 2019). The key to success in the development of sustainable green space is the selection of appropriate ornamental plant species that are adapted to the urban area (Asgatzadeh et al., 2014).

One of the very common ornamental species on public green areas throughout Novi Sad (Serbia) is the Rose of Sharon (*Hibiscus syriacus*), which occurs as woody and shrubby species. *Hibiscus syriacus* is a flowering shrub from the Malvaceae family, native to Asia. The Rose of Sharon is the main ornamental species on green surfaces due to its resistance to very low temperatures, the wide range of flower colors and unique floral characteristics that include species with single, double, or multiple flowers (Contreras & Lattier, 2014). It often appears as an ornamental species that occupies a major place in the green

infrastructure of a city consisting of parks, squares, urban gardens, urban pockets as well as decorative greenery within residential complexes. According to the typology, it appears in the form of a hedge, tree line, solitary, as well as in decorative alleys on various green areas. Rose of Sharon is of great importance in the pharmaceutical and chemical industries, it has long been used in nutrition as a spice and folk medicine.

In this chapter, we will briefly describe the general characteristics of the Rose of Sharon and the assessment of its decorativeness on public green areas from the aspect of its invasiveness. We will give a brief overview of the genetic methods used to create new genotypes of the *Hibiscus syriacus* L. and their application on decorative green areas. We will discuss the ecosystem services that *H. syriacus* provides according to the Millennium Ecosystem Assessment (2005) and define its positive and negative impact on the environment and human health.

1. HISTORY OF INTRODUCTION AND DISTRIBUTION OF THE GENUS *HIBISCUS* SP.

Hibiscus is a polymorphic genus of flowering plants in the mallow family (Malvaceae) (Van Laere et al., 2007). The genus is quite large, encompassing about 250-300 species that originate from warm, temperate, subtropical and tropical regions around the world (Akpan, 2007). The genus species is known for its large ostentatious flowers, whose name is derived from the Greek word *ibiskos* (gr. *ἰβίσκος*) - mallow given by Pedanius Dioscorides about 40-90 years A.C. (Lawton, 2004), while the epithet of the species *syriacus* means that it

originates from Syria although it is not its natural range. Many species of *Hibiscus* are valued as ornamental species that are planted in gardens and green areas, while other species of this genus have long been used as a folk remedy. The species is known for its ability to attract bees and butterflies, which is why it is often planted in gardens to help other flower species attract lure pollinators (Salib, 2014). The flowers have a wide range of different colors that indicate the country of origin from which the species originated. *Hibiscus syriacus* L. is the national flower of South Korea, *Hibiscus rosa-sinensis* (red *Hibiscus*) is the national flower of Malaysia, and the species that has a burgundy flower is the flower of the Hound goddess Kali and often appears in her depictions in Bengal art in India, depictions speak of the beauty of a goddess and a flower merging into one (Figure 1) (Minahan, 2009).

In the Philippines, gumamela, which is the local name for *Hibiscus*, was used by children as part of the fun to create bubbles. They crushed the flowers and leaves until the sticky juice came out, then they took hollow papaya stalks which they then dipped into that sticky hibiscus juice, and they used those papaya stalks as straws for blowing bubbles. Together with soap, hibiscus juices produce more bubbles (Salib, 2014). *Hibiscus* flowers were proudly worn by women from Hawaii and Tahiti. If they wore a hibiscus flower behind their left ear, it meant that the woman was married or had a boyfriend. While the women who wore the hibiscus flower behind their right ear gave a sign that they were free. Yellow *Hibiscus* (*Hibiscus brackenrdgei*) is a national flower in Hawaii. *Hibiscus* is the national flower of Haiti, and it is also the

national flower of that nation, including the Solomon Islands and the islands of Niue. *Hibiscus* species have a wide application and are successfully used for all types of landscaping to achieve summer and autumn colors.



Figure 1: a) *Hibiscus syriacus*; b) *Hibiscus rosa-sinensis* (Original by Pušić)

2. GENERAL CHARACTERISTICS OF THE ROSE OF SHARON

2.1. Origin

The Rose of Sharon is a flowering shrub from the Malvaceae family, native to Asia (Figure 2) (Yoon et al., 2021). In the 16th century, it began to be grown in gardens, it was primarily introduced in America and later transferred to other parts of the world through trade exchanges. By the end of the 17th century, it was already known that the species is very resistant to cold and winter, and since then it has started to be planted as a highly ornamental species. In the 18th century, the Rose of Sharon became a common species that can be seen in English gardens, and North American colonies, it is also known as the Sharon rose. It was introduced very early to the Middle East and Europe, due to its

decorative value and power to be planted on green areas in the form of hedges, solitary or in decorative flower alleys. In some states, the United States is considered a very invasive species, where it is recommended to prune the fruit so that the seed does not spread further. In Serbia, it is considered a potentially invasive species, in accordance with which control measures are not yet recommended, but it is proposed to establish monitoring of this species on public green areas.

Taxonomic review of the Rose of Sharon according to Valdés (2011):

Kingdom: *Plantae*

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Malvales Bercht. & J. Presl

Family: Malvaceae Juss.

Genus: *Hibiscus* L.

Species: *Hibiscus syriacus* L.

Hibiscus syriacus L. is also called the Korean rose because it is the national flower of South Korea. The flower appears in national symbols (flags, coats of arms) and is often compared to the flower in the South Korean anthem. The symbolic meaning of the hibiscus flower comes from the Korean word *mugging*, which means eternity or inexhaustible abundance. People from South Korea generally consider the *Hibiscus syriacus* flower to be a symbol of the Korean people and culture.

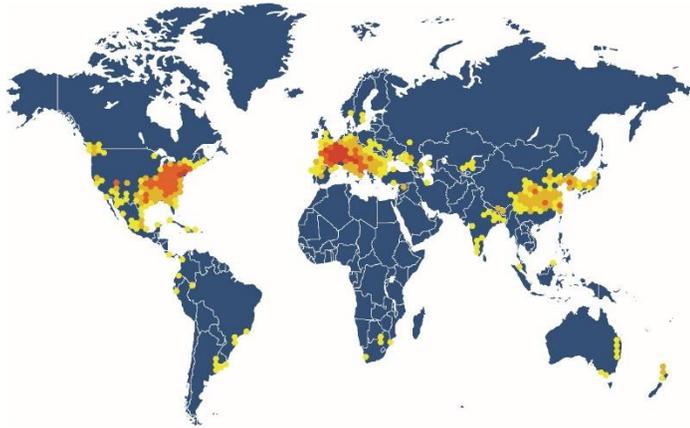


Figure 2: The Geographical Distribution of the Rose of Sharon (Original by GBIF)

2.2. Morphological and Biological Characteristics

Hibiscus syriacus L. is a monoecious deciduous shrub. It is of upright growth with a vase-shaped canopy, ie it forms a canopy that has an inverted ovoid shape. It reaches a height of 2-5 m, it is suitable for formal and informal planting. In the last couple of years, it has been used to form a hedge, because, with its branched canopy and large flowers, it nicely covers and enriches the space. Also, the Rose of Sharon is often found in parks, both in group and individual planting. The trunk and bark are thin, gray with lighter lenticels. The branches do not branch strongly if regular pruning is not performed, because regular pruning encourages the growth of shoots, after which they become thicker and stronger, and pruning also encourages a larger number of flowers as well the size of the flower.

The leaves usually appear on the twigs a little later, usually in May. The leaves are simple, ovate, spirally arranged on the branches, 5-10 cm

long. They have three different lobes with coarsely serrated edges of the leaves. The flowers are single and bisexual, about 6 cm wide. They are usually bell-shaped, with 5 petals (Figure 3). From the base of these five petals, a pistil extends in the middle while surrounded by anthers. The basic characteristics of the *Hibiscus syriacus* flower and its many varieties give it a recognizable shape. The species can bloom continuously during the summer, i.e. from July to September. There are different forms with flowers of different colors (purple, white, pink, blue, yellow, red), and the flower can have a simple or complex crown, i.e. single or double (Vukićević, 1996).

The Rose of Sharon has green or brown decoratively unattractive fruits in the form of capsules with 5 compartments inside the capsule in which the seeds are placed. The fruits are kept on the branches for most of the winter. Eventually, the capsule will open and spread easily germinating seeds around the base of the parent plant, thus colonies will form over time. The seeds appear in a larger number in the capsule. The seed is kidney-shaped, 5mm long, 4 mm wide, while the seed thickness is 2.5 mm (Figure 4). The length of the hair is 3 mm. The weight of one seed is between 0.05-0.09 g, while the weight of 1,000 seeds is 15.9 g.

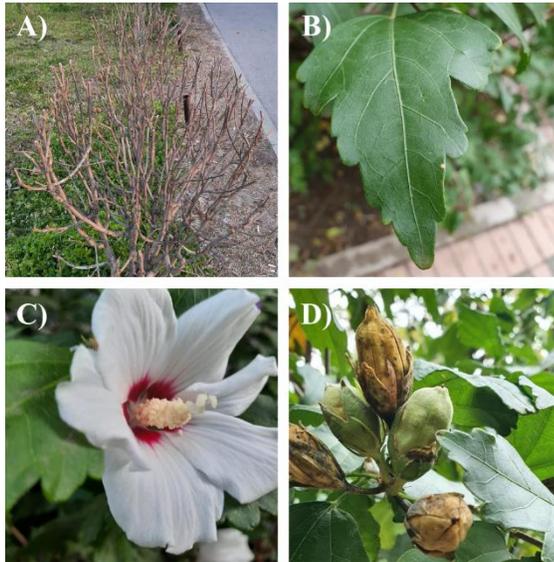


Figure 3: A) Woody Twigs; B) Leaf; C) Flower; D) Fruit of *H. syriacus*
(Original by Pušić)

The Rose of Sharon has tiny seeds that are very light and covered with tiny ashy hairs that help it to be easily dispersed by the wind and very easily propagated by seeds. Also, the seed is not dormant which is another relief when it comes to generative propagation.

Vegetative propagation of Rose of Sharon is done by cuttings (mature and green cuttings), grafting, and by micropropagation. The rooting success of the Rose of Sharon varies from clone to clone species. The rooting process itself is better if the shoots used for cuttings are ringed or strangulated only a few weeks before the cuttings are prepared.



Figure 4: A) Open Fruit Capsule; B) Collected Seeds; C) A Mass of 1 000 Seeds; D) Single Seed (Original by Pušić)

Mature cuttings are taken during February and March and placed in the ground or substrate of a mixture of peat and sand. Scarring is mainly performed in cold or warm beds, with prior treatment with phytohormone (2% IBA).

During the summer period, green cuttings are taken, after treatment with phytohormone (1% IBA), their rooting takes place under plastic foil. Grafting was mainly done in the winter on rootstocks with bare roots. Grafting in the form of lateral joining is most often used, and saddle grafting, triangulation, or gap grafting is used less often (Stilinović, 1987). The shoots are most often taken from the parent trees, which are formed in the form of a hedge and which were previously abundantly and roughly pruned during the fall, to obtain stronger shoots. While one- or two-year-old seedlings or parts of the

root of the basic form were used as a base (Grbić, 2004). Also, the Rose of Sharon can be propagated by aerial laying, which is the most common way of propagating *Hibiscus syriacus*.

2.3. Ecological Characteristics

The Rose of Sharon can tolerate a wide range of environmental conditions including frost, drought, and urban pollution but thrives best in a warm and sheltered area, as well as on neutral and well-drained soil with plenty of sun considered one of the most strong and durable ornamental species (Da-Costa-Rocha et al., 2014). Although it easily adapts to all habitat conditions, it is best suited to sandy, loamy and clayey soils that are rich in organic matter, whose pH value is 5.6-7.5. It requires moist soil that is well-drained because it does not tolerate soil staining. *H. syriacus* does not tolerate low temperatures from -15 °C to -20 °C.

It tolerates pruning very well, which is why it is often used for hedges. Pruning is usually done every year in late February and early March, to create the desired shape on public green areas, also pruning encourages faster growth of new shoots and greater flower size (Grbić, 2004).

Like all other flower species, this species is attacked by diseases and pests that need special attention. The most common pest is aphid (*Diaspidiotus perniciosus*). The infestation of the aphid is easily noticeable because it forms numerous colonies on the leaves and twigs, sucking the sap from the vegetative parts of the plant leads to weakening and gradual drying of the species itself (Kereši et al., 2016).

It very often occurs in urban areas, where it can cause great damage, to protect the Rose of Sharon from these pests, it is necessary to remove the infected part and apply appropriate insecticides. The disease can often develop into trunk cancer, but only on older specimens of the Rose of Sharon. In addition to this pest, the species is susceptible to downy mildew, rust on the leaves, as well as spots that appear on the flowers and leaves.

Although it has no autumn color and looks like an awkward shrub, the Rose of Sharon certainly remains one of the most decorative ornamental shrubs with large flowers of different colors. Since the Rose of Sharon is mainly grown for its gorgeous flower, it easily adapts to balconies, gardens, and small urban spaces, and can also be easily grown in pots.

3. GENETIC METHODS OF BREEDING OF ROSE OF SHARON

Biological invasion is caused by favorable site characteristics, climate change, lack of natural enemies as well as biological characteristics of the species itself (hairy or sticky seeds, small seeds, secretion of growth inhibitors, scattering of seeds by wind and animals, etc.) leading to their rapid spread and adaptation to new conditions (Nikolić et al., 2010). Invasive alien species have the power to change natural ecosystems, acting as edictory that suppress autochthonous plant species and causing a socially negative impact on the environment (Tmušić et al., 2018). Since the cultivation of ornamental allochthonous woody species has led to an increased number of invasive alien species, for this reason, various methods are sought to reduce the spread and adaptation

of invasive alien species (Vining et al., 2012). The genetic resources of invasive alien species have been very little researched, but they can provide insight into the mechanisms of invasion and their pathways. The easy spread and adaptation of these species has gained significant attention in the international community and has raised many environmental issues. The genetic characteristics that characterize these species greatly affect their capacity to spread and adapt to new habitats (Eunmi Lee, 2002). The influence of natural selection and genetic characteristics of these species could change the genetic structure of invasive populations, and they would be able to modify and control their adaptation and tolerance to new conditions. Knowledge of the genetic structure of invasive alien species is very important to successfully form programs to reduce their invasion (Eunmi Lee, 2002). Invasive alien species spread very quickly and easily adapt to different environmental conditions, so eradication of these species is often impossible, extremely expensive and greatly disrupts the ecosystem, so all investments in preventing and reducing invasiveness through conventional breeding methods are justified.

Hibiscus syriacus is one of the most popular species of the genus Malvaceae, it has about 40 different varieties that differ in color and shape of the flower. The basic number of chromosomes in *H. syriacus* is $k = 20$, and most cultivars are tetraploid ($2n = 4k = 80$) (Skovsted, 1941). Conventional methods of breeding ornamental Rose of Sharon have contributed to the creation of new varieties and hybrids that offer unlimited possibilities for decorative landscaping of various public

green areas. Conventional breeding methods of *H. syriacus* are mainly focused on reducing sexual fertility, however, these ornamental species have great success in vegetative propagation. The prevention of the vegetative spread of *H. syriacus* is affected by regular maintenance and cultivation of areas where the ornamental species are present, although these methods do not completely prevent the spread of Rose of Sharon, in which case the generative parts (fruit and seeds) of this species can be used for raw material production, which will be used for alternative purposes (Vining et al., 2012). Conventional *H. syriacus* breeding methods include polyploidy and interspecific hybridization, interspecific hybridization is most commonly used to create new varieties of ornamental species, and these methods can be applied to a large number of different species, and have been shown to produce commercially successful varieties.

3.1. Interspecific Hybridization

Interspecific hybridization is a major source of genetic variation whose main goal is to combine traits from different genes that do not exist in a single species. Interspecific hybridization is used in various programs to grow a large number of different ornamental species such as chrysanthemums, gladioli, lilies, roses, orchids as well as hibiscus and many other ornamental species (Van Tuyl & Lim, 2003). Interspecific hybridization of *H. syriacus* plays a role in the creation of new varieties and hybrids that have high endurance during the winter period (Van Tuyl & De Jeu, 1997; Kuligowska et al., 2013; Tapeç et al., 2021). Interspecific hybrids provide qualitative and quantitative traits of the

parent pairs from which they are crossed (Van Laere, 2007). The greater the phylogenetic distance between two parents, the more difficult it is to create interspecific hybrids (Sharma, 1995). The resulting offspring are often sterile, resulting from structural differences in the parent chromosomes due to improper chromosome pairing during gametogenesis (Vining et al., 2012). The appearance of sterility in this way is manifested as the appearance of meiotic abnormalities called hybrid sterility or chromosomal hybrid sterility and represents an opportunity for growers to develop different ornamental varieties of *H. syriacus* with new phenotypic characteristics which also have a reduced risk of invasiveness in green landscaping. *Hibiscus syriacus* was successfully crossed by interspecific hybridization with *Hibiscus sinosyriacus* and *Hibiscus paramutabilis* (Eeckhaut et al., 2004; Van Laere et al., 2007), crossbreeding compatibility was one-sided and successful only when *H. syriacus* was used as a parent- mother (Kuligowska et al., 2013). Also, the success rate of the cross depends on the variety itself. Although interspecific hybridization is considered to be very successful in invasive alien species, it can pose a high risk of increasing invasiveness in public green areas, producing offspring that are incompletely or partially sterile leading to an increasing prevalence of invasive alien species and their spread, are new genotypes with stronger genetic variants (Ellstrand & Schierenbeck, 2000).

Although this method of breeding leads to the creation of offspring with an extremely high level of sterility, it is desirable to obtain offspring with complete sterility, especially in hybrids that arise from highly

invasive species, because this will prevent their spread (Vining et al., 2012).

3.2. Polyploidy

Polyploidy is a widely accepted technique for breeding ornamental plants. The genome structure in *H. syriacus* confirms that this ornamental species is tetraploid compared to other closely related diploid species (Kim et al., 2017). In addition to tetraploid forms of *H. syriacus*, varieties with a higher degree of ploidy were discovered, such as hexaploid forms ('*Aphrodite*', '*Diana*', '*Helene*', '*Minerva*', '*Melrose*', '*Pink Giant*' and '*Shimsan*') and octoploid ('*Purple CV*', '*Purple CV2*', '*Red Heart CV*') (Eeckhaut et al., 2004; Van Laere et al., 2007; Lattier et al., 2019). To induce polyploidy in *H. syriacus*, meristems are usually treated with antimitotic agents such as colchicine or dinitroanil herbicides such as oryzalin or trifluralin (Van Laere et al., 2007; Contreras et al., 2009). There are several techniques for applying these agents to various plant species, such as soaking seeds, soaking shoot tips, covering shoot tips with agar or lanol solutions, spraying with atomized solutions, and the single drop method. In the National American Arboretum, the hexaploid forms of '*Aphrodite*', '*Diana*', '*Minerva*' and '*Helena*' of *H. syriacus* were crossed with seedlings of '*William P. Smith*' treated with colchicine to improve phenotypic characteristics. Also, Van Laere et al. (2006) created hexaploid forms of blue-flowered *H. syriacus* derived from '*Blue Bird*' and '*Woodbrige*' cultivars treated with colchicine. Unlike oryzalin, colchicine is more effective in the application of chromosome doubling in the Rose of

Sharon (Lattier et al., 2019). In varieties produced by polyploidy, anatomical differences of morphological character have been observed, which exhibit a "giant" effect, including larger flowers, a longer flowering period, and reduced seed production (Van Huylbroeck et al., 2000).

In some cultivars of *H. syriacus*, changes in plant height, leaf size, internode length, crown volume, pollen diameter, and differences in wood fiber anatomy were observed (Lee & Kim, 1976; Shim et al., 1993; Contreras et al., 2009). The creation of *H. syriacus* cultivars through polyploidy may prove extremely useful in the study of polyploidy in ornamental shrubby and woody species (Lattier et al., 2019). Polyploidy involves the production of clonal replicas of each taxon to which this type of conventional breeding is applied as well as the use of mature plants to measure anatomical, morphological and physiological characteristics such as drought tolerance, cold resistance, flower size and other parameters aimed at improving the growth of ornamental species on public green areas and enrich the space with their decorativeness and make it more pleasant to stay.

3.3. Ornamental Varieties of Rose of Sharon

The species *Hibiscus syriacus* has a large number of varieties that mainly differ in color and structure of the flower, and less often in the shape of the leaves. Varieties of *H. syriacus* can be:

- *Hibiscus syriacus* var. '*Diana*'- white flowers
- *Hibiscus syriacus* var. '*Helene*'- white flowers

- *Hibiscus syriacus* var. '*Minerva*'- lavender-pink flower
- *Hibiscus syriacus* var. '*Aphrodite*'- pink flower
- *Hibiscus syriacus* '*Variegata*'- burgundy flowers with branched leaves

Certain ornamental varieties of *H. syriacus* have received awards as species most commonly planted in gardens and orchards by the Royal Horticultural Society such as:

- *Hibiscus syriacus* var. '*Blue Chiffon*'- blue half-full flowers
- *Hibiscus syriacus* var. '*Hamabo*'- light pink with a red center
- *Hibiscus syriacus* var. '*Lavander Chiffon*'- light lilac flower
- *Hibiscus syriacus* var. '*Meehanii*'- pink with panned leaves
- *Hibiscus syriacus* var. '*Olseau Bleu*'- blue purple with burgundy center
- *Hibiscus syriacus* var. '*Red Heart*'- white flowers
- *Hibiscus syriacus* var. '*White Chiffon*'- white and full flowers
- *Hibiscus syriacus* var. '*William R. Smith*'- white and simple flowers
- *Hibiscus syriacus* var. '*Woodbridge*'- dark pink flowers

As shown in Figure 5, the represented varieties of *H. syriacus* on public green areas in the territory of Novi Sad. As the flowers appear in different colors, it is a species that can give a lively note and enrich the decorativeness of the public green area.



Figure 5: A) *Hibiscus syriacus* var. 'Blue Chiffon'; B) *Hibiscus syriacus* var. 'Pink Chiffon'; C) *Hibiscus syriacus* 'Variegata' (Original by Pušić)

4. INVASIVENESS RISK ASSESSMENT

A large number of wild ornamental vegetation appears on public green areas in cities, they are mostly inhabited by invasive and allergenic species that have an adverse impact on biodiversity. Invasive alien species represent allochthonous vegetation that was introduced by man to an area where it was not naturally distributed (Lakušić, 2005). The global invasion of allochthonous species is expanding very rapidly and inhabiting other green areas, leading to ecosystem degradation (Pyšek & Richardson, 2010). Invasive alien species cause biodiversity degradation has a major impact on the environment and reduce the number and diversity of ornamental species in public green spaces. One part of the introduced ornamental species exist in cities as cultivated ornamental plants in botanical gardens, garden centers and nurseries, which intentionally or unintentionally spread to the natural environment under the anthropogenic influence and today represent astonishing vegetation, while the other part was introduced as a commercial product. horticultural markets such as various fairs, horticultural exchange, sales, and /or other ways (Vining et al., 2012; Gavrilović,

2016; van Kleunen et al., 2018). Invasive alien species are generally considered harmful species that pose a global threat but can offer numerous economic and environmental benefits because they provide a large number of positive ecosystem services (Olszańska et al., 2016; Crowley et al., 2017; Vaz et al., 2017; Zengeya et al., 2017; Bach et al., 2019; Villatoro et al., 2019).

Weber & Gut (2004) proposed a methodology to determine the invasive potential of an ornamental plant species in an area. The methodology takes into account all important characteristics that identify ornamental species as strongly, moderately, or weakly invasive. By knowing the biological characteristics, data on the geographical distribution of the species itself, as well as the characteristics of the plant community of the Rose of Sharon, the invasive potential of the species itself can be determined. The criteria used to determine the risk of invasiveness of the Rose of Sharon are climatic area, geographical prevalence and distribution, history of invasiveness, taxonomy, viability and seed reproduction, life form and population density of the species. According to the proposed methodology, ornamental species rated 3-20 are considered potentially and weakly invasive species that are not considered a threat, while species rated 21-27 are characterized as species with a moderate risk of invasiveness, it is necessary to observe and establish appropriate monitoring to monitor these species and species rated 28-39 are considered highly invasive, posing a major threat to the natural community (Weber & Gut, 2004).

Based on the described methodology, the sum of *Hibiscus syriacus* scores on public green areas throughout Novi Sad (Serbia) was 26/39 (Table 1), which represents a moderate risk of invasiveness, the species requires further observation and monitoring to prevent it from becoming highly invasive. The Rose of Sharon does not originate from Europe and originates from a climatic coincidence. The species occurs as an ornamental species in more than five countries and has a wide range of global distribution, while *H. syriacus* has not been reported as a weed or spontaneous dendroflora elsewhere. On the seedlings that have been observed on public green areas throughout Novi Sad, they produce large amounts of seeds, which are small and rich in small hairs, which is why they are easily dispersed. Spontaneous seedlings of Rose of Sharon were observed in the immediate vicinity of the mother plant as well as from the surrounding greenery, where they appear as spontaneous seedlings (Figure 6).



Figure 6: Spontaneous Seedlings of *H. syriacus* (Original by Pušić)

The Rose of Sharon is a perennial shrub species with woody vegetative organs, which is mostly planted on public green areas as a shrub species in the form of hedges, for this reason it is marked as a woody perennial in the risk assessment. The Rose of Sharon is a highly valued ornamental species in public green areas with great economic and environmental potential. An ornamental species such as *H. syriacus* provides large amounts of quality seed that can be used as a potential green solution, thus turning poor service into a significant ecosystem service (Vaz et al., 2017; Ljubojević et al., 2021).

Table 1: Invasiveness Risk Assessment of *H. syriacus* in Public Green Areas

Risk assessment	
<p>1. <u>Climatic match</u> Does the known geographical distribution of the species include ecoclimatic zones similar with those of the risk area?</p>	<p>– No0 – Yes.....2</p>
<p>2. <u>Status of species in Europe</u> Is the species native to Europe?</p>	<p>– Yes.....0 – No2</p>
<p>3. <u>Geographic distribution in Europe</u> In how many countries does the species occur?</p>	<p>– Species occurs in 0 or 1 country..... 1 – Species occurs in 2-5 country.....2 – Species occurs in >5 country.....3</p>
<p>4. <u>Range size of global distribution</u> How is the size of the global range (native and introduced)?</p>	<p>– Range is small, species is restricted to a small area within one continent0 – Range is large, extending over more than 15 ° latitude or longitude in one continent or covers more than one continent.....3</p>
<p>5. <u>History as an agricultural weed elsewhere</u> Is the species reported as a weed from somewhere else?</p>	<p>– No0 – Yes.....3</p>
<p>6. <u>Taxonomy</u> Does the species have weedy congeners?</p>	<p>– No0 – Yes.....3</p>

<p>7. <u>Seed viability and reproduction</u> How many seeds do the species approximately produce?</p>	<ul style="list-style-type: none"> - Few seeds or n viable seeds 1 - Many seeds 3 - Do not know 2
<p>8. <u>Vegetative growth</u> Allocate species to one of the following. If more than one statement applies, take the one with the highest score.</p>	<ul style="list-style-type: none"> - Species has no vegetative growth that leads to lateral spread 0 - If a tree or shrub, species has the ability to resprout from stumps or steam layering or stems root if touching the ground 2 - Species has bulbs or corms 1 - Species has well developed rhizomes and/or stolons for lateral spread 4 - Species fragments easily, fragments can be dispersed and produce new plants 4 - Other or do not know 2
<p>9. <u>Dispersal mode</u> Allocate species to one of the following. If more than one statement applies, take the one with the highest score.</p>	<ul style="list-style-type: none"> - Fruits are fleshy and larger than 5 cm in diameter 2 - Fruits are fleshy and larger than 10 cm in diameter 0 - Fruits are dry and seeds have well developed structures for long-distance dispersal by wind (pappus, hairs, wings) 4 - Fruits are dry and seeds have well-developed structures for long-distance dispersal by animals (spikes, thorns) 4 - Species has mechanisms for self-dispersing 1 - Other or do not know 2
<p>10. <u>Life form</u> What is the life form of the species?</p>	<ul style="list-style-type: none"> - Species is a small annual (<80 cm) 0 - Species is a large annual (>80 cm) 2 - Species is a wood perennial 4 - Species is a small herbaceous perennial (<80 cm) 2 - Species is a large herbaceous perennial (>80 cm) 4 - Species is a free floating aquatic 4 - Other 2
<p>11. <u>Habitats of species</u> Allocate species to one of the following. If more than one statement applies, take the one with the highest score.</p>	<ul style="list-style-type: none"> - Riparian habitats 3 - Bogs/swamps 3 - Wet grasslands 3 - Dry (xeromorphic) grasslands 3 - Closed forests 3 - Lakes, lakeshores, and rivers 3 - Other 0

<p>12. <u>Population density</u> What is the local abundance of the species? If the species is present in the risk area, this question refers to plants within the risk area. If the species is present in Europe, this question refers to plant within the European range. If the species is not present in Europe, this question refers to the native or introduced range of the species.</p>	<ul style="list-style-type: none"> – Species occurs as widely scattered individuals.....0 – Species forms occasionally patches of high density2 – Species forms large and dense monocultures.....4
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5. OIL PROPERTIES OF SEEDS DERIVING FROM ROSE OF SHARON

Every year, urban dendroflora generates large amounts of waste — a byproduct of urban greenery that is usually neglected (Shi et al., 2013). Urban dendroflora biomass such as leaves, fruits, and seeds is not lost back every year (Ljubojević et al., 2021). The green raw material of urban dendroflora on public green areas has great potential in the production of new green solutions that have the ecological and economic benefit of ecosystems (Raud et al., 2017). Due to increased urbanization and industrialization, increasing efforts are being made to reduce global warming and emissions, and the focus is on renewable energy sources that represent alternative fuel-biodiesel options (Fawzy et al., 2020). Biodiesel is considered a promising alternative to fossil fuels derived from biological resources in the production process of which edible or inedible oils, animal fats, or recycled edible oil are used (Tomić et al., 2020; Ljubojević et al., 2021). Biomass sources for biodiesel production located all around us can be a reliable and renewable energy source that replaces non-renewable energy sources, such as urban dendroflora in public green areas (Jain & Sharma, 2010). In recent years, biodiesel has become more attractive due to its environmental benefits and its great impact on the preservation of the

environment and ecosystems. Biodiesel is considered one of the most important options for preserving sustainable energy development and for reducing greenhouse gas emissions that cause global warming (Kanth & Debborma, 2021).

Rose of Sharon is one of the most common ornamental species that occurs in urban dendroflora in public green areas of Novi Sad and is therefore considered an excellent source of new raw material for the production of green solutions because it comes from shrubs that grow on-site and no need to take additional land or for investing in its care and maintenance (pesticides, fertilizers, insecticides) because it has adapted very well in our area (Tomić et al., 2020; Ljubojević et al., 2021). Collecting the Rose of Sharon seeds reduces the risk of its invasiveness in public green areas and creates new ecosystem services that will have a positive impact on preserving the environment and the green economy.

The Rose of Sharon seeds contains 11.5% oil. The oil is extracted from this seed in two phases. The first phase involves the extraction of oil from the seeds by a mechanical press, while the second phase involves the separation of oil using a catalyst and a solvent. Rose of Sharon seeds requires some preparation before the oil squeezing process itself, due to their tiny hairs that prevent adequate oil squeezing using a mechanical press. It is necessary to remove the hairs in the seed grinder, in this way its membrane will break, which will lead to better extraction. For this type of extraction, a mechanical press is mainly used on the principle of pulling seeds that pass through the auger and a roller that is heated at

a certain temperature but never over 60 °C, under certain pressure and speed of rotation of the auger. At the same time, oil is extracted and the cake is thrown out (the rest of the ground seed). The cake retains a certain amount of oil that can be obtained chemically using N-hexane. In order to obtain biodiesel from Rose of Sharon oil, it is necessary to mix the oil obtained by mechanical pressing and solvent extraction and undergo transesterification (Tomić et al., 2020).

Before transesterification, it is necessary to clean the obtained oil from impurities and solid impurities. Transesterification is performed in alcohol (methanol) and the presence of a basic potassium hydroxide catalyst (KOH). This chemical alcoholysis is the technologically simplest process of biodiesel production and can be applied to smaller quantities of oil for biofuel production. Potassium hydroxide was taken as a catalyst due to the ecological aspect that it has when mixed with phosphorus, and produces K_3PO_4 (potassium phosphate) and can be successfully used as a fertilizer and thus affects the preservation of ecosystems and does not produce waste materials (Tomić et al., 2020). Transesterification was performed at a temperature of 62°C on a chemical stirrer with a speed of 600 min^{-1} / rpm, in a molar ratio of alcohol and oil in a concentration of 6:1, 0.7 w% of potassium hydroxide pelate was added to 6 moles of methanol (Mičić et al., 2013). After transesterification was complete, the reaction mixture was poured into a decantation vessel and left for 8h to allow gravity separation of biodiesel and glycerin (Figure 7).

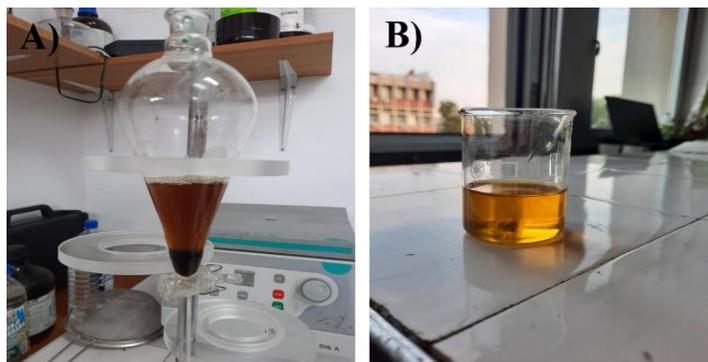


Figure 7: A) Decantation of the Reaction Mixture; B) Biodiesel of *H. syriacus* (Original by Pušić)

5.1. Physico-Chemical Characteristics of Oil Rose of Sharon

The Rose of Sharon oil contains a high level of polyunsaturated fatty acids, of which linoleic and stearic acid are the most common. The high content of monounsaturated and polyunsaturated fatty acids in Rose of Sharon oil maintained the iodine number (IN) at an optimal level making this oil suitable for biodiesel production according to EN 14214. High IN values are associated with several problems when using biodiesel in engines as fuel, low IN values have negative effects on the cetane number (Bouaid et al., 2007). With the increase of the chain length of fatty acids and the degree of saturation, the cetane number increases, while with the increase of the degree of unsaturation, its number decreases (Ljupković, 2014). The importance of density as a characteristic of biodiesel stems from the fact that the control of the injection of the housing into the engines is performed based on volume or time. Variation in fuel density above the limit values set by the standard cause's disturbances in engine operation, which results in high exhaust emissions and fuel consumption (Tomić et al., 2020). *H.*

syriacus oil has a slightly increased density value compared to the standard. The acid number is a measure of the content of free fatty acids in biodiesel. The high content of polyunsaturated and saturated fatty acids in the Rose of Sharon oil itself maintains a high value of the acid number. A higher value of the acid number affects the extraction process at high temperatures, which results in an increased content of free fatty acids (FFA). Free fatty acids in biodiesel cause fuel degradation during storage due to oxidative reactions with oxygen from the air (Tomić et al., 2020). A higher value of the acid number affects the extraction process at high temperatures, which results in an increased content of free fatty acids in *H. syriacus* oil.

One of the predominant problems that arise during the production of biodiesel is its low oxidative stability. During the process of oxidative decomposition, various chemical products are formed, such as alcohols, aldehydes, carboxylic acids, and other sediments, which affect the preservation of the produced biodiesel (Monirul et al., 2015). Low oxidative fuel stability is closely related to the high content of polyunsaturated fatty acids and it increases with the increase of double and triple bonds per molecule (Alptekin & Canakci, 2009; Varatharajan & Pushparani, 2018). The content of polyunsaturated fatty acids in *H. syriacus* oil occurs in larger quantities, for this reason, it is considered a fuel of low oxidative stability, but has high-temperature characteristics. Biodiesel that has high-temperature characteristics has its advantages, because it has the possibility of use during the winter and summer period because the possibility of its transition to a solid-

state at very low outside temperatures is reduced. Due to the high content of linoleic acid, biodiesel has good lubricating properties. While monounsaturated acids occur in smaller numbers, oleic acid occurs. Saturated fatty acids occur in concentration, where stearic and palmitic acid are the most common. The importance of density as a parameter for the characterization of biodiesel whose value occurs above the maximum values set by the standard causes great disturbances in the operation of the engine, which leads to high emissions and fuel consumption (Tomić et al., 2019).

Based on the physical and chemical characteristics of biodiesel, Rose of Sharon seed is a new raw material for urban dendroflora for the production of renewable energy, which will raise urban greenery to a higher level and encourage the development of a green economy. The use of green raw material *H. syriacus* for the production of biodiesel represents the transition of ecosystem disservices into a new ecosystem service that will have a positive impact on the environment and the preservation of ecosystems.

6. ADVANCING ECOSYSTEM SERVICES OF ROSE OF SHARON

Ecosystem services are the benefits provided by ornamental dendroflora and that provide optimal and functional living conditions in the city (Hamilton & Yang, 2017). Dendroflora can provide a variety of positive and negative ecosystem services in public green spaces. Assessing and identifying ecosystem services in urban green spaces is rare, but crucial for planning, managing, regulating, and setting up

monitoring to monitor ornamental species on public green areas that can have a major impact on the urban ecosystem (De Groot et al., 2010; Haase et al., 2014a; 2014b). Due to increasing urbanization and industrialization, the Millennium Ecosystem Assessment (2005) declared that "cities and human settlements should be made sustainable, safer and as functional as possible for life", considering this their main goal of sustainable urban development that will be of global importance (Klopp & Petretta, 2017; Srđević et al., 2019).

In this chapter, we will explain the new services acquired by the presence of the Rose of Sharon in public green areas throughout Novi Sad. In order to improve the ecosystem services that the Rose of Sharon provides on public green areas, it is necessary through the process of evaluating the representation of green areas, to determine four main groups or services that it provides, namely: provisioning, regulating, supporting and cultural services. The Rose of Sharon provides several positive services in public green spaces. This ornamental species was originally introduced in the urban environment due to its great resistance to very low temperatures and the expressive decorative value of flowers. However, it is necessary to take into account the negative aspects of the use of *Hibiscus syriacus*, such as invasive potential and vegetative reproduction.

Uncontrolled seed dispersal and vegetative reproduction, as well as inadequate management of Rose of Sharon in green areas, have led to its widespread distribution in urban areas in the form of stunning vegetation that disrupts the appearance of public green areas throughout

the city. As shown in Figure 8, the benefits that this ornamental species provides outweigh its negative impacts on urban green spaces. It should be taken into account that all the positive services that the Rose of Sharon provides on public green areas can be outweighed by the negative services if this species starts to spread uncontrollably and acquires the status of a highly invasive species.

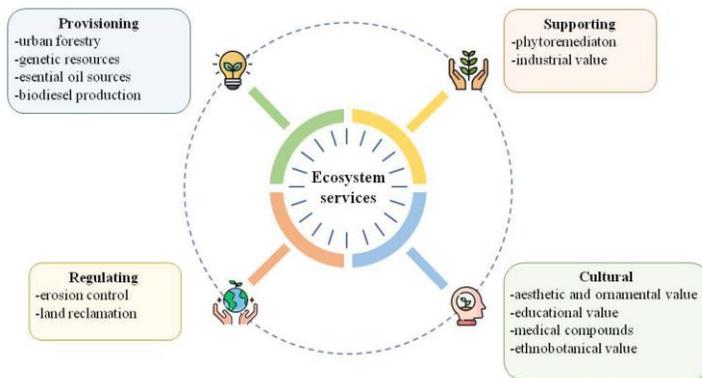


Figure 8: Ecosystem Services of Rose of Sharon (Original by Pušić)

6.1. Agricultural Effects

The Rose of Sharon provides several ecosystem services such as urban forestry, genetic resources, biodiesel production, erosion control, land reclamation and phytoremediation on public green spaces. It is considered ideal for landscaping public green areas due to its aesthetic-decorative value and rapid growth, which is why it is a suitable ornamental species for urban forestry (Vaz et al., 2017). The ornamental species, such as the Rose of Sharon, is characterized by great resistance to low temperatures, different types of soil and other urban conditions,

and also influences the regulation of climatic disasters. *Hibiscus syriacus* promotes soil stabilization and prevents landslides or erosion (Gomez-Baggethun & Barton, 2013). Some research has shown that certain varieties of the genus *Hibiscus* have the power to absorb and transport heavy metals, i.e. to provide phytoremediation (Dhar et al., 2015). Phytoremediation using an ornamental species such as *H. syriacus* easily stabilizes and absorbs heavy metals and is considered an easily sustainable and environmentally friendly method by which renewable energy sources are obtained (Harindintwali et al., 2020). Although the Rose of Sharon is considered moderately invasive in public green areas throughout Novi Sad, it produces large amounts of fruits and seeds that represent a green raw material for a new potential ecosystem service in the form of green solutions based on nature - biodiesel. The collection of green raw materials of this ornamental species for the production of biodiesel encourages the development of the green economy and the preservation of urban greenery in urban areas (Hoseini et al., 2018; Ljubojević et al., 2021). The Rose of Sharon is considered an excellent source of genetic material, which is shown by the fact that a large number of varieties and cultivars of this species occur. Genetic resources of *H. syriacus* are used not only to improve the impact of environmental factors (resistance to diseases and pests, drought, pollutants, etc.) but also to improve and obtain new phenotypic characteristics such as new flower color, larger flower size and length of the flowering period, then completely or incompletely sterile seeds, higher production of seeds and fruits that will contribute to the greening of public green areas (Zhang et al., 2007). The creation of new varieties

through genetic methods is a borderless game where there is the possibility of constant production of new varieties and hybrids of the Rose of Sharon, thus providing the ecosystem service that it continuously provides. It is represented in large numbers on public green areas in the form of hedges, solitary or in decorative alleys, it is characterized by exceptional aesthetic, decorative and educational value, creating the identity of one place but promoting mental and physical health through various passive and active activities on green areas on which it appears (Chan et al., 2012).

The ecosystem services provided by the Rose of Sharon represent new potential green solutions based on nature that have a positive impact on preserving the environment and green areas in the city, also reduce the negative impact of ecosystem disservices such as invasiveness of this species and establish a natural balance between ecosystems and biodiversity.

6.2. Ethno-Medical Use of the Rose of Sharon

Ornamental species belonging to the genus *Hibiscus* thrive in different habitat conditions and produce a variety of natural compounds with different bioactive compounds (Holser et al., 2004). The pharmacological properties of *H. syriacus* indicate that this ornamental species provides various biological compounds that have antibacterial, anti-inflammatory, antihypertensive, antifertility, hypoglycemic, antifungal and antioxidant effects (Vasudeva & Sharma, 2008; Gomes Maganha et al., 2010). A large number of species of the genus *Hibiscus* have been investigated and found to contain secondary metabolites such

as flavonoids, anthocyanins, terpenoids, steroids, polysaccharides, alkaloids, amino acids, lipids, sesquiterpenes and other metabolites. Rose of Sharon has great use-value, from various beverages and teas, various alternative and traditional medicines to industrial value.

The Rose of Sharon is a source of a wide range of biochemicals that can improve the health of each individual in the cosmetics industry as well as in the food and pharmaceutical industries. *Hibiscus syriacus* is a plant that is known in Asia for its antiseptic, antibacterial and antifungal properties. The Rose of Sharon extract has been proven to have antioxidant and antiproliferative effects on lung cancer cells in humans (Di Martino et al., 2017). This decorative type helps in wound healing, which is a dynamic physiological process by which the skin regenerates after an injury. The healing properties of *H. syriacus* stimulate the regeneration of injured tissue, which is an interaction between several different cellular processes and components of the extracellular matrix that lead to the edges of the wounds regrouping together and healing faster (Midwood et al., 2004).

Yoon et al. (2017) proved that natural substances contained in *H. syriacus* have a great dermatological effect on skin recovery from wounds and other skin diseases. Plants used as alternative medicines have a lower degree of toxic substances with much fewer side effects than standard conventional medicines (Ohnishi & Takeda, 2015). However, the leaves of this species have been declared a traditional hair tonic in the Indian medical system, and it has been determined that topical application of this extract stimulates hair growth and prevents

hair loss (Di Martino et al., 2017). Rose of Sharon has small seeds that are rich in fatty acids, which have great potential for the production of edible oils or for feeding livestock. However, Rose of Sharon seed oil contains two unusual fatty acids, dihydrosterculinic acid (DHSA) and vernolic acid (VA) (Wang et al., 2012). When humans or animals consume the Rose of Sharon oil, which is rich in DSHA (similar to cottonseed oil) can cause major adverse health effects such as toxicity or physiological disorders, while VA-rich oil has great potential benefits in industrial applications such as the production of plastic or glue (Wang et al., 2012). Chemical examination of seeds revealed the presence of different groups of flavonoids such as dihydroquercetin, herbaceous, kaempferol, saponaretin and saponarin, which have a great influence on the reduction of tumorigenesis and erythema due to the occurrence of various diseases (Di Martino et al., 2017). In addition to being rich in fatty acids and flavonoids, the Rose of Sharon seed also contains large amounts of anthocyanins and a large number of different pigments (Di Martino et al., 2017). Rose of Sharon from seed to flower is a species that is traditionally used in folk medicine and is predominantly represented in the cosmetics industry because it is a source of useful bioactive compounds for various cosmetic products on a natural basis. In traditional Korean medicine, *H. syriacus* root is widely used because of its active components such as lignins, coumarins, and phenolic components, it acts to reduce fever or as an antidote for poisoning by chemicals or poisonous fungi (Gomes Maganha et al., 2010; Yang et al., 2019). The flowers of the Rose of Sharon are attributed to numerous benefits for making folk remedies.

Rose of Sharon flowers act on stomach and digestive problems and calm the nerves, and its flower is considered one of the main ingredients in making hot or cold hibiscus tea around the world (Salib, 2014).

Due to the large number of positive medicinal properties that *H. syriacus* contains, various studies are constantly being conducted to discover new potential benefits and medicinal properties that this highly decorative species provides.

6.3. The Impact of the Rose of Sharon on the Environment

Biological and ecological characteristics make *H. syriacus* a competitive species in public green areas that is able to thrive in a variety of ecological conditions. Due to the large production of seeds that are sustainable and very easy to spread, as well as due to vegetative reproduction, this ornamental species is very capable of scattering seeds and planting new seedlings near the parent plant. Examination of the Rose of Sharon on the natural environment is very scarce, but it is very easy to combine with other ornamental plants, invasive populations or wild vegetation. To date, it has not been discovered whether the Rose of Sharon causes changes in the chemical and physical properties of the soil. Although considered a moderately invasive species, it has a positive impact on biodiversity and the ecosystem.

6.4. Other Uses

In its rural area and later in other distribution centers, *H. syriacus* was primarily used due to its decorative characteristics and greening of gardens and green areas. Today it is an extremely ornamental species

that provides numerous services on public green areas. The Rose of Sharon is considered an excellent source of food for bee pollen because of its large flowers and a large amount of pollen. Flowers are often used for dyeing fabrics and food, as well as for culinary purposes to decorate dishes (Salib, 2014). The stems and roots of *H. syriacus* are considered an excellent source of fiber in the pulp, paper and twine industries (Gomes Maganha et al., 2010). Rose of Sharon seeds are neglected in public green areas and are a by-product of green waste of urban greenery, but have great importance for the development of natural green solutions and great potential for commercial exploitation of seeds as raw materials that can be used for alternative purposes (Chan & Ismail, 2009; Dhar et al., 2015).

CONCLUSION

Promoting the development of new green solutions based on nature offers new potential ecosystem services that will raise urban greenery to a higher level and affect the sustainability of biodiversity and ecosystems.

The Rose of Sharon is an ornamental species that provide many ecosystem services that are not fully utilized. Although it is characterized by exceptional aesthetic and decorative value, the Rose of Sharon on public green areas provides many more services than just aesthetic and decorative. This ornamental species on public green areas throughout Novi Sad has proven to be moderately invasive in conducting an invasive risk assessment and is characterized by the production of large amounts of seeds that are neglected and scattered

each year to form spontaneous seedlings around the mother plant. The Rose of Sharon in an area affected by human activities such as maintenance and care does not pose a major threat and its invasive properties are outweighed by the potential ecosystem services it provides. In order to prevent the deposition of Rose of Sharon seeds in the soil and the formation of invasive seedlings, it may be recommended to collect seeds and fruits that will have the function of green solutions or green raw materials. Collected green raw material *H. syriacus* is considered a renewable energy source - biodiesel that provides fuel for machines used for urban landscaping of public green areas, also vegetative and generative biomass of this species is very often present in the pharmaceutical and cosmetic industries.

A significant contribution to further research would be a database that also contains other cities where the Rose of Sharon appears as an ornamental species in public green areas. An innovative database would encourage the utilization instead of eradicating *H. syriacus* and the intrinsic disservice that species provides will turn into positive ecosystem services that will foster the development of a green economy that aims to preserve the environment

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CHAPTER V

TRUE BUGS (HETEROPTERA) AS PESTS IN ORNAMENTALS

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INTRODUCTION

Insects are amazing creatures. They have many wonderful strategies to survive in this huge and diverse world. Being relatively small and tiny they can easily hide, move and spread, and they can be found in any kind of environment, from the air, ground, water, plant and animal tissue, to a sack or a jar in our homes. They also usually develop fast, have the ability to leave a lot of offspring, and relatively easily repeat the cycle again and again, many times. These are just a few general characteristics that make insects strong and provide them survival in spite of many natural predators and measures taken by humans to reduce the number of pest and disease vector species in the environment and/or agricultural fields.

There are also many beneficial insect species, of course, not all of them should be considered as the “bad ones”, and insects have their purpose in nature, as parts of the food chains, as plant pollinators and animal scavengers, e.g. Therefore, we have to respect them and admire them, although they can be a nuisance sometimes, they can endanger our health and even destroy our crops. Our mission should be the find the best strategies how to live with them in peace. Ornamentals, by definition, are a hugely diverse and big group of whole plants or just a part of plants that are grown for decorative purposes (Elhadi, 2019).

Ornamental crops can be classified as floriculture and nursery plants, trees, shrubs, and foliage plants (Saraswathi et al., 2018) which are produced with the purpose of decorating or enhancing the environment.

They exclude plants deliberate for commercial food production, like fruits and vegetables, which have demands for, sometimes serious, plant protection and pesticide use. But, when we talk about insects, they don't recognize definitions and classifications written in books, administrative borders, and other kinds of limitations and, in nature, they instinctively choose plants following their own aspirations for their homes and/or food sources. This is the link between agricultural crops that are very well observed and protected, and ornamentals which are sometimes neglected for insect and/or disease monitoring, but in the vicinity of the crops they might serve as reservoirs, being the primary or alternative plant hosts, and ultimately, enable insects to enlarge their populations and cause damage which leads to economical loses.

1. WHY TRUE BUGS?

The “true bugs”, or Heteroptera, are insects classified in order Hemiptera. Actually, heteropterans are the only insects that are correctly called “bugs”, what has ancient etymological roots (Schaefer & Panizzi, 2000). The word originates from the Anglo-Saxon *bugge*, which means wraith or specter that was blamed for mysterious itching welts on people's skin after a night's sleep. Those kinds of symptoms causes the bed bug or very small insect named by Linnaeus as “the bug of a couch” (*Cimex lectularius* L.), a tiny insect that lives in cracks and crevices near the bed, including mattresses and bedclothes, and under the cover of the night, specimens feed on the blood of the sleeping hosts, remaining invisible during the day. Awakenning people seem to have had no cause for symptoms, which are therefore described as attack of

wraith, specter, or *bugge*. Later on, when the real causing agent was identified and described, the word bugge was applied not only to bed bug, but also to all its relatives, all heteropterans.

The suborder Heteroptera represents the largest and most diverse group of hemimetabolous insects (Henry, 2009). This monophyletic group is generally characterized by the flattened, shield-shaped body, elongate mouthparts designed for piercing and sucking fluids from plant or animal tissue, four to five segmented antennae, forewings partially sclerotized (2/3) and partially membranous (1/3), which are lying flat over the body, with large, well-developed scutellum (“little shield”, lying just between the front of the wings). Perhaps the most important characteristic for certain representatives of the suborder is paired metathoracic scent glands in adults, and dorsal abdominal scent glands in both adults and nymphs, which are responsible for another nickname of some of the true bugs – the stink bugs (Fam. Pentatomidae).

As mentioned above, heteropterans are hemimetabolous insects that have three developmental stages: eggs, nymphs or larvae, and adults/imago, pupa doesn't exist. Immature instars, nymphs, more or less look like their adults and feed in the same way. They have no wings and genitalia, and when they finish their development they moult into adults. Many authors agree that Heteroptera is the most abundant group of insects with incomplete metamorphosis and it is also the most diverse (Henry, 2009).

Thanks to their wide adaptive abilities, developed during the period of a long evolution, true bugs are widespread in all natural zones of the globe, except the Polar Regions. Terrestrial species can be found from tropical zones to tundra, and even in the Himalayas, at an altitude of 5000 m above sea level (Hutchinson, 1934. cit. Vinokurov & Kanjukova, 1995). In the aquatic environment, true bugs inhabit rivers, lakes, streams, and there are also species that live on the surface of the ocean, at a considerable distance from the shore, such as the genus *Halobates*, family Gerridae (Kirichenko, 1951 cit. Vinokurov & Kanjukova, 1995). The importance of the migratory possibilities of true bugs is evidenced by the permanent findings of the species *Nabis capsiformis* (Germar, 1837) over the ocean (Keržner, 1981, cit. Vinokurov & Kanjukova, 1995) and its flight far to the north.

Hemiptera as a highly diverse group of insects includes a large number of true bugs that feed on different types of food. There is no other group of hemimetabolic insects whose representatives show such diversity in terms of the food they eat. Among them, there are many polyphagous species that barely choose food, but also narrowly specialized species that feed only on a certain plant or animal species. Thus, for example, the species *Corythucha ciliata* (Say), the sycamore lace bug, which feeds exclusively on sycamore trees (*Platanus occidentalis*), is cited as a typical monophage (Glavendekić & Mihajlović, 2006). An extreme example of the ecological adaptation of the species is the true bug from the family Nabidae, the species *Arachnocoris albomaculatus* (Scott), which is adapted to live in a spider web and feeds on caught insects

(Myers, 1925). There are species that specialize in feeding on ants; others feed on centipedes, or fungi under the bark of trees, while a large number of representatives feed on various plant species. Sometimes they might feed on plants and other animals at the same time. Example is the species *Nesidiocoris tenuis* (Reuter), which sucks sap from vegetative and generative plant parts, causing symptoms like necrosis of trees and leaves, flowers falling, and fruit stunted, but it is also a predator of various preys, through cannibalism and feeding on dead insects on the host plants (Protić, 2011). Varieties are numerous.

However, for ornamental and any other plants, the most important are the plant feeders, which make about 60% of all Heteroptera. Many bugs, especially Pentatomorpha, during their diet choose the reproductive organs of plants, like flowers, immature and ripe grains. Other species, such as mirids and lace bugs (Cimicomorpha section) feed on non-reproductive plant organs, and the plant responds by redistributing nitrogen (Schaefer & Panizzi, 2000). With this way of feeding and the preference of the species towards the reproductive organs of plants, true bugs directly "compete" with man, who, also due to the high nitrogen content, wants seeds and fruits of plants for himself. Therefore, many true bugs species pose a danger to cultivated plants and are labelled as pests of cereals, vegetables, fruits, and industrial plants, including ornamentals and landscapes. Any polyphagous species, either invasive or not, may cause significant damage to the attacked plants, and besides their direct damage, when they reduce the

aesthetic and market value of ornamental plants, they can also cause nuisance problems in urban and residential areas.

2. SPECIES IMPORTANT TO ORNAMENTALS

Different species might occur in gardens and landscapes. As mentioned above, during the feeding process hemipterans suck juices from leaves, fruits, or nuts, forming pale circles around feeding points and deforming tissue. At the same time insects' excrement usually speckles the leaves and both symptoms reduce the aesthetic appearance of plants and impair the function of urban greenery. Although true bugs rarely seriously harm woody plants, their activities in forests and landscapes are undesirable, especially if occurring in high abundance.

The highly invasive sycamore lace bug, *Corythuca ciliata* (Hemiptera, Tingidae) is native to North America where it is described as an annual, chronic pest of *Platanus occidentalis* L., the sycamore, and of its hybrid, the London plane tree (Schaefer & Panizzi, 2000). Both adults and nymphs of this species (Figure 1) feed on the underside of the leaves and produce chlorosis. Their injuries reduce photosynthesis and respiration of host plants causing sometimes huge effects on aesthetical value of the trees. As a result foliage becomes bronzed and leaves may fall earlier, in late summer (Halbert & Meeker, 1998). The first records of this species in Europe date back to 1964, from Italy, and more than 20 years later it has been reported in many other countries as France, Hungary, Austria, Spain, Switzerland and Yugoslavia (Maceljski, 1986; Protic, 1998; Kment, 2007). Recent studies conducted in

Romania, revealed annoying behaviour during August, when adult specimens of this species were observed flying without a specific target and pinching the human skin (Grozea et al., 2020).



Figure 1: Adults of *Corythuca ciliata* on the Bark of a Tree
(Original by Z. Gavrilovic/URL-1)

First generation adults in Europe appear in June and second generation appears around July/August. Species overwinter as adults under loose bark, leaf litter and crevices, and tolerate low temperatures. As the average daily temperature rises above 8 °C in spring, the adults emerge. Mating pairs of *C. ciliata* initiate colonies by laying eggs along the leaf veins, where a single female can lay up to 350 eggs. There are five immature instars. Nymphs stay close together at first, only moving to new leaves after they reach the fourth instar. Three weeks after the nymph development has been finished, the second egg-laying-period starts mainly in the second half of July with a number of 80–160 eggs/female. Approximately in the middle of September, the adults of

the second generation emerge, and they start wintering at the end of October (Tatu & Tăușan, 2011).

Very similar, sibling species, *C. arcuata* (Say), the oak lace bug (Figure 2), is also native to North America (Drake & Ruhoff, 1965). Since its first record in Italy in 2000 (Bernardinelli & Zandigiaco, 2000) the fast spreading is evident all over the Europe, with latest records in Slovakia in 2019 (Zúbrik et al., 2019). In Serbia it has been recorded in 2013 for the first time (Pap et al., 2015). Feeding on the cellular content of the oak leaves, species causes chlorosis typical for this group of insects (Figure 2). Discoloured, pale, molted leaves are the results of both nymphs and adult feeding process. Also, on the underside surface of oak leaves black spots of insect excrements are clearly visible and usually numerous (Figure 3).



Figure 2: Symptoms of Chlorosis Caused by *Corythuca arcuata*
(Original by Z. Gavrilovic/URL-1)

This very tiny insect, 3 mm long, overwinters in bark cervices or in the litter. Oak lace bugs are small, rectangular, dorsoventrally flattened insects. Adults have transparent, lace-like textured wings that are held

flat over the insect's body. The adults are cream coloured with black or brown patches. Nymphs are gray to black, with some white spots from the third nymphal stage, and many spines are present on their bodies. They are seen as clustered among their dark faeces and cast nymphal skins on lower leaf surfaces. The head of adults is covered entirely by the pronotal hood what is not the case with nymphs. Hemelytra are areolate and some brown spots are present across the apex. Oak lace bugs overwinter as adults on or near their host in the crevices of bark, branches, or other protected places.



Figure 3: Adults of *Corythuca arcuata* (Original by Z. Gavrilovic/URL-1)

Overwintered adults move to oak leaves as soon as leaves begin to appear in spring. Adults begin feeding on young leaves of the host plant and females deposit their black and elongate eggs on the underside of the leaves, ranging in number from 15 to more than 100 per female. The average number of eggs laid in Italy was about 100 eggs per cluster. From egg to adult, *C. arcuata* passes through the five nymphal stages and full development takes 4-6 weeks. In northern Italy there are three

generations per year (Mutun, 2003 and citations within). Although adults of oak lace bugs are not good fliers, they are obviously successful in fast spreading by wind or different ways of transportation. As stated by Neimorovets et al. (2017) oak lace bug was able to overcome 250-270 km in two years. Most probably human activities and passive ways of transportation are usual pathways of its spreading.

Human activities and transportation as stowaways are the most important ways of spreading one of the most important worldwide pest true bug species, the Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* Stål (Hemiptera: Pentatomidae). *Halyomorpha halys* (Figure 4) is native to East Asia where it has been reported to feed on at least 106 ornamental host plants as the Princess tree, mulberry, elm, willow, Chinese arborvitae, Chinese milk vetch, and similar (Wang & Wang, 1988; Lee et al., 2009). Frequent damage caused by *H. halys* have been reported on many agricultural plants too, and the list includes many vegetables, field crops, ornamentals and even medicinal plants. Major economic losses were recorded on crops in families Fabaceae and Rosaceae (Lee et al., 2013). Surveys conducted at a semi-landscaped horticultural garden revealed all stages of brown marmorated sting bug on *Paulownia tomentosa*, *Fraxinus americana*, *Liquidambar* spp., *Viburnum opulus* var. *americanum*, *V. prunifolium*, *Pyrus* sp., and *P. pyrifolia* (Nielsen & Hamilton, 2009). These ornamental plants very often serve as host plants where the population grow is usually undisturbed, and from where specimens spread and attack cultivated plants.

The harmfulness of this species in agriculture is reflected in the fact that it feeds on a large number of plants, among which are economically important crops, fruits, vegetables, field and ornamental plants. In Japan, it attacks primarily fruits and soybeans, and in China, apple fruits (Funayama, 2012). In the U.S. it is harmful to apples, peaches, tomatoes and peppers, pumpkins and cucumbers, sweet corn, soybeans, and beans (Leskey, 2012). In Switzerland, economically significant damage was reported in pepper crops in 2012 (Sauer, 2012), and in Italy, large damage were recorded on pears and other fruits (Maistrello et al., 2017).



Figure 4: Adult of *Halyomorpha halys* Feeding on Hazelnut (Original by Konjevic)

This extremely polyphagous species was for the first time recorded in Serbia in October 2015 (Šeat, 2015). Soon after these first findings this invasive species become established pest species in the country (Musolin et al., 2018). Monitoring of BMSB, using pheromone traps, was conducted soon after the first findings, in both urban areas and

arable land. Trapping was conducted from May to October, in three consecutive years, and more than 20 traps were set in both urban areas and in orchard or crop vicinity. Pyramid dead-in traps (AgBio Company) with aggregation pheromone (Tréce lures) were set in order to get information on whether the BMSB is present in a certain area or not. Results of the trapping revealed the continuous spread of the species almost all over the country, and also an increase of the recorded population abundance at most of the trapping points. Based on monitoring and visual observations, together with information obtained from citizens, it can be concluded that today, the named invasive pest, established stable populations in the region of Serbia, with an increased abundance of the species in the central and northern parts of the country. Field monitoring, which revealed crop, vegetable and fruit damage at a different stages of ripening, and at a different times of the year, showed that the species starts to become a serious, economically important pest in different cultures. Mid to high-level damage were recorded on hazelnut in 2020, but also in other cultivated plants as soybean, apple, cherry, peach, pear, tomato, and pepper, with most probably enlarged list of hosts expected in the future (Konjevic, unpublished data). In 2021 blueberry is added to the hosts list as the sensitive one, e. g. Among urban hosts the most important are catalpa and paulownias trees which serve as hosts for both adults and nymphs, and which are usually excluded from any kind of control program.

Halyomorpha halys is a very mobile insect, the adults are good fliers and nymphs move very quickly on the plants in both vertical and

horizontal direction. This allows them to switch from one to another host quickly and easily, and thus avoid an unfavourable situation when there is no desired cultivated host plant in the field. Very often the number of laid eggs is lower in orchards or crops than in the surrounding plants, although the given cultivated host meets the needs of specimens' development and provides favourable conditions (Zhang et al., 2007). For this reason, Zhang et al. (2007) proposed, as one of the measures to reduce the number of these true bugs, to chemically control the surrounding forest or ornamental plantations, as well as to use the technique of attract and kill, isolation of ripe fruits, and also the use of natural enemies. However, the control of surrounding plants is not fully justified due to the care of non-target organisms as well as the effect on beneficial insects. Although BMSB is sensitive to a number of insecticides (pyrethroids, organophosphates, carbamates, neonicotinoids (Lee et al., 2013)), the good flying abilities of adults allow them to escape from the treated plant zone and move to other hosts (Watanabe et al., 1994), and then re-inhabited the treated crop/orchard, which greatly impacts the successful protection.

During monitoring in urban and semi urban areas all developmental stages of BMSB were observed at different heights of catalpa, apple and hazelnut trees. Based on the Figure 5, which is the result of a survey conducted in 2020, it can be seen that adults of *H. halys* were present at all examined heights, while they were most abundant at 1.2 m above the ground. In contrast, the lowest number of adult specimens was recorded at 2 m. As for the nymphs of the BMSB, they were also found

at all examined heights, except 1.6 and 2 m, while being dominated at 1m height. The largest number of egg clusters was found at 1.2 m while only one egg cluster was founded at 1.8m above the ground. Here it must be emphasized that this test was performed only up to 2 m height due to the inaccessibility of the canopies.

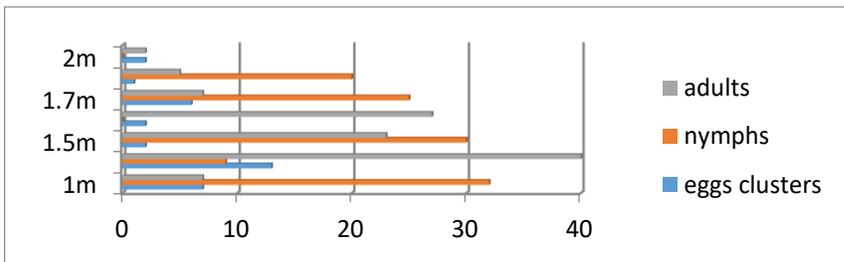


Figure 5: Heights of the Canopy Where Eggs, Nymphs and Adults of *H. halys* Were Observed

Another pentatomid species *Acrosternum heegeri* (Fieber, 1861) (Hemiptera: Pentatomidae), very often found in urban areas, has been recently recorded on thuja trees in Novi Sad vicinity, in Serbia (Kereši & Konjević, 2020). This also polyphagous species is a well-known pistachio pest in Iran (Ribes & Pagola-Carte, 2013, cit. Károlyi & Rédei, 2017), while it can be found on deciduous trees, conifers (Pinaceae and Cupressaceae) and other plants too (URL-2). This Mediterranean species has been observed expanding to the north. In Serbia, the first specimens were photographed back in 2013, but this was not published. In the Alciphron database (URL-3) there are 22 locations where this species was photographed or recorded (13 of them are in the northern part of country - Vojvodina province). Large number of nymphs and adults of this species were observed during September-

October 2019, on *Thuja* trees at the outskirts of Sremski Karlovci showing its potential to further spreading and importance to ornamental and landscape plants.

Biology of this species has not been explained in detail in literature, most probably due to its small importance to plant production. Among the species of the genus *Acrosternum* the species *A. hilare* (Panizzi et al., 2000) is described as the most significant harmful species, which is polyphagous and can cause damage to more than 30 plant species in the northern hemisphere, including ornamental ones, while in the southern areas of distribution (below 40 ° north latitude) represents a pest of soybeans, beans and other legumes. This species is semi-migratory and has one generation per year in the northern range. Similarly, most probably species *A. heegeri* has also one generation per year, with the possibility of developing two generations in warmer conditions (McPherson 1982 in Panizzi et al., 2000). The nymphs usually end their development during August, going through five nymphal stages. Adults appear from mid-September to early October (Javahery, 1990 in Panizzi et al., 2000) and overwinter in hidden places, similar to other pentatomids. During October and November 2019, at the site of the find in Sremski Karlovci, adults of *A. heegeri* were noticed on the walls of the house, together with specimens which entered the house (Kereši & Konjević, 2020) while searching for suitable overwintering place. Thus, this species has joined the group of nuisance species which, in recent years, disturbed humans in urban and suburban settlements, such as *Nezara viridula* L., the green vegetable stink bug, and *H. halys*, but also the Asian ladybug, *Harmonia axyridis* Pallas (Coleoptera:

Coccinellidae) too. For now, however, population density of *A. heegeri* is not as numerous as the above mentioned species.

CONCLUSION

Ornamental plants, with all its morphological and physiological diversity, offer suitable residential and feeding properties to a numerous number of insects, amongst which are also some heteropterans. These insects, with their sucking mouthparts, might cause different kind of damage, which is usually chlorosis, discoloured, pale and molted leaves, flower falling and fruit deformation. All these symptoms disrupt the appearance of urban greenery and its functionality. But, not only are this direct damage important for ornamental plants. At the same time those attacked plants offer suitable conditions for pest species to increase their population densities and then migrate to cultivated plants, where their impact becomes even bigger. Most of the times on ornamental plants insects are free from insecticides treatments and other ways of monitoring and/or control. Species as *Halyomorpha halys* or *Nezara viridula* overcome obstacles such as the chemical control by moving from field to ornamental plants hosts and vice versa. Some other, as *Corythuca ciliata*, *C. arcuata* and *Acrosternum hegerii* hide high in the tree canopy and remain relatively safe while causing damage to plants.

These strategies are obviously good strategies for their survival in space and time. It is now up to us how to find a way to better understand such species and find a way to live with them in peace.

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CHAPTER VI

INVASIVE PATHOGENS AND PESTS ON WOODY ORNAMENTALS

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INTRODUCTION

“On a global basis...the two great destroyers of biodiversity are, first habitat destruction and second, invasion by exotic species”

E.O. Wilson, Harvard biologist

Urban green spaces are recognized as important contributors to the health and well-being of the citizens, influencing the quality of life by bringing natural elements and wildlife habitats into urban settings, providing a beautiful and interesting place for relaxation. Their main benefits are physical, psychological, social, economic, and environmental (Dorst et al., 2019). Their importance is especially emphasized through urbanization resulting in an increasing proportion of the population living in the cities. It is estimated that 66% of the world's population will live in cities by 2050, compared to 30% in 1950 (UN, 2014). Sufficiently large tree plantations can provide crucial environmental benefits since the trees regulate rainwater runoff, alleviate high greenhouse gas emissions, improve air, soil, and water quality, reduce the urban heat island effect, create specific microclimates through decreasing wind and solar irradiation, providing shade and reducing the noise (Russo & Cirella, 2018). At the beginning of the development of most cities, their environment was not as degraded as it is now. With the cities becoming more urbanized, tree planting in urban planning and sustainable urban development, such as garden cities or green belts, has become more crucial (Czaja et al., 2020).

At the same time, the urban environment negatively affects the vital functions of trees, influencing their physiological and morphological features and general fitness. Plant growth conditions in cities are worsening due to high urbanization rates and stress factors derived therefrom. Dense buildings, heavy traffic, construction works with deep excavation, and the widespread use of concrete and glass materials lead to the degradation of the environment for plants growth. Dense buildings and limited space for root growth (Jim, 2019) and upper branching result in unfavorable factors, enhancing plant stress. Basic requirements for plants, such as soil, rainwater supply, air, and light, are significantly modified in urban areas compared to rural conditions (Czaja et al., 2020). In an urban environment, longevity and growth of plants are decreased, as well as their tolerance to stress as drought, cold, pests, and diseases (Tubby & Webber, 2010). Additionally, in recent decades, urban trees have come under increased threat as a result of aging in the majority of main European cities (Pauleit et al., 2005) and damage by invasive pests and diseases (Paap et al., 2017; Santini et al., 2018).

Plant pathogens and pests have always posed a challenge to plant growth, notably for trees. The severity of threat caused by these pests and pathogens varies from weak and mild symptoms to massive rapid plant mortality, depending on the aggressiveness of the pathogen or pests, host resistance, environmental conditions, duration of infection, and other factors (Paap et al., 2017; Panzavolta et al., 2021).

The origin, as native distribution range of ornamental, urban trees is important when considering their health status and vulnerability. According to Pyšek et al. (2004), native species are species that have evolved in a given area or that arrived there by natural means from an

area where they are (or were) native. Introduced, non-native species in a given area are those whose presence is a consequence of intentional or accidental introduction due to human activity (Gassmann & Weber, 2006). Initially, non-native tree species were mainly introduced out of curiosity and for ornamental purposes, and hundreds of tree species have been introduced to Europe over the last few hundred years as a result of international trade of plants, mainly ornamental (Liebhold et al., 2012; Santini et al., 2018). Due to their limited distribution, their health status is different from the native ones. According to the enemy release hypothesis (Keane, 2002), non-native trees may be more vital because their natural enemies are absent and thus give them competitive advantages. But also, a lack of adaptation to European pests and pathogens could pose a high risk of attack for non-native trees. Due to the lacking long-term co-evolution of the host and pests, particular traits related to plant defense may cause different responses of pests and pathogens compared to native trees (Pötzelsberger et al., 2021).

Invasive pathogens are those introduced to an area where they were not known to occur and where they "have overcome a series of barriers to be able to spread into novel areas in which they become dominant" (Valéry et al., 2008). More than half of the emerging infectious plant diseases in the world over the last few decades have resulted from the arrival of previously unrecognized pathogens (Bandyopadhyay & Frederiksen, 1999), and the risk of plant disease outbreaks caused by alien invasive pathogens is rapidly increasing with the intensification of international trade (Eschen et al., 2017). The majority of trees are

especially vulnerable to invasions of alien pests (Santini et al., 2018). Alien invasive species negatively impact the forest sector in economic, ecological, environmental, social, and health terms (Aukema et al., 2011). Invasions by plant pathogens and pests are considered one of the main threats, not only to tree species and ecosystems but also to biodiversity as a whole. In stressed urban environments, both native and non-native urban trees become more predisposed and vulnerable to pests and pathogen attacks.

It is worth mentioning that non-native ornamental trees can usually serve as a tool for introducing forest pests by international and inter-regional trade of plants for planting or plant propagation material. Thus, it is very important to monitor the spread of alien plant pathogens and pests, predict their potential ranges and spread dynamics, and elaborate measures to mitigate the negative ecological and economic consequences they can cause (Eschen et al., 2017; Santini et al., 2018). Efforts aimed at protecting plants against pests and pathogens often depend on our knowledge of the identification and ecology of the pests and their potential impact. That is particularly true for alien pests, which are occasionally introduced with, for example, the intercontinental trade in living plants or seed exchange between botanical gardens (Eschen et al., 2017).

A plant health policy aims to safeguard and improve the health and quality of commercially produced plants and plant products. In the epoch of intensive and unstoppable international trade, the protection of plants requires continuous caution and efforts to prevent the

accidental introduction of alien pests or pathogens. International phytosanitary agreements were initiated for this purpose. Within the Euro-Mediterranean region, the European Plant Protection Organization (EPPO) was established in 1951 (URL-1), requiring strict controls of traded goods. Many biosecurity protocols for plant protection measures have been developed and set up nowadays. Their purpose is to prevent the introduction and spread of harmful, non-native plant pathogens and pests in new regions, as well as to assist in their eradication (Luchi et al., 2020). They also imply the implementation and enforcement of knowledge of the phytosanitary status of a country through monitoring and control. Quarantine measures are established on a national level by government authorities, particularly by National Plant Protection Organizations. About 250 non-present and restricted distribution plant pests and pathogens in Europe are regulated (Anonymous, 2016) on the basis of specific pest risk analyses (A1 list - pests regulated as quarantine pests (URL-2); A2 list- pests locally present in the EPPO region, and hence still regulated as quarantine pests (URL-3); and Alert List - with a purpose to draw the attention on certain pests possibly presenting risk and achieve early warning (URL-4).

Regulations apply to pests or pathogens included in the list, while all other consignments not included in the list can be introduced without any limitation.

The recent history of invasions has revealed the difficulties in stopping the spread of invasive species at the borders, and minimizing the intensity of international plant turnover and transportation is of great

importance. The best solution would be to prevent invasive species, and the most effective measures are early detection and eradication before they become widespread or problematic. However, the majority of them are detected years after establishment, which makes eradication nearly impossible, particularly in the case of pathogens.

The majority of pathogens and pests threatening ornamental trees nowadays, not only in open urban greeneries but also during their nursery cultivations, belong to invasive species. The combination of unfavorable environmental conditions and damage caused by pathogens and pests might escalate the tree health problems in the urban green spaces in the future, while it is becoming increasingly apparent that the new and existing plantings will need careful maintenance in the years to come.

An urban tree is defined by Roy et al. (2012) as "a woody perennial plant, growing in towns and cities". The definition includes the individual trees as well as trees occurring in stands, patches, and groups within publicly accessible green spaces. In the context of this publication, we consider "woody ornamentals" mainly the same. Our intention is to review a selection of important invasive pathogens and pests associated with noteworthy woody ornamentals, primarily on urban trees at the European scale. Therefore, we will provide information about invasive pests and diseases on woody ornamental plants in Europe, aiming to maximize our chances of finding and eradicating an invasive pest before it grows and spreads. We provide examples from Southern and Eastern Europe, the regions we come

from. This has already been experienced, with harmful organisms moving from these regions toward the North (passing through central Europe). This pattern has been partly, but not exclusively, driven by global warming (Woods, et al., 2016).

1. INVASIVE PATHOGENS AND PESTS ON WOODY ORNAMENTALS

According to existing European databases (Rice, 2008; Santini et al., 2013; Roques et al., 2017), our experience and analysis of scientific papers, about two hundred pathogens of trees were preliminarily determined as the most important. It was considered that the species from the genera *Pinus*, *Picea*, *Abies*, and *Larix* were the most vulnerable among conifers, while the highest number of different invasive pathogens were determined in deciduous trees of *Quercus*, *Fagus sylvatica*, *Alnus*, *Populus*, and *Salix*. Besides, due to their wide prevalence in urban zones all over Europe, woody ornamental from cypress family - *Juniperus* spp., *Thuja occidentalis*, *Chamaecyparis nootkatensis*, *C. lawsoniana*, *Cupressus* spp. as well as *Platanus*, *Juglans nigra*, and *J. cinerea*, were detected as potentially important reservoirs for transfer of invasive pathogens.

1.1. Pathogens from the Family Botriosphaeriaceae on Evergreen Broadleaves, Conifers, and Other Hosts

Fungi from the family Botryosphaeriaceae (Dothideomycetes, Ascomycota) are well-known and important pathogens of fruit and forest trees (Slippers et al., 2017), but several species of Botriosphaeriaceae have also been found causing disease on ornamental trees (Zlatković et al., 2016; 2017; 2018; 2019a; 2019b). They are

considered stress-associated pathogens, acting as entophytes that infect healthy tissue of woody plants and remain dormant until the onset of stress conditions, when they can contribute to tree death (Kovač et al., 2021). The incidence of diseases caused by Botryosphaeriaceae species appears to have increased in Europe in recent decades due to climate changes (Piškur et al., 2011), and ornamental plants are additionally stressed by urban conditions. Species of Botriosphaeriace caused numerous external symptoms resulting in leaf spots and holes (Figure 1), necrotic lesions, canker on branches, resin bleeding (Figure 2, 3, 4) They are often associated with top dieback and death of whole trees (Figure 2.1, 2, 4), while internal symptoms on wood ranged from circular or irregular browning or reddening of the vascular tissue to brown vascular streaking visible as spots in cross-sections (Zlatković et al., 2016).

The Botryosphaeriaceae include a large number of phylogenetically closely related and morphologically similar cryptic species (Phillips et al., 2013), which makes morphological identification of species unreliable. Differentiation within the family Botriosphaeriaceae now relies on a combination of morphology and multiple gene sequence data (Phillips et al., 2013; Slippers et al., 2017). More than 10 species have recently been detected in an extensive study on ornamentals in the

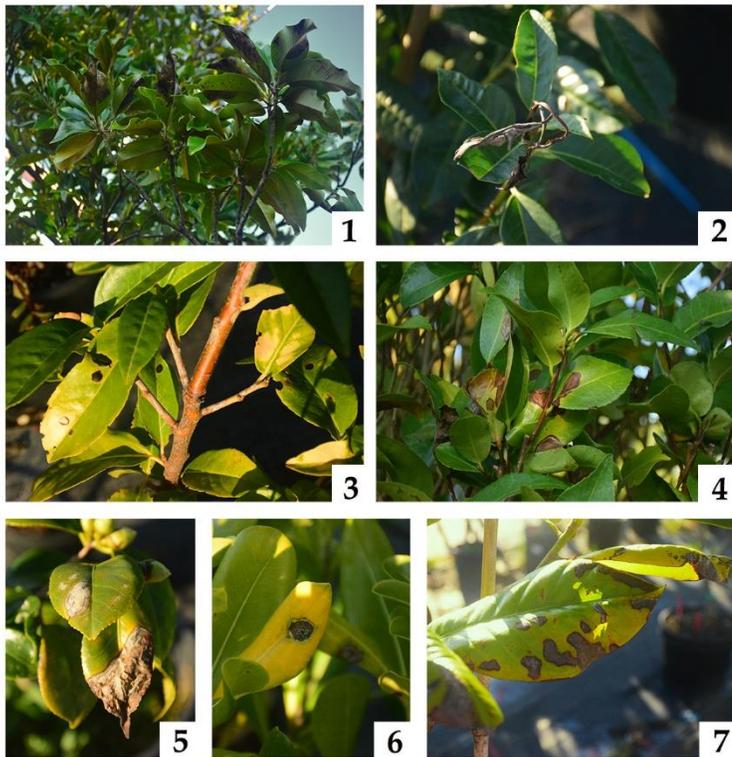


Figure 1: Symptoms of *Botriosphaeriaceae* Infections on Different Broadleaved Evergreens: *Magnolia grandiflora* (1, 7), *Prunus laurocerasus* (2, 3), *Camelia japonica* (4, 5), *Pittosporum tobira* (6) (Montenegro, Original by Lazarević)

Western Balkan Region (Zlatković et al., 2018). Also, the complex of *Botriosphaeriaceae* sp. could be present in plant material, acting differently, from endophytic to pathogenic. Fungi from *Botriosphaeriaceae* are widely distributed also in the Mediterranean and Submediterranean regions in Montenegro, where the disease can develop rapidly in wet and warm autumns and similar seasons. Host species range includes broadleaves, conifers, and evergreen broadleaves, and infection affects both old and recently established trees, hedges, and other ornamental plantings (Figure 3). Among many

Botryosphaeriaceae pathogens, *Botriosphaeria dothidea*, *Diplodia sapinea*, and *Neofussicocum parvum* could be considered the most frequently recorded species on different ornamental plantings.

Botryosphaeria dothidea is a generalist reported from numerous tree hosts worldwide (Hesse et al., 2017). It is considered more frequently associated with broadleaves (Zlatković et al., 2016), affecting even fruit tree species and fruit production (Phillips et al., 2013).

Diplodia sapinea is the most common conifer pathogen causing *Sphaeropsis* shoot blight or *Diplodia* tip blight and stem canker disease. It occurs in urban areas and natural forests of various ages in ornamental plantations in different continents. It has been present for a long time in South Europe (Milijašević, 2002; Luchi et al., 2014) with emerging distribution in North Europe (Adamson et al., 2015; Stenlid & Oliva, 2016; Brodde et al., 2019). This pathogen has recently also been isolated from *Cedrus atlantica*, *C. deodara*, and *Picea omorika* planted as ornamentals, as well as from *Abies*, *Chamaecyparis*, *Juniperus*, *Picea*, *Pseudotsuga*, and *Fagus sylvatica* in Serbia and neighboring countries (Zlatković et al., 2017; 2018).

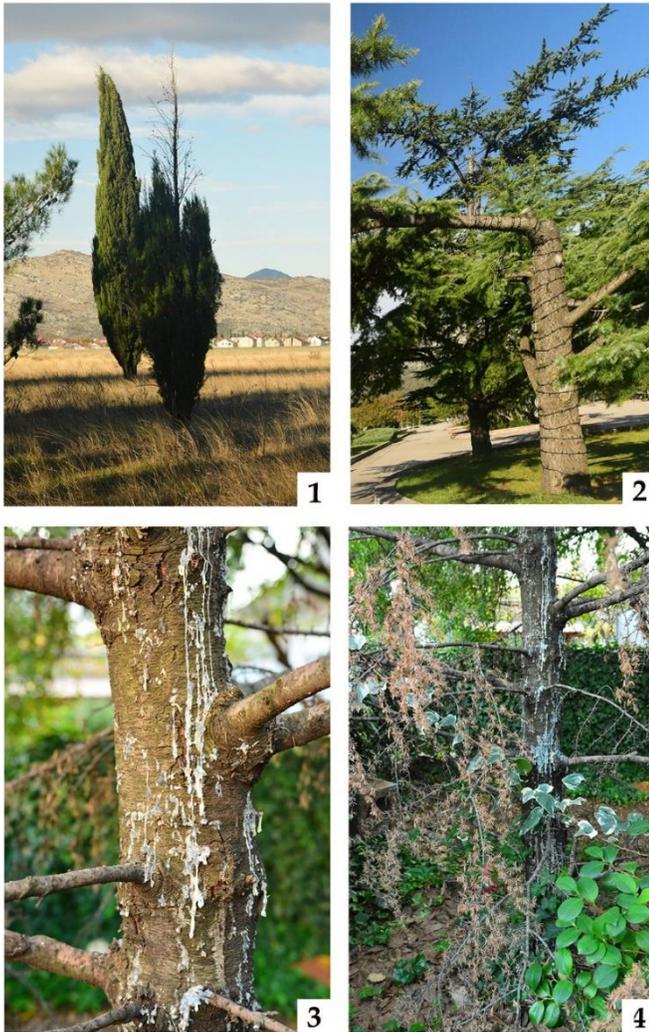


Figure 2: Top Dieback of *Cedrus atlantica* (1) and *Cupressus sempervirens* (2) in the Mediterranean Region (Montenegro); *Cedrus atlantica* Dieback (3), Typical Resin Bleeding in case of *Botrio spheriaceae* Infection (4) (Original by Lazarević)



Figure 3: *Abies concolor* Seedling (1, 3) Inoculated with *Botriosphaeria dothidea* Mycelium, 5 Days Old (2) Cause Fast Dieback. Symptom Developed 60 Days After Inoculation (Original by Lazarević).

Diplodia corticola is a canker pathogen of oaks (*Quercus* spp.), which has recently been reported from North America, but also from Portugal (Pintos Varela et al., 2011a), Italy, France, including Corsica (Linaldeddu et al., 2017), and Spain (Barradas et al., 2016). The fungus may enter branches through wounds and possibly natural openings. Asexual fruiting bodies are produced within 24 weeks and continue to produce spores for about two years. Trees affected by *Diplodia* have completely killed branches with all brown, dead leaves.

Neofussicocum parvum is a plurivorous and cosmopolitan species reported from six continents (Phillips et al., 2013). It seems well associated with evergreen broadleaves species, such as *Camelia japonica*, *Prunus laurocerasus*, *Pittosporum tobira*, *Rhododendron* spp, *Magnolia grandiflora*, as well as *Eucaliptus* sp., *Thuja pilicata*, *Sequoiadendron giganteum*, *Chamaecyparis lawsoniana*. A broad host

range is additionally expanded with *Populus* and *Salix* species, *Aesculus hippocastanum*, and *Ribes* (Pintos Varela et al., 2011b; Phillips et al., 2013; Mansilla et al., 2014; Zlatković et al., 2016; 2019b).

There are few data on preventive and protection measures against *Botryosphaeria* species on ornamental plants. Since Botryosphaeriaceae species are causing swelling and vascular wilt, and fungi penetrate the tree xylem, it is considered that chemical control in case of already established infections on urban trees is not efficient (Mehl et al., 2013) and also not applicable in cities due to the prohibition on the use chemical fungicides in many EU countries. Chemical control is possible in ornamental nurseries, especially in the case of leaf infection, where the experiences from agricultural crops could be applied (Sosnowski & Mundy, 2019).

1.2. Dutch Elm Disease and Other Invasions on Elms

(*Ulmus* spp.)

Due to their resistance to harsh conditions and very good recovery capacity from mechanical damages, elms were considered an ideal tree for urban environments (Buiteveld et al., 2015). Unfortunately, they were affected by Dutch elm disease (DED), which during the last 100 years has destroyed billions of elm trees worldwide (Santini & Faccoli 2015). DED is a lethal vascular wilt disease caused by three distinct fungal pathogens from the genus *Ophiostoma* (Ascomycota): less virulent *O. ulmi*, highly virulent *O. novo-ulmi*, as well as *O. himal-ulmi*, endemic to the western Himalayas (Menkis et al., 2016). These fungi

infect the bark and xylem tissue of elm trees, which exhibit symptoms of leaf wilting and yellowing on branches and twigs that the bark beetles have colonized (Figure 4.1, 2). Further, symptoms of brown streaking that runs in the direction of the grain of the wood and tylosis formation by the tree as a reaction to the fungal infection are typical of this disease (Figure 4.3, 4). Large elm bark beetle *Scolytus scolytus* and small elm bark beetle *S. multistriatus* are the most common and important insect species spreading the DED (Kirisits, 2013). Conidia are normally affixed to the bark beetle body surface and transmitted from the beetles into the tree (Menkis et al., 2016). *Scolytus* species usually oviposit in the phloem of stressed or weakened trees, and hatching larvae immediately construct larval galleries, which extend from the maternal gallery, and emerging young adults can infect new healthy trees during maturation, feeding, and mating. The decline of trees that have contracted the disease via root grafts is often much quicker because the whole tree is affected at the same time.



Figure 4: Elm Tree (*Ulmus* sp.) Wilting Caused by Ophiostomatoid Fungi (3, 4), Brown Streaking in Xylem Tissue, As a Consequence of Tylose Formation Due to Fungal Infection (1, 2) (Original by Meshkova V.)

Since the first DED outbreaks, more than several decades ago, different methods to control DED have been applied with different efficiency (Postma & Goossen-van den Geijn, 2016). Today, treating valuable urban trees with the biocontrol product Dutch Trig®, with the fungus *Verticillium albo-atrum* strain WCS850 as an active substance, yields good results. As an elicitor of the elm tree self-defense mechanism against Dutch elm disease, this product successfully protected healthy elm trees. However, it is inefficient in the case of already infected trees or trees connected with diseased trees via root grafts (Postma & Goosen-van de Geijn, 2016; Hauer et al., 2020).

Elm zigzag sawfly *Aproceros leucopoda* is a pest of East Asian origin from the order *Hymenoptera*, whose larvae make a characteristic zigzag pattern on the elm leaves as they feed. In Europe, the larvae can completely defoliate native and non-native elms and may cause at least partial dieback. *A. leucopoda* has been present in Austria, Hungary, Poland, Romania, Slovakia, and Ukraine, at least since 2003, but has been reported also from Belgium, the Netherlands, and Germany (Blank et al., 2014). Its population is growing, but trying to predict the amount of damage it will do in different regions of Europe is difficult. In some areas of Europe, the sawfly has been reported to have caused severe (74-98%), or even complete defoliation, but in other countries, such as Bulgaria, defoliation rates appear to be much lower (1-2%) (Doychev, 2015; Zubrik et al., 2017). Field observations indicate that elms are infested independent of their age and site characteristics. This pest will likely spread into other regions of Europe. Monitoring of *A. leucopoda*

is required to assess future range extensions, its exacerbating effect on Dutch elm disease and also to find a suitable biocontrol agent (Tuffen, 2016). The impact of predators and parasitoids is largely unknown, with very few observations of predation on the sawfly's larvae or pupae. The parasitoid fauna associated with the sawfly is also poorly understood. Pesticide applications are likely to have significant effects on controlling the *A. leucopoda* larvae. Treated trees will almost certainly be re-infested, making repeat applications necessary. Therefore, the use of chemical control seems to be limited to the protection of individual elm trees or groups of amenity or cultural importance.

Erysiphe kenjiana (Erysiphales, Ascomycota), as an example of powdery mildew of an Asian origin, is parasitizing on elm trees' leaves. It was detected in Ukraine in 2007 (Heluta et al., 2009), and 10 years later in Romania on *Ulmus pumila* cultivated in the cities as an ornamental and shade tree (Claudiu Chinan, 2019). In recent decades, the number of powdery mildew species in Europe has increased due to the introduction of many invasive species from eastern Asia or North America. In addition to *Erysiphe kenjiana*, other invasive powdery mildews include East Asian *Erysiphe (Microsphaera) palczewskii*, *E. (M.) syringae-japonicae*, *E. (M.) vanbruntiana*, *E. (Uncinula) arcuata*, and North American *E. (M.) azaleae*, *E. (M.) elevate*, *E. (U.) flexuosa* that have been recorded in many European countries (Heluta et al., 2009).

1.3. Ash Dieback and Recent Insect Invasions on Ash (*Fraxinus* spp.)

Two invasive organisms of East Asian origin have threatened European ash forests and urban plantings in recent decades. They are the fungus *Hymenoscyphus fraxineus*, established in 1996 in Poland, and as of recently, the insect pest emerald ash borer (EAB), *Agrilus planipennis*. They both show high aggressiveness to the host and pose a serious threat to ash trees in Europe and North America, indirectly affecting nature and the economy.

Hymenoscyphus fraxineus has spread rapidly across Europe, affecting mostly *Fraxinus excelsior* stands in many European countries and causing massive ash decline (Enderle et al., 2017; Langer, 2017; Davydenko & Meshkova, 2017), known as ash dieback. Other *Fraxinus* species are currently under threat as well. The symptoms of ash decline include quick crown dieback, leaf and stem necrosis, discoloration of wood and leaves, premature leaf-shedding, and similar symptoms (Figure 5). Following the infection, the fungus grows inside the tree, blocks its water transport systems, and causes dieback after repeated, year-on-year infections. The fungus overwinters in leaf litter, producing fruiting bodies and releasing spores responsible for new infections. It was shown that infected urban or veteran ash trees must not be felled because they can provide many important environmental and social benefits. These trees allow the maintaining of the biodiversity of species associated with ash in the green zones of settlements, but pruning of damaged and dead branches and twigs will help to maintain the ornamentality of these trees (Marciulyniene et al., 2017). For future ash

planting, well-selected material from resistant or highly tolerant individuals from native populations needs to be chosen. Resistant or highly tolerant individuals have been reported from Danish, Swedish, and German ash provenances (Metzler, 2012; Enderle et al., 2017).

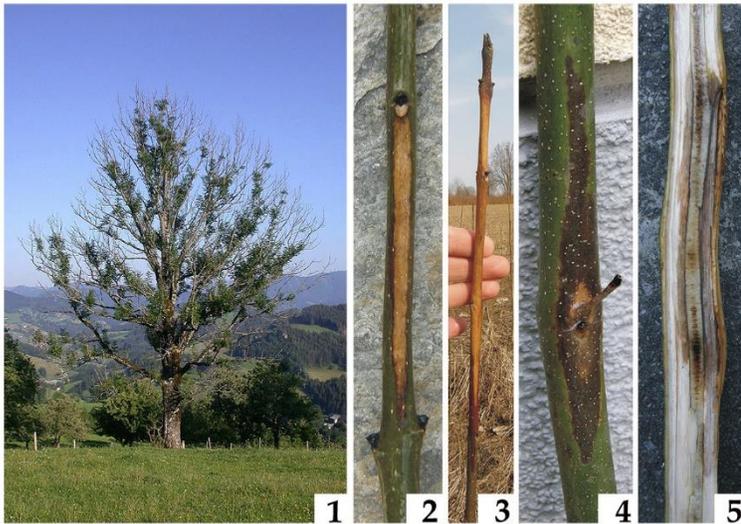


Figure 5: Ash (*Fraxinus excelsior*) Decline Syndrome (1). Appearance of Symptoms on Infected Twigs (2-5), Due to *Hymenoscyphus fraxineus* Infection (Kirisits et al., 2012)

For detection and monitoring of *H. fraxineus*, species specific polymerase chain reaction (PCR) method ITS primers were developed (Johansson et al., 2010), as well as real time PCR tests (Chandelier et al., 2014; Grosdidier et al., 2017).

It has also been indicated that collar and root rots drastically increase the process of ash decline and reduce the stability of the trees, resulting in tree hazards. A previous study demonstrated that collar rot could develop for a long time without any crown symptoms and wilting leaves (Langer, 2017). They are caused mainly by different fungal pathogens

or wood decay fungi as well as *H. fraxineus* itself (Enderle et al., 2017; Langer, 2017). Hence, more detailed and careful monitoring is needed in urban zones.

Agrilus planipennis (Coleoptera, Buprestidae), the emerald ash borer (EAB), is primarily a pest of ash. EAB invasion was first found in the US and Canada in 2002 (Haack et al., 2002), where it was able to infest all 16 known species of ash trees, causing widespread tree mortality, and spreading rapidly through the continent (Herms & McCullough, 2014). It is considered responsible for the destruction of approximately 150-200 million ash trees in the USA until now, and the number keeps growing (Sadof et al., 2017). The damage caused by the destruction of ash trees was estimated at hundreds of millions of dollars (Kovacs et al., 2011). EAB is included in the A1 EPPO list, banning its introduction into the European countries.

The current situation with EAB in European Russia is quite similar to the situation in North America. EAB was recorded in the Moscow region in 2003 (Izhevskii & Mozolevskaya, 2010), and during 2005-2019 EAB spread toward the west (URL-5). The current known EAB range in European Russia is close to the borders of Belarus, and EAB has already been found in Ukraine (Drogvalenko et al., 2019). Obviously, EAB can be expected to cross the western border of Ukraine and Russia and arrive at the EU countries soon.

1.4. Canker Stain of Plane, Plane (*Platanus x acerifolia*) Diseases and Pests

Ceratocystis platani is a destructive pathogen of plane trees (*Platanus* spp.), also known as canker stain of plane. It is considered to be native to the southeastern USA. Since its first detection in 1972, canker stain of plane has killed tens of thousands of plane trees in France, Greece, and Italy, both in natural stands and urban greenery (Ocasio-Morales et al., 2007). It is considered a lethal parasite with the potential to have major impact on plane trees and hence is regulated by the EPPO (A2 list). Recent findings cover Albania (Tsopelas et al., 2015), Turkey and Armenia (Lehtijärvi et al., 2017a).

Platanus species are the only known hosts of *C. platani*, which belongs to the *C. fimbriata* species complex. *P. orientalis* is considered highly susceptible to the fungus, while *P. occidentalis* has probably coevolved with the fungus and is, therefore, relatively resistant, while hybrid *P. acerifolia* is generally intermediate in resistance between its parents. It is widely planted in town and city streets, parks, public and private gardens in both urban and rural areas all over Europe, counting more than 120 000 trees only in London (Panconesi, 1999; Montecchio, 2016). *C. platani* is a wound parasite that rapidly infects plane trees, causing disruption of water movement, causing wilt, and canker on the tree trunk. Cankers are characterized by necrosis of inner bark and bluish-black to reddish-brown discoloration of sapwood. The disease can cause sudden death of a portion of the crown, and trees of 30–40 cm diameter may die within 2–3 years of infection.

The disease continues to spread into new areas in Europe, presenting a serious threat not only to plane trees themselves but with considerable aesthetical and ecological consequences for many urban areas where the plane trees are abundantly present. The movement of the pathogen into new regions is clearly associated with plants for planting. Following its establishment, it can spread locally by natural means, such as waterways, root anastomosis, insects, or by contaminated pruning tools. It can be eradicated when the infection does not occur close to water (Jeger et al., 2016). It is considered that there are no obvious climatic factors limiting the potential establishment and spread of *C. platani* in Europe if hosts are present, and more attention should be paid to the possible spread of *C. platani* into the Mediterranean, but also Central and Northern Europe.

For detection and monitoring of *C. platani* quantitative real-time polymer chain reaction (qPCR) test was developed, particularly valuable due to high diagnostic sensitivity and specificity (Luchi et al., 2013). Moreover, the Loop-mediated isothermal amplification (LAMP) technique is provided. LAMP involves the optical excitation and detection of a pathogen's DNA in an environmental sample; optimized for portable instruments applicable in the field, it can provide rapid detection (Aglietti et al., 2019).

Other pathogens are also involved in the dieback of plane trees in urban zones. As they are usually street trees, branch dieback poses a risk to public safety, and dead or damaged branches require timely pruning.

Splachnonema platani, also known as Massaria disease, has the potential to have a serious impact on plane trees. It is common in a warmer, Mediterranean climate that prevails in the southern USA (Tubby & Perez-Sierra, 2015). It was also recently reported from many European countries, such as Germany (Kehr, 2011), Austria (Cech et al., 2007), the Netherlands and the UK (Tubby & Perez-Sierra, 2015). *S. platani* is usually considered a weak pathogen only capable of causing minor twig cankers and damage such as natural pruning of small diameter twigs. However, the disease is favored by adverse climatic conditions such as summer drought and soil compaction (Kehr, 2011; Tubby & Perez-Sierra, 2015). It was found that the disease primarily affects the lower and middle-order branches of mature trees older than 40 years. It causes large lesions on the upper surfaces of major branches. Infected branches manifest bark and cambial death, affecting up to one-third of branch circumference. Lesions are associated with wood decay, characterized by soft rot, often resulting in fracture of the branch, which can happen within a few months of the first symptoms, but it more typically occurs after one or more years. Tree stems are not usually affected by *S. platani* disease.

Our research suggests that decay fungi such as *Inonotus hispidus* (Figure 6.1), *Perenniporia fraxinea* (Figure 6.2) might play a role in plane tree stem decline (Lazarević, 2004; Perić & Lazarević, 2006), as well as *Phellinus punctatus*, causing decay associated with the branch lesions.



Figure 6: Decay Fungi on *Platanus* Trunk: *Inonotus* sp. (1), Frequent Decay in the Upper Part of the Stem (Bar, Montenegro); *Perenniphoria fraxinea* (2) on the Bottom of the Stem (Belgrade, Serbia, Original by Lazarević)

Another serious problem for plane trees in parks and gardens is associated with the plane lace bug *Corythucha ciliata* (Hemiptera, Tingidae). Native to North America, *C. ciliata* has been spreading in Europe since 1964. Starting from Italy, southern and central Europe, it is now well established throughout Europe (Lakatos et al., 2021). It was also reported in China in the first decade of the 21st century (Yang et al., 2017). Nymphal and adult stages cause leaf damage by biting and sap sucking. Leaves become depigmented, leaden-gray in color, and fall prematurely. Three to five generations can develop per year.

From the same genus *Corythucha*, we indicate *C. arcuata*, oak lace bug (OLB). It is an insect pest of oak and several other broadleaves, including sweet chestnuts (*Castanea* species), maples (*Acer* spp.), hornbeam (*Carpinus betulus*), hazels (*Corylus* spp.), beeches (*Fagus* spp.), whitebeams (*Sorbus* spp.), limes (*Tilia* spp.) and elms (*Ulmus* spp.). Plants from the family Rosaceae are also susceptible, which

includes plants from the genus *Prunus* (as plums, cherries, peaches, nectarines, apricots, and almonds), then roses (*Rosa* spp.), and raspberries (*Rubus caesius*). Oak lace bug's native range is in North America. It was reported from France in 2017, and it became established in more than 20 countries in southern and central continental Europe (Paulin et al., 2020). Although OLB is not known to kill trees, its feeding damage could reduce growth rates and weaken oak trees, reducing their ornamentality. That could make oak trees more susceptible to other biotic threats, such as other pests and pathogens, abiotic threats, and drought. However, there is considerable variation in the reported impacts of *C. arcuata* in Europe. Population levels in Croatia and Hungary have reached levels that are causing concern to plant health authorities about the impact it might have on oak and other plants health. Oak lace bug is already present in several countries and hence is not regulated at the European level.

1.5. Invasive Pests and Pathogens of the Horse Chestnut (*Aesculus hippocastanum*)

Horse chestnut (*Aesculus hippocastanum*) is a valuable ornamental tree, widely used in urban greeneries across Europe. The horse chestnut leaf miner *Cameraria ohridella* (Lepidoptera, Gracillariidae) is an invasive pest that causes significant damage to the appearance of horse-chestnut trees. In Europe *C. ohridella* was first recorded in the territory of today's North Macedonia in 1985, and it had since spread throughout Europe by 2007. The pest seems to be quite monophagous, while its primary host plant is *A. hippocastanum*, other *Aesculus* species and varieties, and occasionally *Acer pseudoplatanus*, mostly when growing in the

immediate vicinity (Kopačka & Zemek, 2017). The larva feeds in a mine in the leaves of the tree, damaging the leaves and causing late summer browning (Figure 7). Chestnut miner has three generations per year (sometimes partial fourth) and overwinters in the puppet stage in fallen leaves (Kereši et al., 2016). Despite the poor appearance of infested trees, there is no evidence that damage by the moth leads to tree death. Seed weight, photosynthetic storage, and reproductive capacity may be reduced (Percival et al., 2011), but research in the UK shows that it does not affect stem radial growth or general tree condition (Straw & Williams, 2013). The situation could be different in the warmer and dry continental climate in the Pannonian plain in Serbia, where already in July and August, 95% drop in leaves were recorded in repeated seasons (Kereši et al., 2016). According to Kereši et al. (2016), damage caused by moths happens in June for the first moth generation, after 35-50 days for the second generation, and further. In case that horse chestnut is well-represented in urban greenery, its aesthetic and ecological role is considerably reduced.

Good results can be achieved in the control of *C. ohridella* by mechanical means, which include collecting and destroying fallen leaves, using sticky belts on the trunk for catching butterflies, then pheromones, and bioinsecticides on early leaves, as well as watering of horse chestnut trees.

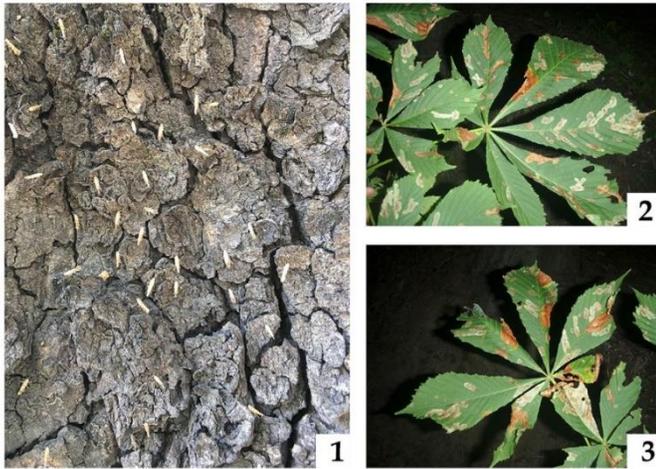


Figure 7: *Cameraria ohridella* on *Aesculus hippocastanum* 1. Imago (butterflies) on the Horse Chestnut Bark, 2. Leaves Damaged by Larvae Feed- “Mines” in the Leaves (Belrade, Serbia, Original by Milanović S.)

Regarding the health status of *A. hippocastanum*, more attention should be paid to invasive bacterial disease caused by *Pseudomonas syringae* **pv. aesculi**. Since the early 2000s, increasing numbers of declining and dying horse chestnut trees were recorded in the Northwest of Europe due to the presence of this pathogen. Affected trees show bark splits and bleeding cankers on the trunk, defoliation and general decline, and tree death can follow 2 or 3 years after the first symptom appearance. It was also evidenced that some trees may survive for many years due to slow disease progression, and some trees might even show signs of recovery (Green et al., 2013; Straw & Williams, 2013; Koskella et al., 2017).

Trees with bleeding cankers on the trunk can still have healthy-looking crowns, but also recently dead branches can be susceptible to sudden

fracture and drop. For public safety purposes, on moderately infected trees, branches that are infected and show dieback need to be removed.

Considering the significant tree mortality which has already been observed in north-western Europe, it is desirable to prevent any further spread of *P. syringae* pv. *aesculi*, and primarily avoid and stop the movement of plants for planting over long distances. Since there is a risk of transmission of *P. syringae* pv. *aesculi* in soil, the tools, boots, and vehicles should be cleaned after working with symptomatic trees. All this is a regular procedure and represents a common good practice we repeat here to increase awareness of its necessity.

Real-time PCR diagnostic tests were developed for *P. syringae* pv. *aesculi*. They facilitated its rapid detection in symptomatic trees and provided a useful tool for studying host infection and survival outside the host (Green et al., 2009).

1.6. Box Tree Moth and Boxwood (*Buxus sempervirens*) Related Diseases

Buxus is a popular and favorite ornamental shrub extensively used for hedges, borders, and topiary forms all over Europe. It is considered one of the most important ornamental plants cultivated in urban greenery, around important objects, and in historical gardens, but also in private facilities. The box tree moth *Cydalima perspectalis* (Lepidoptera, Crambidae) is an invasive pest of East Asian origin, associated with various species of *Buxus*. Box tree moth was first found in Germany in 2006, and by 2018 its distribution covered the whole of Europe (Plant et al., 2019). Its larvae feed on the leaves and shoots of box tree, and

can cause complete defoliation of the boxwood in a very short period of time. Commonly, there are two or three generations per year, while there might be four in the South European Mediterranean climate. It is widely reported in the literature as a pest of ornamental, planted *Buxus*, but increasingly also as a threat to native *Buxus*-dominated ecosystems across Europe (Plant et al., 2019). Substantial or complete defoliation and death of a boxwood plant in the native population were reported from Turkey, southern Russia, Georgia, Albania (Raineri et al., 2017), but also Germany and the UK (Plant et al., 2019). It was noted that box tree would be replaced by co-occurring tree species with faster growth rates, which would permanently alter the character of the woodland ecosystem.

In June 2019, *C. perspectalis* was recorded in *B. sempervirens* native population in central Montenegro (Municipality of Danilovgrad, Krasovine). The intensity of the attack was strong, and the boxwood suffered significant damage (Figure 8). In the following season, the plants were partly regenerated, and the new sprouting happened even following the rainy period during the late autumn and continued during the spring.

Larvae (stages 1 and 2) were recorded in feeding even at the beginning of March, during the *Buxus* flowering, which probably represents the first moth generation (Figure 9). Although many old trees died due to the invasion, some trees still produced the shoots. On the positive side, it was recorded that the *Buxus* population was not evenly infested and

that in one part, plants were in better condition and not seriously infested during the two seasons.

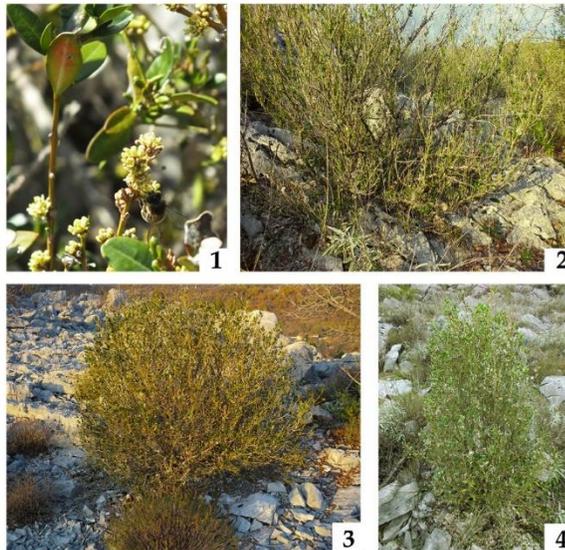


Figure 8: *Buxus sempervirens* in Native Population in Montenegro (Danilovgrad Krasovine) and Heavily Infested Shrubs of Different Ages, March 2020 (1) and December 2019 (2, 3, 4) (Original by Lazarević).



Figure 9: *Cydalima perspectalis* Larvae Feeds on *Buxus Sempervirens* Leaves and Flowers (1), Damages from Upper (2) and Down (3) Leaf Surface. Danilovgrad, March 10th 2020. (Original by Lazarević)

In the area of origin, *C. perspectalis* is regulated naturally by parasitoids and predators. However, it is considered that in Europe, the damage is severe because natural regulation does not occur at a significant level and that the larvae are toxic to most European predators (Wan et al., 2014). Pheromone traps and insecticides are commonly used in *Buxus*-infested areas to control the invasion. When properly applied, synthetic insecticides such as cypermethrin and deltamethrin are efficient, but they are suitable for private restricted areas. For broader application in open and public areas, biological pesticides based on *Bacillus thuringiensis* ssp. *kurstaki* were recommended as highly efficient, as well as natural pyrethrin insecticides (Wan et al., 2014).

Boxwood blight disease is the next significant concern for the ornamental horticulture industry and native ecosystems. The disease is caused by two closely related fungi *Calonectria pseudonaviculata* and *Calonectria henricotiae* (Le Blanc et al., 2018), which are also invasive alien species introduced and spread mainly via the trade of plants. Boxwood blight disease appeared in the mid-1990s on boxwood in the UK and spread throughout Europe. It causes leaf spots, stem cankers, defoliation, and the death of boxwoods (Castroagudín et al., 2020). The fungi can initiate infection from asexual conidia and form long-lived survival structures called microsclerotia (Le Blanc et al., 2018), and it is considered that climatic conditions may play a crucial role in disease epidemiology. The disease affects many commercial boxwood species and their varieties, as well natural populations of the host in Georgia and Turkey, where it causes severe outbreaks (Gorgiladze et al., 2011;

Lehtijärvi et al., 2017b). To prevent *Buxus* blight in urban plantings and native populations, it seems that only chemical control by fungicides could be efficient (Lehtijärvi et al., 2017b) but is not easy to apply due to environmental issues.

Multiple molecular diagnostic assays have been developed for the early detection and quantification of the causal pathogens of boxwood blight (Castroagudín et al., 2020).

1.7. *Dothistroma* Needle Blight and Other Invasive Pathogens on Pines (*Pinus* spp.)

Many different pine species represent a significant component of urban green areas, growing in parks, squares, and gardens. Among pathogens that affect them, *Dothistroma* needle blight (DNB), also known as red band needle blight, is one of the most serious and widespread (Barnes et al. 2014, Drenkhan et al., 2016). This disease has posed a major challenge for pines in the southern hemisphere for a long time but only recently has become more serious in Europe. The known host range of the disease has continuously expanded, and it was recorded also on trees of *Abies*, *Cedrus*, *Larix*, *Picea*, and *Pseudotsuga* (Mullett et al., 2021), which means that DNB affects more than 100 hosts from the family Pinaceae.

DNB is caused by two fungal species: *Dothistroma septosporum*, considered a cosmopolitan, and *Dothistroma pini*, which has a more restricted distribution. These two species are morphologically very similar, and molecular detection tools, which imply polymerase chain reaction (PCR) with species-specific primers, are needed to distinguish

between them (Ioos et al., 2010). DNB has been known in the Balkan Peninsula since the 1980s (Karadžić, 1989), where it was recognized as the main causal agent of the decline of *P. nigra* in forest plantations. The disease has increased in prevalence and severity in areas of Central and Northern Europe during the last decades, where country records also show that most DNB outbreaks occur on *Pinus nigra* and its subspecies. However, in the Mediterranean Region, highly susceptible to DNB are *P. brutia*, *P. pinea*, *P. halepensis*, and *P. pinaster*, as well as *P. mugo*, nowadays frequently present in urban greenery in continental regions (Drenkhan et al., 2016).

The fungus infects the needles, and typical red spots and bands, as well as reddish-brown needle ends develop on the previous year's or older needles, resulting in premature defoliation (Figure 10). If it continues for years, it gradually weakens the tree, causing a reduction in tree growth and in severe cases, tree death. Trees of all ages can become infected, and the infection level could vary in different years and locally (Lazarević et al., 2017). Combined infections with *Diplodia sapinea*, which appears on current-year needles and apical buds, can cause rapid tree decline and death (Milijašević, 2002).

Climate change has been suggested as one reason for the increased severity of the disease (Woods et al., 2016), which is reported nowadays even from high elevation pine forests in the Balkans (Lazarević et al., 2017). Also, it can be expected that with the change of environmental conditions sensitivity of pine species to DNB would be different, which was evident in more stressful habitats (Lazarević et al., 2017).

Unlike many other pathogens that overwinter in assimilation organs, DNB is not suited to life in fallen needles in the long term, and its survival rapidly declines once needles are shed from a tree, which can facilitate disease control. However, the fungi survive much longer in fallen needles caught in the canopy. It has been proved that pruning and thinning of the crowns have a very good effect as a disease control method (Bulman et al., 2016), which is in line with the existing practice of maintaining urban greenery. The removal of lower branches that tend to be most heavily infected results in the decrease of fungal inoculums and reduces the humidity, decreasing the infection level in the crowns. A copper fungicide spray program is appropriate for disease control in plant nurseries or in restricted, private areas (Bulman et al., 2016).

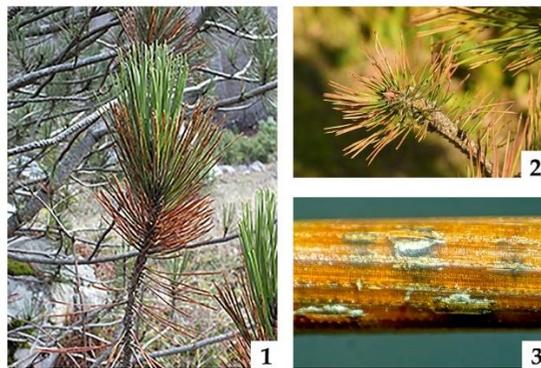


Figure 10: Dothistroma Needle Blight: Infection on Previous Year Needles, the Appearance of Twig During The Summer Months (1), *Pinus heldreichii*; Development of Symptoms and (2) Appearance of Twig During the Winter Months (December), *Pinus nigra*; Fruit Bodies on Heavily Infected Needle, Detail (3) (Montenegro, Original by Lazarević)

The first symptoms appear as needle necrosis, but subsequently, the damage spreads to twigs, branches, and eventually the whole tree. Currently, *C. ferruginosum* causes damages to *Pinus densiflora*, *P.*

thunbergii, and *P. koraiensis* in Korea (Ryu et al., 2018). It was reported from the Balkan Region, where the fungus appeared in epiphyte periodically, causing massive dieback and economic damages, once every several years (1957, 1962, 1987-88) (Jurc, 1986; Karadžić, 2010). A recent outbreak of *Cenangium*-dieback was reported in early spring 2012 from several regions of Slovakia, suggesting a wide geographical spread in the occurrence of the disease, which is alarming (Kunca & Leontovyč, 2013).

Pine pitch canker (PPC) disease, caused by the invasive fungus *Fusarium circinatum*, is one of the most devastating diseases of pines worldwide. *F. circinatum* is present in pine and Douglas fir (*Pseudotsuga* spp.) forests of all ages in the USA, Japan, Korea, and South Africa. Recent records of the fungus in Europe were from Spain, Portugal, France, and Italy (Drenkhan et al., 2020). For example, the fungus has been occasionally found in ornamental pine trees in Italy, in a garden in the Province of Foggia (Carlucci et al., 2007). PPC disease shows typical sunken cankers that produce abundant resin in branches and main stem. Although it was most likely subsequently eradicated (Vettraino et al., 2018), it is considered that the fungus threatens the sustainability of pine forests in South Europe, which is confirmed by climatic data and susceptible host distribution (Watt et al., 2011; Möykkynen et al., 2015). However, taking into account the high susceptibility of young pines to the disease and the ease of infection via seeds or seedlings, the threat of *F. circinatum* to the European pine species has no distribution limits, and the rest of European countries are

also at risk of infection. Global warming may affect the spread of fungus as well.

For detection and monitoring of *F. circinatum*, polymerase chain reaction (PCR), real-time quantitative PCR (qPCR), as well as techniques based on Loop-mediated isothermal amplification (LAMP) were developed (Aglietti et al., 2019; Stehlíková et al., 2020).

Bursaphelenchus xylophilus is a nematode species posing a serious threat to European conifer forests. Three main elements of the disease have been involved - the pinewood nematode (PWN) *Bursaphelenchus xylophilus*, the insect-vector *Monochamus* spp., and the host tree, mainly *Pinus* spp. (Vicente et al., 2012). Being native to North America, it spread in the early 20th century to Japan and in the latter half of the century to other areas of Asia, including China, Taiwan, and Korea (Hussain et al., 2021). It was first recorded in Europe in 2008-2009, in Spain and Portugal, where it is under an eradication regime (de la Fuente et al., 2018). Forests in Portugal have been considered particularly under threat, due to the fact that Portugal's main forest species is *P. pinaster*, susceptible to the nematode (de la Fuente et al., 2018). There, the pest is causing significant damage, despite stringent measures to bring it under control (Gruffudd et al., 2019).

The nematodes live and feed upon fungi (the mycophagous phase of the life cycle) rather than the wood itself, which takes place in dead or dying wood (Li et al., 2021). This small plant-parasitic nematode is capable of feeding on plant and fungal mycelium and pine nematode life cycle consists of four juvenile stages; in vivo pine nematode can complete its

life cycle, usually 4 days while in the laboratory it will be 4 - 6 days on isolated *Botrytis cinerea* Pers. (Vicente et al., 2012). In nature, it reproduces most rapidly in the summer, producing large numbers of individuals that spread throughout the resin canal system of susceptible pines, into the trunk, the branches, and the roots. The nematode must be transported outside the wood by an insect vector (Vicente et al., 2012); hence it is spread by bark beetles and wood borers. Most often, it is associated with beetles of the genus *Monochamus* (the pine sawyers) (Li et al., 2021). Pine sawyers larvae feed on the wood and pupates in wood cavities which become infested by juvenile stages of nematodes. Eventually, nematodes invade the trachea of the adult beetle, which transports the nematode to other trees (Li et al., 2021). The global spread of the nematode is particularly accelerated by exports of infested timber, but also by climate change and ecological disturbance. *B. xylophilus* attack trees of most coniferous genera, but especially pines (Gruffudd et al., 2019). Among native and common European pine species, *P. nigra*, *P. mugo* are considered highly susceptible, while *P. pinaster* and *P. sylvestris* belong to moderately susceptible species (Rodrigues, 2008).

Species of the genus *Bursaphelenchus* are difficult to distinguish because they are similar in morphology. Positive identification can be made with molecular analyses such as restriction fragment length polymorphism (RFLP) (Ribeiro et al., 2012; Vicente et al., 2012).

1.8. Invasive Longhorn Beetles (Cerambycidae) on Broadleaves

Longhorn beetles *Anoplophora chinensis* (black and white citrus longhorn) and *A. glabripennis* (Asian longhorned beetle) are highly destructive insect pests that pose a serious threat to a wide range of broadleaved forest and ornamental trees outside of their natural range in China and neighboring countries. They spread over long distances by plants for planting. Both species are listed as EPPO quarantine pests, included in the IUCN global invasive species database (URL-6).

Anoplophora chinensis feeds on over 100 hosts in at least 30 plant families, both broadleaved and coniferous. It seriously damages Citrus trees and harms apple, pear, and other fruit and nut trees, forestry plantations, and woody ornamentals. Its first finding in Europe was on natural vegetation in 2001 (Colombo & Limonta, 2001), although it is suggested that the pest may have been present since 1997. *A. chinensis* is now established in Italy, France, and Turkey, where efforts to eradicate it have been underway, and it is considered eradicated in the Netherlands and no longer present in the UK.

Anoplophora glabripennis, introduced in the USA in 1996, is now considered a species with the potential to become a major pest there. The first European invasive population was detected in Austria in 2001. Since then, populations have been detected in several European countries and Canada (Javal et al., 2019). It was considered eradicated in Austria, Belgium, Finland, Montenegro, the Netherlands, and the UK

and is no longer present in Russia. It is narrowly distributed and under eradication in France, Germany, and Italy (URL-7).

The beetle is polyphagous; it infests and can eventually kill hardwood trees in more than 15 plant families. Its preferential hosts include poplars (*Populus* spp.), willows (*Salix* spp.), maples (*Acer* spp.), birches (*Betula* spp.), elms (*Ulmus* spp.), and plane tree (*Platanus* spp.) (Sjöman et al., 2014), but *A. glabripennis* has never been found on conifers, nor apparently on oaks (*Quercus* spp.) and beech (*Fagus* spp.). In the native range, most populations are found in urban environments, mainly in green belts and in parks (Pajović et al., 2017; Javal et al., 2019).

Longhorn beetles adults feed on the tender bark of young twigs, and females damage the surface of the trunk and exposed roots when making slits for oviposition (Haack et al., 2010). The main damage is caused by larvae tunneling in the lower stem and roots of hosts. The adult beetles of the two species (*A. glabripennis* and *A. chinensis*) are very similar, about 20-40 mm long and shiny black with variable white markings (Haack et al., 2010). The most obvious symptoms of their presence are the circular huge exit holes made by the emerging adult beetles in the trunks and branches about 12 mm diameter. Other signs include sawdust at the base of infested trees, scraped bark, feeding damage on the bark of smaller branches and shoots, broken and dead branches, and oval 'grub holes' in the wood (Javal et al., 2019).

1.9. Invasive Aphids (Aphididae) on Coniferous

In recent decades, there has been an increasing number of alien aphid (Hemiptera, Aphididae) species in Europe on non-native ornamental plants (Havelka et al., 2020). It was reported that 102 invasive species of Aphididae have been introduced into Europe, making about 7% of the European aphid fauna (Petrović-Obradović et al., 2010).

Here we give an example of *Cinara tujaefilina*, developing on *Thujas* and *Cinara splendens*, recorded on ornamental Douglas fir (*Pseudotsuga menziesii*). Although they both represent minor pests, under certain climate conditions, with abundantly established colonies, they could affect the esthetical value and fitness of their reference hosts.

Cinara tujaefilina was reported as profusely present on cultivars of *Picea*, *Chamaecyparis* and *Thuja* (Kereši et al., 2016), as well as on *Cupressus* and *Juniperus* (except *J. communis*).

Aphids and larvae of anholocyclic species *C. tujaefilina* suck saps from leaves and twigs, causing dieback of branches or parts of the crown, thus reducing photosynthesis and host plant growth. As such, the appearance of *Thujas* and plenty of small conifers from the cypress family could be considerably reduced. Aphids tolerate low winter temperatures well unless the temperature drops below -10°C (Kereši et al., 2016). In the areas where the populations of *C. tujaefilina* had expanded, it demonstrated its adaptation for surviving colder winters (Durak et al., 2021), and it is further shown that *C. tujaefilina* adapts to

the cold winter conditions not only behaviorally but also physiologically (Durak et al., 2021).

Thus, the risk of massive occurrence of *C. tujaefilina* increases in warmer regions of South Europe and the Mediterranean, as well as in mild winters in continental Europe.

Successful survival was recorded also for another aphid, *Cinara splendens*, after the introduction from North America to Europe. *C. splendens* was strictly monophagous on *Pseudotsuga menziesi*, and research in the Czech Republic shows that its population density remained quite low in normal abiotic conditions, not causing damage to cultivated plants (Havelka et al., 2020). Higher population density of *C. splendens* occurred due to a synergistic effect of the dry weather on plants already infested by other pests, such as wooly conifer aphids *Gilletteella coweni*, a key pest on Douglas fir.

1.10. *Phytophthora* Species on Evergreen Broadleaves and in Ornamental Plant Nurseries

Species from the *Phytophthora* genus are fungi-like organisms within the kingdom Chromista (Stramenopiles) and SAR supergroup (Beakes et al., 2012) that inhabit the soil. Phytophthoras are able to lie dormant in the soil and survive unfavorable environmental conditions such as temperatures extremes, droughts, and absence of host for several years due to thick-walled and long resting structures they form. They are characterized by the so-called "multicyclic" nature, the persistence of the resting spores, high aggressiveness, and wide distribution.

Phytophthoras infect different tissues of roots, stems, shoots, leaves, and fruits of native and cultivated plants. They are considered the most notorious plant pathogens, which cause damages to plants in agriculture and forest nurseries, in the fields, forests, and urban greeneries. They present ecological and economic threats to forestry and biodiversity (Jung et al., 2016). There are around 150 described *Phytophthora* species, with possibly another 400–500 undescribed species awaiting discovery in under-researched ecosystems (Jung et al., 2021).

Phytophthora infections were recognized as an important factor in the oak decline phenomenon in forest ecosystems in Europe (Jung et al., 2018). They are usually linked with the other tree species growing on wet habitats and nearby water flows, such as *Alnus* and poplars (Brasier et al., 2004; Milenković et al., 2018), dispersing through forest soil and water flow. The pathogens present in rhizosphere soil causes damage to fine roots, while crown damage occurs as a consequence, often resulting in tree dieback.

The other important and widespread group of *Phytophthoras* is related to forest and ornamental plant nurseries, where they present common pathogens (Figure 11, Figure 12.1), causing root rot in wet, poorly drained nursery soils, affecting many different woody and herbaceous plant species (Jung et al., 2016). Some *Phytophthoras* are specialists, affecting only certain plant hosts and being inferior in the case of the others, such as *P. quercina* (Jung et al., 2018). Others, such as *Phytophthora citricola*, *P. plurivora*, *P. cactorum*, *P. cinnamomi*, *P. ramorum* are plurivorous, infecting plants from different genera and

families in forests, plantings, fields, and nurseries as well (Peterson et al., 2015). In addition to causing mortality, the disease can significantly reduce the survival rate of outplanted stock.

Phytophthora ramorum is capable of causing serious damage to a wide range of ornamental plants. *It* produces three types of disease symptoms: shoot dieback, when shoot or stem infections result in wilting and dieback of affected parts (Figure 11- 3,4; Figure 12- 2, 3); leaf blight, resulting in discolored lesions on leaves due to foliar infection (Figure 11-1, 2); and sudden oak death, characterized by lethal cankers with dark, reddish ooze on tree trunks.

In Europe, *P. ramorum* mainly causes leaf and twig blight. It was first described in Europe in 2001 (Werres et al., 2001). In oak forests of coastal California, it has been causing serious damage since 1995, while tree death may occur within several months to several years after initial infection (Rizzo et al., 2005).

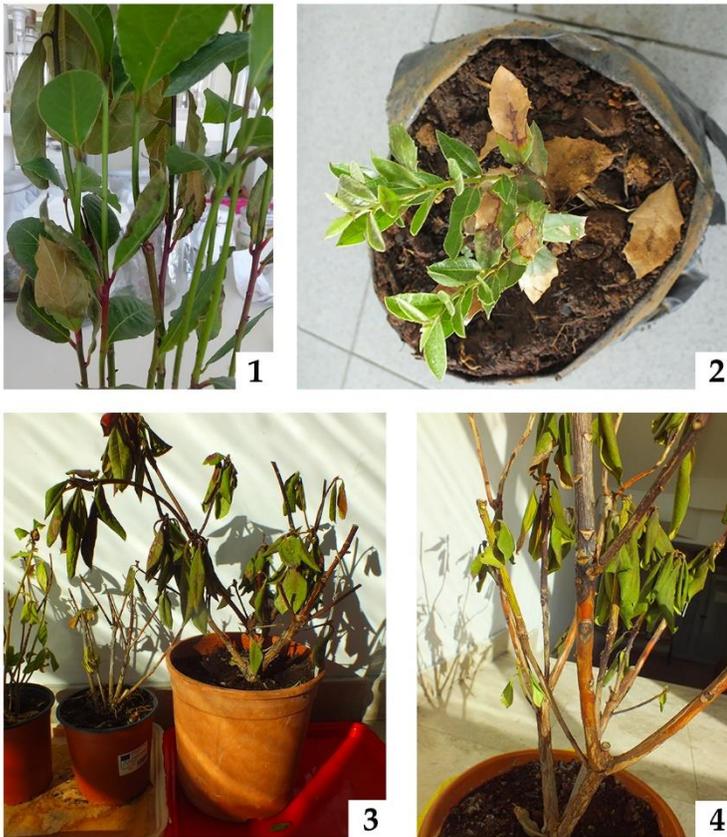


Figure 11: *Phytophthora* Dieback on Nursery Plants of *Laurus nobilis* (Imported Plants), *Quercus ilex* (Local Nursery Production), and *Rhododendron hybridum* (Imported) (Original by Lazarević)

Large and important components of European forests, heathland flora, and ornamental plantings are considered under threat from *P. ramorum*, and it is regulated within EPPO. *P. ramorum* host range includes hardwoods, softwoods, and herbaceous plants. It has been detected on plant species in over 70 genera representing 33 families, which can either be infected by *P. ramorum* or facilitate its spread. The host lists are being continually updated, but in Europe, *P. ramorum* is mainly

found in nurseries, plant markets, and already established urban greeneries, mainly on container-grown varieties of *Camellia*, *Rhododendron*, *Viburnum*, and *Pieris*. Among the more common hosts, *Arbutus*, *Camellia*, *Hamamelis*, *Kalmia*, *Leucothoe*, *Magnolia*, *Pseudotsuga*, *Sambucus*, *Syringa*, and *Taxus* are also listed. It has been reported from established *Rhododendron* plantings, principally in public and historic gardens and more rarely in unmanaged forest sites in the UK (DEFRA, 2008). Thus, the pathogen's wide and economically significant host range is of particular concern for nurseries and garden plant markets. Frequent movement of plants between different nursery sites, garden centers, landscape plantings, and home gardens enables its quick spreading in new areas.

Other *Phytophthora* species regulated by the EPPO include *Phytophthora kernoviae*, *Phytophthora lateralis*, as well as *P. fragariae* (on strawberries), and *P. rubi* (on raspberries).

P. kernoviae is currently recorded only in the UK, though considered to be of exotic origin. It appears to have a more limited host range than *P. ramorum*, but causes more serious disease on *Rhododendron*. It causes disease also on beech (*Fagus sylvatica*). *P. lateralis* is a virulent and aggressive pathogen that primarily affects *Chamaecyparis lawsoniana* and some other species of the cypress family (Cupressaceae), usually killing the trees it infects. Nowadays, it is present in the UK, the Republic of Ireland, the Netherlands, Belgium, France, the West Coast US states and Taiwan. Being connected with many varieties of *Chamaecyparis*, *Thuja*, and *Juniperus* widely used in parks and

gardens, it could pose a threat to the ornamental plant industry if it became widely established (Brasier et al., 2012).



Figure 12: *Phytophthora* Symptoms on Leaves of *Prunus laurocerasus* Cuttings (1) (Local Nursery Production); *P. ramorum* on Shoots of Imported Containerized *Taxus bacata* Seedlings (2, 3) (Original by Lazarević).

For fast and reliable detection, molecular tools have been developed for *P. ramorum*, based on Loop-mediated isothermal amplification (LAMP), Polymerase chain reaction (PCR), and Real-time quantitative PCR (qPCR) (Ioos et al., 2006; Aglietti et al., 2019; Migliorini et al., 2019); and also for *P. lateralis*, based on qPCR (Schenck et al., 2016).

It is considered that *Phytophthoras* root rot problem can be treated but not completely eradicated. Once contamination has been confirmed, the

infected plant should be removed and destroyed. One of the most effective ways to prevent *Phytophthora* is effective soil drainage.

CONCLUSION

SUSTAINABLE PRACTICE IN INVASIVE PATHOGEN AND PEST CONTROL ON WOODY ORNAMENTALS

Woody ornamentals in urban areas are threatened by many different pathogens and pests. Some of them, such as fungi from the family Botriospherioaceae, are generalists, infecting plants from different genera and families, while the others are mainly specialists, affecting only specific hosts and being inferior to the others. Outbreaks of destructive tree diseases caused by introduced pathogens and pests are increasing due to the growing international trade in plants. We are already facing health problems on many favorite tree species that we are accustomed to in urban areas, such as plane trees, ash, horse chestnut, bux tree, but also cedars, *Thujas*, and different cypresses, *Prunus laurocerasus*, and so on. It is important to be aware that the plant health situation is not static, and new or more severe attacks may occur for different reasons, being caused abiotically or as the consequence of pests and pathogens naturally spreading or introduced. Environmental changes will also provide a more favorable environment for many native and non-native, potentially harmful organisms.

Early detection of emerging threats is crucial for successful control since it offers the only realistic prospect of eradication of newly established pathogen and pest populations. For that purpose, increased monitoring is needed. Biosecurity protocols for plant protection and

new detection tools based on molecular techniques have been developed. Being more sensitive and rapid, they play an important role in plant health monitoring, surveillance, and quantitative pathogen risk assessment (Luchi et al., 2020).

It could also be considered that many organisms with the capacity to devastate certain plant species will most likely have a visible impact in urban areas, where they could be detected during the initial stages of establishment. This is highly significant not only for trees present in urban green spaces but also for forests, natural ecosystems, and the environment (Tubby & Webber, 2010).

On the scientific side, a lot remains to be learned about the dynamics of pest and pathogen impacts on non-native trees. But for the practice, it is important to raise public awareness about the risks associated with tree health problems and invasive species introductions. Knowledge transfer between the plant health specialists, nursery owners, persons involved in plants trade, decision-makers, local authorities, and the citizens should be facilitated.

Taking action to control pests and diseases is a very important but not always easily achievable goal. For diseases caused by pathogens that develop in inner tissues of stem and branches, as well as in soil, chemical control is almost impossible or impractical. Due to the negative impact on the environment in urban and open areas, the possibility of the use of chemical control measures is questionable also in the case of pests and diseases causing all the other types of damages. Biorational methods of pests and disease control could be a solution if

they are available and practical. The biorational methods include living organisms that can kill the pest, or naturally occurring biochemicals that are harmful to the pest, but often harmless to the other living organisms. Insect pests frequently have natural enemies, predators, or parasites, which could be used as their control agents without causing any risk to the rest of living nature. An example of a method using a naturally occurring biochemical is the bacterium *Bacillus thuringiensis*, already mentioned as a possible control agent for box moth, which contains a protein poisonous to specific insects yet harmless to the other organisms. Also, different types of pheromones have been developed for monitoring of insect population.

Considering prevention as the best strategy to protect plants from diseases, it is important to make an effort to reduce plant stress by regular watering during drought and periods of extremely high temperatures and, if possible, mulching the plants. Injuries caused by pruning or other unintentional mechanical injuries should be treated appropriately. In the case of pruning, tools need to be disinfected between the two uses. Dead shoots, branches, and trees must be removed and destroyed timely before producing fruit bodies or before insects emerge and the infection spreads.

As woody ornamentals represent an indispensable part of the green areas and the urban environments, efforts are needed to keep them healthy, as well as to ensure their longevity and survival.

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CHAPTER VII

ROOFTOP FRUIT GROWING AS A NATURE BASED SOLUTION TO MITIGATE THE CLIMATE CHANGE

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INTRODUCTION

Awareness of facing humanity with great global challenges (urbanization, climate change, food security) is a key step towards emphasizing the priority of environmental issues. The term globally refers to the need to solve problems at the global level, to confront the whole world with existing problems, and that the key to the solution lies in the global movement to raise awareness of the real size of the problem, and insufficient adequate solutions.

The demographic explosion accompanied by the seizure of large-scale territories from the treasures of nature in order to raise various types of facilities is considered the most important factor of climate change, it can accelerate problems such as pollution or extreme weather sensitivity. Worldwide urbanization led to an increase demand for new buildings, water, land, and energy (Felseghi, 2015). Urban growth, the density of which is continuing increasingly with the population increase that has taken place in urban areas in the recent years, leads to the vanishing of an extremely limited number of natural resources and to the occurrence of irregular and unsound urban areas, along with impairing the agricultural lands (Brueckner et al., 2001).

Urbanization, industrialization, unsustainable agriculture and the continued expansion of gray infrastructure are increasingly eroding our natural fabric and natural capital. Over the years landscapes have become more and more fragmented and polluted which in turn has disrupted the state of ecosystems and the patterns and level of biodiversity (Mazza et al., 2011).

In this context, green roofs represent an adequate measure based on nature, providing numerous environmental, social and economic benefits. However, it is important to mention the preconditions and obstacles of an economic nature, in the form of a large initial investment that requires large investments and thus limits green roofs to break into the market more easily.

Although they are becoming an increasingly present architectural feature in many cities, the history of green roofs indicates their existence since ancient times. The existence of Hobbit houses whose roofs are covered with grass indicates that the relationship between man and nature is not only epic fiction, but also the announcement of architectural styles that require the implementation of landscape structures on built buildings. The Hanging Gardens of Babylon, one of the seven wonders of the ancient world, was the first known green roof. As modern technologies have made green roofs cheaper and more efficient, modern green roofs began to appear in Germany in the 1960s. Since then, they have been praised as a smart urban response to climate change and spread around the world.

1. GREEN ROOF CONCEPT

Green roof concept can be most effective in urban areas where the lack of green-open spaces is a common phenomenon. Roofs are important components of buildings (Maier & Marusciac, 2011), accounting for nearly 20–25% of the overall urban surface area. Therefore, efficiently designed and integrated green roofs have great potential to affect the building and urban environments, replacing the lost green spaces and

habitats in modern cities. Specifically, green roofs are engineered roofing systems, planted with different kind of plants on the top of a growth medium (Cascone, 2018).



Figure 1: Green Roof without Plant Material (Down) and Green Roof with Plant Material (Up) (Original by Grubač)

The term "green roof" means a built-up area that is completely or partially covered with plant material (Figure 1). There are also terms: ecological roof, eco-roof, fifth facade, natural roof and vegetative roof.

Simply put, a green roof is one that encourages the growth of vegetation through several layers. A green roof component varies according to the location and requirements shown in Figure 2. Typically green roofs comprise following components, i.e; (1) Structural support, (2) Roofing membrane, (3) Membrane protection, (4) Insulation (Optional), (5)

Drainage layer/Water reservoir, (6) Growing medium, and (7) Vegetation. For long term environmental benefits, the selection of each layer according to the location and climatic conditions is very important. Every component of green roofs is very important and should select appropriate to achieve the optimum results.



Figure 2: Typical Cross Section of a Green Roof System (Original by Grubač)

They are designed not only to return the natural element to the urban environment, but also to provide solutions to important problems such as the urban island heat effect and atmospheric water treatment, as well as the growing need for food. Green roofs go a step further than important for modern architecture and add new value to the role of buildings in urban planning. In recent years, the application and development of green roofs has become increasingly popular in many countries, as a useful feature for sustainable construction technology (Dunnett & Kingsbury, 2008; Lockett, 2009; Weiler & Scholz-Barth, 2009).

1.1. Green Roof Policies Around the World

The global situation with a green roof, including policies and regulations (greenroofs.com, 2016): The EU recognizes that green infrastructure can make a significant contribution to achieving key policy goals related to sustainability (European Commission, 2013). This confirms that green infrastructure provides many social, economic and environmental aspects and benefits that are mainly related to the quality of urban areas (European Commission, 2016). Green infrastructure is considered a means to achieve EU goals and priorities, including Europe 2020 (European Commission, 2013). In northern European countries, governments and local authorities have invested more than thirty years and integrated green infrastructure into the planning system and put in place regulations to encourage the integration of green infrastructure into urban fabric to benefit from ecosystem services (Ngan, 2004). The EU encourages the integration of green infrastructure in urban areas in order to alleviate the problems resulting from urbanization and the current way of life. Localized flooding, reduced air quality, urban heat island and climate change are some of the issues facing the urban population, which affects the quality of life (European Commission, 2016; URL-1).

Green roofs have become an important addition to the urban fabric and are considered crucial in creating sustainable cities. Germany has the largest area of green roofs with a conservative estimate of about 86,000,000 m² of green roofs in 2004 and with an estimated growth of

8,000,000 m² per year. Figures in other countries may not be as high, but are expected to increase significantly (URL-2).

1.2. The Need for a Green Roof Policy

The EU considers green roofs an important addition to sustainable cities. They encourage their dissemination through high-level directives and policies. When a private individual builds a green roof, they also provide benefits to the public. These green roofs are of national interest and as such deserve intervention through policy, regulation, incentives and laws (Carter & Fowler, 2008).

Although green roofs provide a number of social benefits, it is primarily a private convenience that will entice owners to install green roofs. This highlights the need to implement a national scheme that encourages individuals to install green roofs and thus encourage the expansion of this infrastructure over a wider area. Green roofs will only benefit if the technology is widespread in the territory or district. In most industrialized countries, policies, incentives and regulations have been used to increase the number of roofs installed. In Germany, a country considered a leader in green roofs, the increase in the number of green roofs is attributed to legislation related to collective benefits. Relying on the good will of building owners to install green roofs has not been shown to be enough to bring about the desired change. Financial incentives and related policies are needed to fully understand the benefits that green roofs can provide (Ngan, 2004).

1.3. Challenges in the Implementation of Green Roofs

Adequate green roof design that can be applied in all locations and in all climatic conditions is one of the biggest challenges. To advance green technologies, performance needs to be better understood. For example, when it comes to warmer climates, it is necessary to choose plants for better green roof performance in the summer season. It is for these reasons that research challenges and problems related to green roofs must be further analyzed in order for the implementation of green roofs to be successful everywhere.

All green roof plants depend on water substrate, physical support and nutrients (Dunnett & Kingsbury, 2010). However, given the limited knowledge of the biological, chemical, structural and mechanical components of the green roof substrate / substrate and its performance as a living dynamic system, the consequence is an over-reliance of the green roof industry on a small selection of plants. In this regard, there is a need to investigate the water retention capacity, permeability and granulometric distribution of the substrate, as well as its biological quality, the use of indigenous plants of greater diversity. Green roof performance will influence long-term public acceptance (Maclvor & Landholm, 2011), and therefore the choice of appropriate plants and their longevity, vitality and decorativeness are important.

Extensive green roofs face harsh climate, for instance high solar radiation, limited precipitation and shallow growing substrate; therefore it limits the choices of plants (Li & Yeung, 2014). The aim is to enrich green constructions by exploring new indigenous plant species, which

have the potential to thrive under different conditions and to improve water retention capacity in the substrate to ensure greater amounts of available water while maintaining low substrate depth and mass. Small-sized vegetation would also ensure low maintenance costs, which is a valued feature for an extensive green roof.

Nowadays, green roofs studied and applied for the food production in many different countries (Whittinghill et al., 2013). Every country has their own objectives, i.e. (rainwater harvesting, disconnect roof drains and direct flows to the vegetated area, conveyance and stormwater art etc.) for the application of green roofs. Countries where the rainfall is abundant in the summer season, they usually applied green roofs to avoid flash flooding. On the other hand, a country, where the drought conditions the selection of green roofs and its components needs special consideration. There should select such a plant that not required watering and enhance water quality. Some countries are applying green roofs for water quality enhancement and reduction in temperature. Due to continued urbanization, many countries are looking the application green roofs for the irrigation purposes. Green roof components should select based on the following required objective (Shafique et al., 2018):

1. To reduce flash flooding;
2. To reduce surface temperature (as a result to reduce energy costs);
3. For food production;
4. For the collection of rainwater;
5. For aesthetic and ecological performance;

6. To improve the air quality and recreational activities.

2. URBAN AGRICULTURE IN ROOF GARDENS

Two different phenomena shape our planet: more than half of the world's human population is urbanized (World Watch Institute 2007); and climate change caused by global warming poses a serious threat. Modern cities, in ecological terms, have become parasitic "abysses" of energy and resources, spending 75% of the world's resources on only 2% of the global surface (TFPC, 1999). Urban expansion inevitably includes agricultural land, which has serious consequences in terms of possible food shortages in urban and rural areas.

In order to reduce the risk of food insecurity, it is necessary to rely on looking for new places or ways of food production. One proposal is to rely on the ability of cities to manage local food systems in an environmentally and socially sustainable way (Gittleman, 2009). Adopting urban agriculture that includes growing plants and raising animals for food near populated centers can help alleviate the problem of food safety (van Veenhuizen & Danso, 2007).

Because food-growing land is often limited in urban areas, the use of fruit and vegetable roofs can improve nutrition and food security in urban areas while reducing dependence on an energy-intensive global food economy (Gorgolewski et al., 2011; Whittinghill & Rowe, 2012; Rowe, 2012).

Urban agriculture includes food production located within cities or on urban outskirts, on terraces and balconies of apartments, spaces

between buildings, courtyards, roof terraces or facade fences (Viljoen et al., 2005). The definition used by the UNHABITAT Urban Management Program belongs to Mougeot (2000: 10), according to which urban agriculture is an activity (intraurban) located within or on the edge of a town, city or metropolis (periurban), which grows, processes and distributes various foods. and non-food products, uses mostly human and material resources, products and services that exist in the city or its vicinity, and in turn supplies the urban area with human and material resources, products and services.

Urban agriculture proves its importance not only through the provision of direct benefits (food), but also because of its ecosystem services (conservation of biodiversity). However, due to the small depth of the substrate, and the limited weight, as well as unfavorable environmental conditions, production can be difficult. Ornamental extensive roof gardens covered with succulent plants, various types of herbaceous perennials and grasses, require that the organic matter is less than 20% of the original substrate mixture. In roof agriculture, maximizing growth and yield is a key goal, while the amount of organic matter as part of the substrate is the main factor.

By installing edible roof gardens or growing them on suitable buildings, it is possible to promote more useful and significant functions for green roofs (Canadian CED Network, 2007; ARGP, 2008). Compared to non-agricultural green roofs, rooftop agriculture has other advantages, different design requirements and implementation considerations. In

short, agricultural green roofs are designed for four main purposes (Brown & Carter, 2003):

1. Food production
2. Active recreation
3. Reuse of waste (compost, atmospheric water)
4. Educational opportunities.

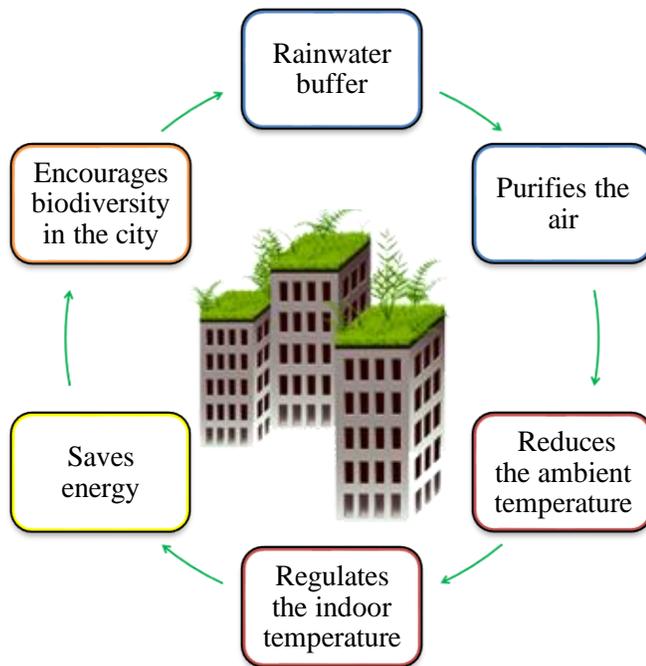


Figure 3: The Main Benefits of Green Roof (Original by Grubač)

Roofs are currently untapped resources and a package of appropriate design, development incentives and public support is crucial to explore their full potential (Bay Localize, 2007). In recent years, environmental concerns have been combined with increased interest in health and

community building issues, leading to support for food systems as an integral part of sustainable development. To achieve a green and productive city, growing food closer to home can help build awareness of and appreciation for food production (van Veenhuizen, 2006). Figure 3 shows how green roofs can contribute to the sustainability of the urban environment.

2.1. Vegetation

The part of the roof garden that completes the life of the green roof is the layer of vegetation. In general, any plant species can be planted on a green roof, provided that it meets the criteria that prevail on that roof. It is important to note that the roofs of various types of buildings do not represent natural places for plant growth, and what must be taken into account when choosing dendro material are primarily the statics of the building, previously assessed by experts, then the thickness of the substrate and nutrient substrate, the climatic conditions prevailing in the area, and the budget and design required by investors that vary from situation to situation. A very important item for vegetation is maintaining plant performance, and the presence of essential nutrients in growth media. Generally speaking, extensive roof gardens use plants that have a shallow root system, which allows them to survive in a thin layer of substrate, while intensive roof gardens due to the greater depth of the substrate provide a wide range of plant material, which means plants located on conventional green areas (trees, fruits, vegetables, shrubs). In addition to usable functions, as well as aesthetic ones, the

vegetation layer plays a significant role in atmospheric water management and thermoregulation of buildings.

Due to the large number of restrictive conditions on roofs, the optimal vegetation/plants for extensive green roofs must have the following characteristics (Shafique et al., 2018):

1. Ability to withstand drought and extreme climate conditions;
2. Easily available and cost effective;
3. No need irrigations regularly;
4. Short and soft roots;
5. Have ability to survive under minimal nutrients conditions;
6. Less maintenance;
7. More evapotranspiration;
8. Can reduce heat island phenomena;
9. Rapid multiplications.

2.2. Nutrient Substrate

Nutrient substrate is not a classic soil that can be used for some other types of green areas. Substrate is critical to the performance of green roofs since plant growth and health directly depends on the substrate (Ampin et al., 2010). It has the specific composition and requires improved structure and nutrients in accordance with the conditions and the choice of dendro materials. For this reason, the composition of the nutrient substrate varies from situation to situation, and it is of utmost importance to be in compliance with the conditions in which it is used

to make green roof vital as long as possible and to minimize the possibility of deterioration of the investment (Penonić & Lazić, 2016).

There are two general types of green roof substrates, commercial (developed by green roof companies) and non-commercial (developed by individuals or research institutions) (Ampin et al., 2010). Non-commercial mixes are usually based on basic guidelines given in the Landscape development and landscaping research society (FLL), however commercial mixes are often confidential and therefore not widely known (Emilsson & Rolf, 2005). Despite this, most green roof substrates are specified to vary little in their physical characteristics as they all need to be lightweight, stable, well aerated, free draining and able to retain nutrients (Friedrich, 2008).

The substrate has to perform the role of an artificial soil for plant growth and therefore must provide moisture, nutrients and physical support to plants, whilst also being lightweight, chemically stable, aeratable, and free draining (Friedrich, 2008; Ampin et al., 2010).

3. FRUIT ROOFS

Fruit roofs include the use of fruit species on roofs, as a technology that helps produce food in urban areas, while contributing to cleaner air and increasing biodiversity. Such systems consist of fruit species, growing medium and waterproofing membrane, which covers the traditional roof.

Installation of roof orchards is possible on various infrastructure facilities, with the statics of the facilities dictating which fruit species

can be planted (Figure 4). Extensive and intensive are the two most common categories of green roofs (Snodgrass & McIntyre, 2010).

Extensive green roof needs a growing medium between 50 and 150 mm to support plant life. This confines the size of plants that can be used on the roof, thus, confining the weight of the green roof on the building structure (Molineux et al., 2009; Bianchini & Hewage, 2012). Extensive green roofs have weight in saturated state 23 - 30 kg / m² and they involve the use of plants with shallow root systems, such as sedum, grasses, low-grass plants and medicinal plants, which do not require constant maintenance.



Figure 4: Roof Garden with an Example of Extensive and Intensive Type and the Use of Fruit Species (Original by Grubač)

The use of extensive roof gardens is much higher compared to other types due to their performance, low cost and independence from low

maintenance. When choosing fruit species, priority is given to species with a shallow root system (Figure 5), so that the containers with the substrate would not be too heavy (strawberry, currant and raspberry, blueberry..).

Semi-intensive roof gardens allow users to access and use a larger selection of plant species, and these are their advantages over extensive ones. They are characterized by a substrate thickness of 100 - 200 mm, their weight is less than 250 kg / m² in the saturated state, and require a concrete roof structure that can withstand heavy loads.

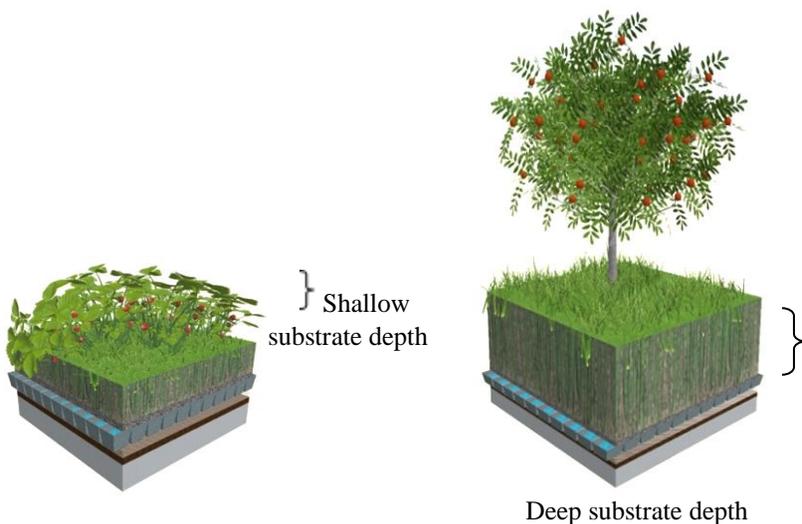


Figure 5: Shallow Substrate Depth (Left) and Deep Substrate Depth (Right)
(Original by Grubač)

Various plant material is used, lower ornamental trees, shrubs, annual or perennial flowers, grasses, and from fruit species strawberries, raspberries, blueberries, currants, columnar forms of apples, pears,

cherries, sour cherries, etc. Due to the diversity of plant cover, the realization of drainage and irrigation systems is necessary, and thus enables them to be resistant to higher atmospheric waters. This type of roof garden can be used for rest, growing fruits and vegetables, as stated.

Intensive green roofs are often designed as public places and generally require substrate depths between 150 - 200 mm. Therefore, they may comprise of trees and shrubs similar to landscaping found at ground level (Snodgrass & McIntyre, 2010). The name "intensive" indicates the more complex construction and structure of this type of roof garden, the wide possibility of use, and thus requires greater maintenance and higher costs. Intensive roofs have a surface load weight of about 1200 kg / m² not counting plants. When designing an intensive type of roof garden, we have the freedom to use almost any plant species, even in its natural size and shape (oaks, maples, pines, hedges and flower beds), and we can form smaller landscape structures. This type of roof garden offers the possibility of growing a wide range of fruit species with a deeper root system (Figure 4), which requires a large amount of substrate (apple, peach, apricot, kumquat, khaki, pomegranate, banana, citrus, etc.). Large concrete or wooden "beds" are often used, filled with enough soil for unhindered growth and development of the root system. Also, the intensive type requires the installation of an irrigation and drainage system, as well as constant maintenance.

As cities become overcrowded, green spaces per capita are far more needed. Therefore, a return to nature is inevitable, and urban gardens,

designed with edible ornamental and fruit species, recently forged "ornamental fruits" (Sahin, 2020), are a growing trend. Constantly exposed to a high intensity of noise, gases, surrounded by concrete and enclosed spaces, citizens strive to incorporate a part of nature into their everyday living space. As much as the space is limited, own cultivation of plants whose fruits will be picked and eaten brings joy and satisfaction to the citizens. According to Al-Mayahi et al. (2019), the main motives of gardening in Oman are aesthetics, shading, joy from hobbies, food source, physical exercises and environmental protection.

Many urban cities around the world are trying to improve sustainability by promoting urban greenery and promoting urban agriculture. By installing green roofs in urban agriculture, it is possible to achieve ecological, social and economic sustainability of buildings in urban cities, because it can contribute to mitigating environmental problems, improving community functions and developing urban food systems. An important means of responding to these challenges is to increase greenery by establishing roof and vertical gardens, especially if they are not just ornamental, but truly urban horticultural gardens that provide healthy and nutritious food. Urban horticulture provides a wide range of services, including the provision, regulation, support and cultural services. Among those who are first supplied, food and honey production appear, cultural would be recreational, educational and physical or mental well-being, while the most important habitat-related service is increasing biodiversity (Galluzzi et al., 2010).

The type of green area that can be built, in terms of size and content, primarily depends on the type of building, the load-bearing capacity of the building and its roof structure. Before establishing an orchard roof, it is necessary to calculate the weight of the entire material from which the garden will be built, and adjust it to the load-bearing capacity of the roof in order to avoid cracking of the roof structure or building walls. When calculating, it is necessary to take into account several specifics related to the perennial character of the orchard. The maximum load varies during one year due to the variation of precipitation and the retention potential of the substrate in which the plants are planted (organic and clay substrates retain water longer than sandy ones). Variation also occurs during the years as fruits grow and develop, as well as by changing the juvenile stage to reproductive (the fruits of most fruit species and the total yield are significantly higher than the mass of leaves and branches). The load carried by the roof structure is divided into two groups: fixed and movable. The fixed load includes the weight of the roof structure itself and all permanent elements that are part of that structure, elements for hydro and thermal insulation, but also mechanical equipment, such as insulation systems, and snow in colder regions. Moving load includes people, furniture, water and snow after precipitation, the influence of wind as well as all equipment that is temporarily on the roof, maintenance equipment and the like (Ognjanov, 2012).

3.1. Ways of Growing Fruit on the Roof

There are many ways of growing fruits, vegetables, various types of plants on the roof, which can be combined together if it is an intensive type of roof garden (Figure 6).



Figure 6: Different Ways of Growing Fruit on Intensive Green Roof
(Original by Grubač)

By introducing greenery on roofs, buildings and other facilities get a new look that, in addition to covering gray, provides numerous environmental benefits for cities. One of the solutions and important steps is vertical landscaping, which is implemented in order to ensure the use of the maximum potential of the garden space. With planning and adequate selection of materials, vertical gardens are formed for growing a wide range of plant material selections. There are various ways of vertical cultivation of plants, one of them is containers filled

with soil and seeds, and arranged in rows in relation to the vertical carrier (Figure 6). There are also shelves, scaffolding and various constructions with the help of which we form flat surfaces along the vertical axis at different intervals and add plant material or pots with plant material to them (Figure 7).



Figure 7: Example of Vertical Breeding of Strawberries (Original by Grubač)

If we have a smaller terrace, corners in the yard that crave a little liveliness or to grow vegetables and fruits in interesting ways, then a vertical garden is a fantastic solution. Replacing the garden with a horizontal system with a vertical one allows people to do less work and benefit more. It is also easier to maintain, there is no effort when picking fruits, the plants are healthier, have better sunshine, the yields are much higher, and the way of use is more interesting. Fruit species such as strawberries, raspberries, blackberries, have proven the possibility of planting in wooden boxes with support grates. Another name for crates with trellis is a trellis, which can be of different sizes and shapes

depending on the volume of growth of plant species and is an excellent solution for both vertical and horizontal cultivation in smaller spaces in urban areas (balconies, roof terraces, small gardens).

3.2. Advantages of Growing Fruits and Vegetables in Containers

Instead of covering the roofs with so-called "raised beds", which are quite difficult for the supporting structure of the roof, growing fruits and vegetables in containers or larger pots is a much easier, simpler and more economical solution. This method enables the cultivation of species with specific requirements according to the type of soil, and we provide suitable species for fruit species, thus increasing their chance for better and higher yields. A very important possibility is to move the pots, unlike the trees that are rooted in the ground, in this way (by rotating the pots), we provide the fruit species with a sufficient amount of light throughout the year. The orchard on the roofs emphasizes its specificity by the type of greenery - subtropical fruit, planted in pots, which is a movable load, is brought indoors in winter, and taken to the roof in summer.

The formation of miniature plantations represents a new and modern way of vertical cultivation of fruit species (Figure 8). With the help of the use of dwarf trees (minarets, pillars or super-pillars), we save space, the application is attractive and fruitful, perfect for growing in small gardens. Columnar fruit species are planted at a distance of 60 to 90 cm and are ideal for use in pots or jardinieres, on balconies, roof gardens.



Figure 8: Examples of Miniature Fruits: Citrus, Dwarf Plum, Dwarf Cherry
(Original by Grubač)

Due to the lack of available garden space in urban areas, the need for urban agriculture through the implementation of roof systems on which food will be produced is huge. In this regard, it is not necessary to strive for even growth of trees, as is the case in commercial fruit growing. This means that pruning is much freer, canopies are formed which, in addition to their decorativeness, provide a rich yield. This way of pruning creates columnar and dwarf forms of fruit trees that can be grown in various ways: in the form of trellis, palmettes with horizontal, oblique and upright branches, crowns in the shape of candlesticks, bows, various forms of reeds, spindles, vases and cordons.

CONCLUSION

Urbanization is one of the most important human factors that directly and indirectly affects the change of land use and as a result of the change of landscape patterns. However, does staying in an urban environment have to be a complete detachment from nature?

Research work on green roofs in response is a challenge and provides ample space for researchers for future research. This chapter provides an overview of the literature on the properties of green roofs, environmental, social and economic benefits, challenges, opportunities and potential applications of green roofs (among other things for food production). Nowadays, the use of roof gardens with the aim of improving the environment and the quality of life of residents in urban areas is becoming increasingly important. The combination of greening and agriculture is an important step in building an environmentally friendly society. Urban agriculture is considered a new concept of a sustainable city, in terms of creating numerous opportunities and benefits that it brings with it. By applying this approach, we help the city to become a richer, healthier and more fruitful place, and raise awareness of the need for its implementation in order to produce food in urban areas. It is important to point out that by doing so, we encourage the building of respect for the Earth and the environment in which we live, and on which we all depend. Roof gardens for food production (fruits, vegetables, aromatic, spicy and medicinal plants) provide us with many practical and significant ways to use empty spaces in urban areas and brings with it numerous economic and non-

economic benefits (contributing to mitigating environmental problems, improving community functions and development urban nutrition systems). A wide range of edible plants can be grown in containers on roofs, of course, depending on the type of roof garden, while respecting the conditions of that space and economic possibilities. Of the fruit species for all types of roof gardens, strawberries, raspberries, blackberries and currants stand out. These species bear abundant fruit and what sets them apart from other species is the possibility of surviving in containers in the open in the winter months. Intensive type of roof garden provides the possibility of using almost all types of fruit (pomegranate, kiwi, kumquat, banana, apple, cherry, sour cherry, etc.), of course with a multidisciplinary approach.

Interest in the application of urban agriculture on the roofs of buildings is growing around the world and many cities are trying to improve life in urban cities by promoting urban agriculture and urban greenery. It is important to point out the benefits of green roofs, ways and growing needs of their application nowadays. They should not be viewed only in the context of implementation by economically rich countries, but from the point of view of the possibility of becoming a tendency of all regions when planning a green system.

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CHAPTER VIII

DROUGHT STRESS IN FRUIT TREES – A REVIEW (MORFOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL BASIS OF TOLERANCE)

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INTRODUCTION

In the natural environment, plants are subjected to a variety of abiotic stresses that cause adverse effects on their growth, development, yield, and quality. These stressors are expected to increase in the near future as a result of global climate change, potentially affecting and limiting the yield of agricultural crops (Peters et al., 2013). According to data from the World Meteorological Organization (WMO), (2021) the main causes of global warming over the last 50 years have been greenhouse gases and an increase in carbon dioxide concentration. Although an increase in carbon dioxide concentration has a number of negative consequences, the most evident is an increase in global average temperature. National Oceanic and Atmospheric Administration (NOAA) reported that average global temperature has increased by about 0.8 °C over the past 100 years. It is estimated that the average global temperature in the coming decades will be 1.5 °C warmer than preindustrial levels (Masson-Delmotte et al., 2018). The consequences of increasing temperatures are the reduction of precipitation amounts and changing rainfall patterns, resulting in droughts all over the world (Sheffield & Wood, 2008). Due to climate change, agriculture is one of the most adversely affected activities (Gaspar et al., 2002; Raza et al., 2019). Therefore, in recent years, plant stress has become a common and popular topic in plant physiology and agronomy. Plant responses to abiotic stresses have been extensively studied during plants' vegetative and reproductive stages (Kumar, 2020; Ma et al., 2020). Plant stress can be divided into two main categories: abiotic stress caused by non-

living factors and biotic stress caused by other living organisms such as pathogens, herbivores, etc. (Boyer, 1982). According to Lichtenthaler (1996) abiotic stress refers to all environmental factors that negatively affect the physiological and metabolic processes of plants or inhibit a plant's growth or development. Plants evolved some resistance to stress during their evolution, such as specific physiological and metabolic mechanisms that allow them to adapt and survive in unfavorable environments (Hirayama & Shinozaki, 2010). Those plants which have the capacity to tolerate a particular stress or adjust and acclimate to the stress condition are considered to be stress-resistant plants. Some plants exhibit stress resistance or stress tolerance because of their genetic capacity to adjust or to acclimate to stress (Martínez-García et al., 2020). Aside from genetic predisposition, plant responses to stress vary according to the tissue or organ affected by the stress as well as developmental stage (Robinson & Alberts, 1986). Abiotic stress is also one of the major constraints to fruit production worldwide since many processes like vegetative growth, flowering, fruiting, and fruit quality are highly vulnerable to different abiotic stresses, particularly in arid and semiarid areas (Mattos et al., 2014). Every year, the quality and yields of fruit trees are seriously affected by abiotic stresses like drought, high or low temperatures, excess light, and salinity. As perennial crops, fruit trees are more exposed to these environmental stresses for a long time with slower recuperation. Reduced yield and slower growth are not only visible in the current year, but can also be seen in the following year. Among the environmental stresses, drought stress is one of the most adverse factors to the growth and productivity

of fruit plants in many orchards. Drought affects 45% of the world's agricultural lands, according to Bot et al. (2000). Although in many orchards fruit trees are irrigated, to achieved maximum yield and excellent fruit quality, fruit production could be compromised in near future. Due to climate change and higher temperatures, water availability is already changing and worldwide water resources may be insufficient for optimal irrigation and maximum yield (Fererres & Soriano, 2007). These changes also cause more frequent periods of drought, and fruit trees will be more frequently exposed to water shortages. Fruit species make adjustments to adverse environmental conditions by using a different mechanism for protection, including morfological, physiological, and anatomical adaptations, in order to adapt and minimize stress-induced damage (Abobatta, 2021). They use various mechanisms to survive and overcome water shortages, such as deeper root systems, reductions in leaf area, stomatal closure, osmotic adjustment, cell wall plasticity, and accumulation of different compatible solutes or osmolytes. Assessing fruit stress resistance to drought stress is crucial for the continuity and sustainability of fruit production. Because climate change has the potential to increase abiotic stress factors, it is important to understand how fruit production will be affected and how fruit species detect stress signals and adapt to adverse environmental conditions.

1. YIELD AND FRUIT QUALITY RESPONSES TO DROUGHT

Drought stress first of all affects the growth of fruit trees and further affects fruit yield and quality. It has been reported that the drought

directly decreases the yield of peach (Rahmati et al., 2015a) and pear (Marsal et al., 2008) due to a reduction in fruit size or number of fruits, as was also observed in mandarin trees (Romero et al., 2006). Also, yield could be reduced in the following season due to a reduction in fruit number, flower initiation, and return bloom (Lopez et al., 2014). Regarding fruit quality, insufficient irrigation mostly resulted in a reduction in fruit size (Naor et al., 1997; Perez-Pastor et al., 2014). Furthermore, the impact of water deficit on fruit yield and quality is not so simple, as moderate drought has been shown to be beneficial in improving fruit quality. An increase in total soluble solid (TSS) concentration under water constraint has been observed in several fruit species like peach (Faci et al., 2014) and pomegranate (Rad et al., 2015). Some authors noticed positive impact of water deficit on soluble sugar accumulation in fruits. They have reported individual sugar behaviors under drought stress conditions, such as higher concentrations of sorbitol (Wu et al., 2014), glucose, and especially fructose, which have a significant contribution to the improvement of apple fruit quality (Wang et al., 2019). Reduced irrigation has also been related to the enhanced acid content of peaches (Alcobendas et al., 2013). Lack of water increases the dry mass content of fruits by the reduction of water accumulation (Cui et al., 2009). Buendia et al. (2008) detected accumulation of phenolic content, mainly anthocyanins and procyanidins in peaches subjected to deficit irrigation. Drought deficit during the vegetative phase may affect the reproductive period and negatively affect yield but improve fruit quality. When oranges are exposed to drought stress, the increase in fruit quality was accompanied

by a decrease in yield (García-Tejero et al., 2010). Reid et al. (1996) reported that a mild drought stress during early kiwi fruit growth caused better fruit firmness during storage, but a decrease in fruit size.

2. MORPHOLOGICAL RESPONSES TO DROUGHT

Limited water supply affects the vegetative characteristics of trees, including height, the fresh and dry weight of the organs, the number and surface area of the leaves. Previous research has shown that drought stress has a greater effect on vegetative growth affecting vegetative characteristics by reducing trunk cross-sectional area and leaf area by more than 60% (Choi et al., 2020) and reducing shoot length and canopy volume in young sweet cherry trees (Livellara et al., 2011). Reduction in plant growth is one of the first responses to drought stress. Various studies have reported the negative impacts of drought stress on many fruit species like pear (Morandi et al., 2014), olive (Boughalleb & Hajlaoui, 2011), and cherry rootstock (Sivritepe et al., 2008). Plant morphological adaptations are one of the adaptive mechanisms under drought stress conditions. Tatari et al. (2019) reported that pear species with less sensitivity to drought stress exhibit less growth reduction under drought conditions. Drought caused a decrease in shoot elongation, leaf area, and shoot mortality in peach trees (Rahmati et al., 2015b). Roots, as the main water absorbing organ, are also seriously affected by drought. It has been shown in previous research that drought reduces root growth in many fruit species, including peaches (Abrisqueta et al., 2008). Also, drought can induce stress defense mechanisms in roots and some structural changes in root architecture.

Water scarcity may cause root adaptation to drought through greater root growth, smaller diameter roots, increased root hair density and length, to improve water uptake from the deep (Comas et al., 2013). A large root mass is advantageous during drought because of the greater contact between the roots and soil, which causes better extraction of available soil water (Wright et al., 2019). Romero et al. (2006) found that the root system of citrus rootstock "Cleopatra" is more effective at water uptake because of a higher root density and a deeper and more branched root system compared to "Carrizo" rootstock. One of the avoidance mechanisms is the reduction in leaf area to reduce water loss by transpiration and keep the leaves photosynthetically active (Dichio et al., 2002). Karimi et al. (2018) showed reduced leaf expansion as an adaptive response to reduce water loss in drought-stressed olive cultivars. Leaf rolling has been observed in apple leaves as a useful drought-avoidance mechanism (Nemeskéri et al., 2015). They found that the leaf rolling was related to the carbohydrate content of the leaves, where cultivars with higher leaf rolling contained a high accumulation of carbohydrates, while those with moderately rolled leaves contained fewer carbohydrates. To survive a severe drought-stressed period, some trees reduce plant height and leaf area to reduce water loss through transpiration and evaporation, as was observed in mango rootstock (Shanthala et al., 2021). This is a typical characteristic of drought-avoider plants through increasing water acquisition or conservation of water. Bolat et al. (2014) showed that the water-limiting conditions resulted in a large decrease in total fresh and dry weights in quince rootstock. Since shoot growth is mostly inhibited under drought

conditions, plants often re-allocate assimilates from shoot to root growth, thereby causing root growth into deeper soil layers with greater potential for water uptake. Thus, greater plant fresh and dry weights in the absence of water are desirable traits. Improved water absorption by increased accumulation of dry matter in the root was observed in apple rootstock (Sakalauskaite et al., 2006). Shafqat et al. (2021) showed that some anatomical changes may occur to protect the citrus rootstock under drought, like increases in epidermal cell thickness, vascular bundle length, xylem thickness, in the phloem cell area, and in cortical thickness. Bai et al. (2019) reported that apple cultivars with thicker cuticle, longer palisade cells, and thicker spongy parenchyma had superior drought tolerance.

3. PHYSIOLOGICAL RESPONSES TO DROUGHT

Other than negative impact on vegetative growth and fruit quality, drought also changes various biochemical and physiological processes in fruit plants. Plants' physiological and biochemical responses to water deficits differ depending on the severity and duration of drought stress. Although drought stress disturbs many physiological processes (stomatal conductance, transpiration, respiration, photosynthesis, chlorophyll synthesis, cell turgidity, and antioxidant activity), the most affected process is stomatal conductance. To avoid water loss from the leaf surface through transpiration, reducing stomatal conductance is one of the first anatomical adaptations of a plant to overcome drought stress (Abobbata, 2021). Reduced stomatal conductance is a mechanism of water conservation that provides avoidance of the adverse effects of

drought. A lower maximal stomatal conductance was observed in the cultivar "Braeburn" than in "Fuji", indicated that this cultivar is more water-conserving (Massonnet et al., 2007). To cope with drought stress, plants adjust the relationship between water and water use efficiency, transpiration, and photosynthesis through stomatal changes. Water stress often reduces whole-plant carbon assimilation and transpiration, usually causing stomatal closure, as it was observed in drought stressed peach leaves (Rahmati et al., 2015b). Studies of deficit irrigation on almond trees have reported reductions in plant water status leading to a progressive decline in stomatal conductance, net CO₂ assimilation rate, and transpiration rate (Romero et al., 2004). Water use efficiency (WUE) represents the ratio of CO₂ uptake to transpiration rate (Chartzoulakis et al., 1999), and it can be improved by limiting transpiration and stomatal closure (Liu et al., 2012). An increase in WUE represented an important plant adaptation under drought stress. Bacelar et al. (2007) noticed increased values of WUE in olive cultivar, compared to other cultivars under severe water stress, which makes this cultivar the most promising for production in drought areas. Bielsa et al. (2018) found that wild-relative species of almond and peach had the higher WUE in comparison with hybrid genotypes and *Prunus* rootstocks. Zhou et al. (2015) noticed that higher photosynthesis contributes to higher WUE in apple trees, making them more tolerant to drought conditions. Leaf relative water content (RWC) represents the balance between water supply to the leaf tissue and transpiration rate, reflecting cellular osmotic adjustment under dehydration (Hamann et al., 2018). It is considered the most important and commonly used

parameter to assess drought tolerance in plants. Previous studies have found a significant decrease in RWC under drought condition, in different fruit species, including pistachio trees (Khoyerdı et al., 2016), olive trees (Boughalleb & Hajlaoui, 2011), and plum rootstock (Bolat et al., 2016). The higher values of RWC were associated with better drought tolerance in pistachio rootstock (Ghasemi et al., 2021), apple rootstock (Wang et al., 2012), mulberry cultivar (Cao et al., 2020).

4. PHOTOSYNTHESIS AND CHLOROPHYLL CONTENT UNDER DROUGHT CONDITION

With respect to drought, the negative impact on photosynthesis is well-documented for many plant species, but there is still a lack of information about fruit species. Photosynthetic rate could be limited by stomatal limitations and non-stomatal limitations. During drought, trees reduce water loss by closing their stomata, which also limits photosynthesis, which is one of the primary processes affected by water stress. The effects of drought on photosynthesis include the restriction on CO₂ diffusion into the chloroplast, limitations on stomatal opening, changes in leaf photochemistry, and carbon metabolism (Chaves et al., 2009). Rahmati et al. (2015b) noticed water stress significantly reduced gas exchange and affected tree C assimilation due to reduction in leaf photosynthesis and in total leaf area in peach trees. Higher photosynthetic performance, stomatal conductance, and transpiration rates allowed drought-tolerant olive trees to maintain better plant water status (Ben Ahmed et al., 2009). Stomatal closure could be provoked by increasing levels of abscisic acid content as it was observed in orange leaves (Gomes et al., 2004). Non-stomatal limitations, such as

mesophyll conductance and biochemical limitations to photosynthetic efficiency, may also result in the reduction in photosynthesis (Grassi & Magnani, 2005). According to Flexas & Medrano (2002), stomatal closure is the earliest response to drought and the dominant limitation to photosynthesis at mild to moderate drought, whereas non-stomatal limitation occurs at severe drought, where inhibition of photosynthetic CO₂ assimilation is caused by metabolic processes leading to decreased Rubisco activity. Wang et al. (2018) found that reduced photosynthesis activity in apple leaves might be caused by non-stomatal limitation due to a shortage of adenosine triphosphate (ATP) and impaired Rubisco activity. Ma et al. (2015) also suggested that decreased photosynthetic capacity in apple leaves results from impaired Rubisco, which is the key enzyme in the Calvin cycle. Measuring chlorophyll *a* fluorescence is a non-destructive technique for analyzing the structure and function of the photosynthetic apparatus, particularly the photochemical efficiency of PS II under various abiotic stresses including drought stress (Viljevac et al., 2012; Kalaji et al., 2016). Fast chlorophyll *a* fluorescence kinetics are usually measured and analysed on dehydrating intact leaves by the JIP-test (analysis of O-J-I-P fluorescence transient) (Strasser et al., 2004). The most commonly applied photosynthetic parameters from the JIP test are the photosynthetic performance index (PI_{ABS}) and the maximum quantum yield of PSII (F_v/F_m). They are very sensitive parameter to the unfavorable environment, thus very often used for screening genotypes for the selection of drought-tolerant cultivars (Gomes et al., 2012). In previous studies on fruit trees, it was observed that the photosynthetic performance index (PI_{ABS}) in the leaves of sour

cherry decreased under drought conditions (Viljevac et al., 2013), and apple cultivars with better photosynthetic efficiency (PI_{ABS}) were more tolerant to the absence of water (Mihaljevic et al., 2021a). Wang et al. (2018) reported decreasing of parameter (F_v/F_m) in drought-stressed apple leaves. Faraloni et al. (2011) showed that the (F_v/F_m) values decreased in the sensitive cultivars of olive, whereas the tolerant olive cultivar did not show any decrease in these parameters, indicating better efficiency of PSII photochemistry and better tolerance to drought. It was observed that drought causes a decrease in electron transport rate and damage to the oxygen-evolving complex (OEC) in sweet cherry and passiflora leaves (Mihaljević et al., 2021b; Gomes et al., 2012). Chlorophylls, as the main light-absorbing photosynthetic pigments in plants, are also very sensitive to environmental stresses. They reflect the growth status of plants and the degree of stress in plants. Drought stress significantly reduces the content of chlorophyll, where chlorophyll decreases could be a symptom of oxidative stress under drought conditions. It was reported that a decline in photosynthesis is directly related to the loss of chlorophyll in apple leaves, where drought stress also affects the composition of photosynthetic pigments (Bai et al., 2019). More tolerant cultivars show elevated chlorophyll content when exposed to drought stress. Previous studies have reported resistant varieties of pistachio and peach rootstock that contain significantly higher amounts of chlorophyll than sensitive varieties (Jiménez et al., 2013; Khoyerdi et al., 2016). Drought caused less degradation in total chlorophyll content in the more drought-tolerant "Fuji" apple compared to the more sensitive "Hongoro" apple (Bhusal et al., 2019). Similarly,

Garciasanchez et al., (2007) noticed no loss of chlorophyll in response to stress, implying that leaves of Cleo citrus rootstock were more tolerant to stress compared to another one with higher chlorophyll content. Besides, carotenoids play essential roles in photosynthesis; these pigments are also non-enzymatic antioxidants since they neutralize the action of free radicals formed during oxidative stress. Therefore, the genotypes with higher carotenoids content are more resistant to drought (Abbasi et al., 2014). It was observed that rapid increase and higher carotenoids content enable better tolerance to drought, in pistachio and cherry cultivars (Khoyerdi et al., 2016; Mishko et al., 2021).

5. OXIDATIVE STRESS

Drought stress in plants induces oxidative stress and damage. During drought periods, the utilization and consumption of absorbed light energy is disrupted because plants absorb more light energy than can be consumed by photosynthetic carbon fixation, causing photosystem damage and the production of reactive oxygen species (ROS) (Farooq et al., 2009). ROS plays an important role in plant acclimatization to abiotic stress by regulating various pathways during acclimatization to protect themselves from ROS toxicity (Choudhury et al., 2017). Although ROS in plants are used as signal transduction molecules, excessive ROS production can negatively impact the normal function of cells, causing lipid peroxidation, protein, DNA, and cellular damage, eventually leading to cellular death (Mittler, 2002; Das & Roychoudhury, 2014). The major ROS produced in plants under

unfavorable environmental conditions are superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH\cdot$) and singlet oxygen (1O_2). Membrane lipid peroxidation is a major damaging effect of ROS. The level of lipid peroxidation is detected by measuring malondialdehyde (MDA), which is a widely used marker of oxidative stress. The malondialdehyde (MDA) content was increased in the leaves of seven citrus rootstocks when they were subjected to drought stress, indicating the oxidative damage within plant cells. However, Brazilian sour orange, which showed a higher level of MDA content, indicates more lipid peroxidation and more sensitivity to water stress than those which produce less MDA content (Hussain et al., 2018).

6. BIOCHEMICAL RESPONSES TO DROUGHT

The increased ROS products induced changes in the activities of antioxidant enzymes like superoxide dismutase (SOD); catalase (CAT); ascorbate peroxidase (APX); and guaiacol peroxidase (GPOD), which can balance the formation and elimination of ROS by detoxification of excess ROS (Gill & Tuteja, 2010). Therefore an increase in antioxidant enzymes is often part of the adaptive response of plants to abiotic stress. Ben Ahmed et al. (2009) showed that increased SOD, APX, and CAT activities may affect the drought tolerance of some olive trees. In order to reduce oxidative damage, apple rootstock *M. prunifolia* exhibits higher antioxidant capacity and a stronger protective mechanism compared to less tolerant rootstock (Wang et al., 2012). Jiroutova et al. (2021) noticed that more drought-tolerant sweet cherry cultivars had the higher antioxidant capacity and accumulation of osmoprotectants.

Drought-tolerant citrus rootstocks had lower MDA and H₂O₂ levels as well as higher antioxidant enzymatic activities to mitigate ROS produced during drought stress conditions (Hussain et al., 2018). Together with enzymatic, plants possess a non-enzymatic antioxidant system (e.g., ascorbic acid, glutathione, α -tocopherol, and β -carotene) which protects plant cells from oxidative damage by scavenging ROS. Ascorbic acid is a plant cell antioxidant which can provide membrane protection by scavenge O₂^{•-} and OH[•]. A role for increased ascorbic acid content in the amelioration of oxidative stress has been reported by Sharma & Sharma (2008), where pyrus grafted on Kainth and BA29 rootstocks accumulated more ascorbic acid. Ascorbic acid, together with soluble sugar and proline content in their leaves, caused higher leaf water potential and showed less reduction in photosynthetic rate. In the study of Gholami et al., (2012), higher α -tocopherol and lower ascorbic acid levels were noticed during water stress in fig cultivars. To avoid water deficit in drought stress, plants also use osmotic adjustment, which is a very important mechanism for maintaining cell turgor and plant metabolic activity (Jiroutova et al., 2021). Among them, two main representatives are proline and sugar. Proline plays a protective role during drought stress and proline accumulation is very often used as a physiological indicator of plant resistance to stress tolerance (Amini et al., 2015). The contribution of proline to drought tolerance mechanisms is demonstrated in many plant species. The superior performance of the cultivar that accumulated the most proline content was demonstrated in pistachio and peach trees (Khoyerdı et al., 2016; Haider et al., 2018). Karimi et al. (2018) discovered similar increases in proline and higher

levels of RWC in the leaves of drought-stressed olive plants, implying that proline accumulation is important for olive drought tolerance. On the other hand, Sircelj et al. (2005) concluded that proline and other free amino acids did not participate in the adaptation to water deficit in apple trees. During drought conditions, accumulation of soluble sugar can mitigate the negative impact of drought by improving the ability of plants to absorb and retain water. In order to maintain the translocation of sugars to the growing parts, the olive cultivars with low photosynthetic activity produced more soluble sugars in olive trees (Ben Ahmed et al., 2009). The accumulation of total soluble carbohydrates in the leaves and roots of pistachio trees is strongly correlated with tolerance to drought stress (Khoyerdi et al., 2016). Escobar-Gutierrez et al. (1998) showed that sorbitol synthesized at low photosynthesis rates acts as one of the components for osmotic adjustment in drought-affected peach leaves. Similarly, Wang & Stutte (1992) reported an increase in sorbitol concentration in drought-stressed apple trees. Accumulation of glycine betaine can improve the drought resistance of plants, osmotic regulation, stomatal conductance, carboxylation efficiency of CO₂ and photosynthesis (Ma et al., 2007). In a previous study of Niu et al., (2021), it was shown that accumulation of glycine betaine is correlated with drought resistance. They found that glycine betaine in drought-stressed pear leaves increases antioxidant activities and reduce ROS and lipid peroxidation, which thus contributes to greater drought resistance. Although limited attention has been given to the effect of phenolic compounds on the fruit tree's water stress tolerance, the production of phenolic compounds is one of the

plant strategies used to avoid and protect plant cells from the oxidative damage caused by drought. Petridis et al. (2012) found that the accumulation of phenolic compounds is a good adaptive mechanism in the olive tree against drought, suggesting their role as antioxidants. The increase in water stress resulted in higher phenol content in apple and quince rootstocks (Bolat et al., 2016) and citrus plants (Hussain et al., 2018). However, Mihaljević et al. (2021a) noticed that phenols do not play a significant role in the defensive reactions of investigated apple cultivars under drought.

CONCLUSION

Drought stress is an important constraint limiting fruit productivity worldwide. Although fruit trees show a wide range of morphological and physiological strategies to cope with the adverse effects of drought, the tolerance of any species to this threat varies remarkably. Aside from adequate agricultural practice with irrigation and fertilization, proper selection of drought-resistant cultivars and rootstocks is one of the strategies to reduce the impact of this stress and contribute to more stable fruit production. Research on the strategies of fruit species helps us cope with drought stress and improves the adaptability of fruit trees to water shortages. Furthermore, breeding programs lack basic knowledge of how fruit plants act and respond to the abiotic stresses in their growing environment. Therefore, screening the current and new fruit species for their potential to mitigate the drought effects is very important for the selection of suitable varieties for future fruit production, and to provide valuable information for future breeding

programmes. One of the useful way to improve drought tolerance in different fruit species is use of autochthonous and wild material. Because of their good biological and economic characteristics, autochthonous and wild fruit varieties are adapted to the local conditions very well. Thus, they should be more thoroughly investigated and used in the creation of new varieties. The adaptive strategies used by drought tolerant fruit species are of major importance for improving fruit yield under water deficit conditions, which will be of great economic importance. Although the mechanisms of tolerance to abiotic stress as well as drought have already been performed on many plants, there is still a lack of studies on fruit species. Therefore, this is a complex process that still needs to be studied and understood.

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CHAPTER IX

POLYPHENOL PROFILE AND ANTIOXIDANT CAPACITY OF RED FRUITS FROM THE CONTINENTAL PART OF MONTENEGRO – A PROMISING SOURCE OF FUNCTIONAL FOOD

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INTRODUCTION

The red fruits have a great nutritional value, which primarily depends on the variety, degree of maturity, yield, and applied agricultural techniques. These fruits are an important source of protective substances that ensure the normal and proper development of the human body. They are suitable for fresh use in the household, for industrial processing, and for deep freezing. Biological-production characteristics, high profitability of cultivation, suitability of climatic conditions as well as the possibility of placing fruits and processed red fruits on the domestic and foreign markets, are the basic preconditions for further expansion and are planned to raise high-intensity berry orchards. Strawberry is one of the most important types of berries in our area. Favorable natural conditions enable the cultivation of this species from lowland to somewhat lower hilly and mountainous areas, which is not sufficiently used. A hindering factor in the further spread of this extremely attractive fruit species is primarily the lack of manpower when performing one of the most sensitive operations, such as strawberry harvesting. By applying mechanized harvesting and solving the problem of high fragmentation of plots, in the future we can count on higher production and consumption of strawberries (Šoškić, 2009). Raspberry is the most economically important type of berry in Serbia. Biological characteristics and technological value of this type of fruit, as well as high adaptability for cultivation in different climatic and edaphic conditions, easy reproduction, relatively high yield, and modest agrotechnical requirements, are the main features of growing

important raspberry varieties. In recent years, significant progress has been recorded in the introduction of new, more fertile, and higher quality varieties, the production of healthy planting material, as well as the increasingly frequent scientific treatment of problems in the field of raspberry growing. Blackberry is a very interesting and biologically and technologically important type of berry fruit, with a wide amplitude of cultivation in different parts of our country. The existing natural conditions enable the cultivation of blackberries from the plains to the foothills. The extraordinary nutritional, dietary and protective value of the fruits of this fruit imposes the need for further expansion and raising of modern plantations of blackberries. In recent years, with the introduction of new American varieties of blackberries without thorns, a trend of mass cultivation has been noticed, so that today, in terms of production and representation, it is in the third place in the group of berries. In addition to these species, attention should be paid to blueberries, which on lands with higher acidity and temperate climate provide regular and relatively high yields.

1. GENERAL BIOLOGICAL CHARACTERISTICS OF RED FRUITS

Due to its specific fruit characteristics, wide use-value, as well as a high degree of adaptability to different cultivation systems, red fruits occupies a special place (Figure 1). Each fruit crop from this group has an intensive work character, ie it requires large participation of live labor, especially in certain work operations. Fruits from this group also have many common features in terms of anatomy and physiology.



Figure 1: Red Fruits (Original by Kolarov)

1.1. Wild Strawberry

Wild strawberry (*Fragaria vesca* L.) is a perennial herbaceous plant from the rose family (Rosaceae) (Figure 2). It blooms from early spring to the end of summer, and the first fruits can be found in May and can be picked until autumn. As with other flowering plants, strawberry organs are divided into vegetative and generative. Vegetative organs serve to maintain the life of the individual and in strawberries are differentiated into root, stem, stolons, and leaf. They are generative reproductive organs and enable the survival of the species. These are flowers, fruit, and seed. The root is an under ground organ that absorbs water and dissolved substances from the soil and conducts them to the tree. He is sinewy and very branched. It also serves for the storage of organic substances that it synthesizes itself (amino acids, proteins, lipids, etc.). The strawberry has a vegetative branch that together with

the leaves forms a shoot. The stem is recumbent or erect, overgrown with hairs. The leaf is a very important vegetative organ of the strawberry because the most important physiological processes take place in it: photosynthesis, respiration, and transpiration. It appears as a growth on a tree and is part of a single shoot organ. Strawberry leaves are divided into three parts. Stolons are above-ground creeping strawberry shoots that serve for vegetative propagation and that lie on the surface of the earth. The flowers are single, mostly bisexual, with five white petals. The fruit of the wild strawberry is a collective nut that appears from the middle of summer to the end of autumn, and it was formed from a large number of individual fruits that are located on a thickened, fleshy flower bed.



Figure 2: Wild Strawberry (URL-1)

Wild strawberries are type of wild fruits of Eurasian origin that, due to their quality properties, have been transferred to all other continents. It is, of course, a very old plant species, which is confirmed by the seeds that were found in the deposits that date back to the Stone Age. The

chronology says that the cultivation of this fruit began in the 15th century. Wild strawberries love woods, bright, sunny places, and semi-shady positions. They are the first plant species to inhabit shrubs and forest fires. They are more numerous and lush in the mountainous areas and grow up to 1500 meters above sea level. There is a lot of truth in the old saying that ants know best the path that leads to wild strawberries. This fruit, above all, is most affected by the type of soil on which ants stay and build their "houses" - moist, rich, and permeable.

1.2. Strawberry Kitnjača

The Kitnjača strawberry (*Fragaria moschata* Dush.) grows under lower trees, in the shade surrounded with tall grass (Figure 3). Like the classic wild strawberries, the Kitnjača strawberry belongs to the rose family (Rosaceae). It is widespread in Europe and Siberia. The plant is very lush, up to 40 cm high, and it gives very little or no hedges. The flower stalk is tall, with large flowers that are usually above the leaves. The Kitnjača strawberries are very similar in appearance to the leaves and flowers of wild strawberries and it is very difficult to distinguish them from the leaves, but already at the time of flowering, the difference is visible. Kitnjača strawberries have slightly larger flowers than wild strawberries. The fruits of kitnjak strawberries are also larger than those of wild strawberries, light to dark red in color or purple-red, round and very aromatic.



Figure 3: Kitnjača Strawberry (URL-2)

At harvest time, the fruits of the wild strawberry are clearly visible above the leaves, while in Kitnjača berries the fruits are hidden under the leaves. The fruits of Kitnjača strawberry are used in the gourmet diet because of their intense aroma and superior taste, which is reminiscent of a mixture of flavors of ordinary strawberry, raspberry, and pineapple. Due to its lushness and aromatic fruits, it is of great importance for breeding. The Kitnjača strawberries ripen only in the second half of June, unlike the wild strawberries, which start ripening as early as the end of May.

1.3. Wild Raspberry

Raspberry (*Rubus idaeus* L.) after strawberry has the greatest economic importance in the group of red fruits. It is widespread in Europe and northern Asia. It grows from mid-summer to mid-autumn, and blooms from May to June. It adapts very well to different climatic and soil conditions, so it can be grown in hilly and mountainous areas at higher altitudes (up to about 1100 m). It has fruits in the first year after planting

and reaches full fertility in the third year. It is easily propagated by root shoots. Wild raspberries (Figure 4) grow on the edges of forests because they need higher humidity and some sun for better fruit ripening. The root system of raspberries is perennial and well developed. The shoot consists of a tree and leaves and consists of an above-ground and an underground part. The wild raspberry leaf is compound oddly feathered and consists of 3 or 5 ovate leaves. The leaves are spirally arranged on a raspberry stem. Wild raspberry flowers are grouped in clustered inflorescences, which are formed on the native twigs in the axils of the leaves or on the tips.

The fruit of the wild raspberry is consists of a large number of small drupes, which are compacted and attached to the flower. Wild raspberry is quite resistant to low winter temperatures, so it grows well in areas with harsher climates. These and other biological production and technological properties and market value of raspberry products make it one of the most profitable crops in plant production.



Figure 4: Wild Raspberry (URL-2)

1.4. Wild Blackberry

Wild blackberry (*Rubus caesius* L.) (Figure 5) is a perennial herbaceous plant that also belongs to the rose family. The Latin name of the genus *Rubus* comes from the word *ruber* (red) because several species of this genus have red fruits. The word *caesius* means blue-green. It is widespread throughout Europe and western Asia, and in some places in North and South America, it is considered domesticated. It grows on neglected terrains, as part of shrubs, along rivers, streams up to 1000 meters above sea level.



Figure 5: Wild Blackberry (URL-3)

Blackberry bush consists of relatively tall shoots that grow from root veins and usually bend in the shape of an arch towards the ground. It reproduces vegetatively, creating new shoots from the root neck. The root is strong, tough, up to 200 cm deep. Due to its strong and deep roots, it tolerates drought well. Its stem is more or less prickly, depending on the variety, and it is woody in places. The thorns serve for climbing, but also as a defense mechanism against animals that feed

on blackberry leaves. It blooms quite late, after late spring frosts, and ripens in August. After flowering, a small berry fruit, red in color, develops from each flower, which later darkens to dark blue and almost black fruit. Compared to raspberries, blackberries are more sensitive to cold, which means that warmer regions or southern positions suits the best, but with a lot of moisture during the growing season. Deep, moderately acidic soils with a lot of clay and sand, which are permeable, are the most suitable for growing blackberries. Otherwise, it is necessary to know that blackberries, as well as raspberries, do not tolerate swampy lands. Soils with a high groundwater level are also not suitable, especially if it reaches the root zone.

1.5. Forest Blueberry

Blueberry (*Vaccinium myrtillus* L.) is a perennial shrub originating from Europe and Asia, where these berries are still mostly grown today. It is a plant from the heather family (Ericaceae). The scientific name is derived from the Latin words *bacca* = berry and *myrtus* (diminutive *myrtillus*) by the similarity of the leaves with the myrtle plant. Shrubby plant up to 50 cm tall with thick and thin twigs with extremely sharp corners and green, shiny bark. The leaves are finely toothed, 1-3 cm long, round-ovate, pointed at the apex, and hairless, glabrous. They are located on short leaf stalks. The stems are erect, with sharp edges. The flowers are hanging on short stalks, white-pink in the shape of a bell. They are bisexual, single and blooms in May and June.

The fruit is a shiny berry of blue-black color and slightly sour taste, which ripens from July to September (Figure 6). It contains a large

number of seeds. Blueberry fruits are very nutritious because they are rich in iron and various vitamins.



Figure 6: Forest Blueberry (URL-4)

Medicinal parts of the plant are the leaves, fruits, and roots. It can grow at altitudes and over 2000 meters. It grows in the zone of deciduous, beech forests and above it on fresh soils. It is a frequent inhabitant of many mountain massifs. Blueberries need humus, moist and acidic soil whose optimal pH value is 4-5. It likes a sunny and semi-shady position. It is resistant to winter and can withstand temperatures from -20 °C to -25 °C without any damage, and if it is covered with snow, it can withstand much lower temperatures.

2. INFLUENCE OF RED FRUITS ON HUMAN HEALTH

Modern production and processing offer the market many foods that are not the best from a nutritional and health point of view. Consumption of refined foods and the increasing use of fats (with trans fatty acids)

and foods with low plant fiber is a significant factor in the development of diseases of modern civilization. Therefore, it is advisable to consume more fresh fruit produced primarily organically or according to the principles of integrated production. The connection between diet and health is becoming more and more obvious, and numerous researches show an exceptional connection between health and consuming fresh berries. The nutritional properties of fruits are based on their chemical composition, where the most important role is played by: vitamins A and C, minerals, carbohydrates, plant fibers, and phytochemicals, many of which show antioxidant properties. These include primarily flavonoids (anthocyanins and proanthocyanidins) and carotenoids (Maksimović et al., 2015). Functional food is a food whose biological characteristics have a positive effect on human health and certain bodily functions. Its goal is to have a beneficial, protective effect on the digestive system, speed up digestion, "clean" the liver, prevent the formation of sand in the urinary tract, etc. At the same time, it cleanses the large intestine and thus frees the body from harmful substances ingested through food, such as additives or heavy metals. The significant role of this food is that it has a great antioxidant potential, important for the fight against free radicals, which are formed in our body every day, depending on the conditions in which we live. Antioxidants are widespread in the food industry, which aims to prevent various diseases, from heart disease to the treatment of various types of cancer. Although initial research into the effects of antioxidants on the prevention and treatment of various types of disease has yielded positive results, overuse can have detrimental effects on health. Given

the above, and having in mind the chemical composition of berries, it can rightly be considered that it is a good source of functional food (Sies, 1997).

2.1. Medicinal Properties of Wild Strawberry and Kitnjača Strawberry

They are a real treasure trove of antioxidants that protect the body from free radicals, which accelerate aging and cause many diseases. Also, wild strawberries are an excellent source of vitamin C, which strengthens the arteries and adrenal glands, which are also known as stress glands, and they require a larger amount of vitamin C from all organs in the body. Studies have shown that strawberries can help regulate blood sugar levels. Blood sugar levels are associated with obesity, mood swings, diabetes, and the like. The fruit of the wild strawberry improves the blood picture, is used against sclerosis of blood vessels and high blood pressure, prevents blood clotting and sticking. Strawberries contain anthocyanins, which help burn fat deposits on the body because the body uses them as fuel. Studies have shown that anthocyanins also have a beneficial effect on short-term memory. They are very helpful in preventing heart disease and arthritis. Strawberry fruits are also used in cosmetics for facial care, whitening, and nurturing of a teeth. This biological value of strawberries is given by the significant content of vitamins, especially mineral, pectin, and tannin substances, fruit sugars, amino acids, as well as other ingredients. They are full of anti-cancer substances. Flavonoids fight cancer cells and prevent their ability to multiply. Phenolic acids from strawberries have anti-allergenic properties, which means that they help reduce

biochemical processes, which are associated with allergic reactions. Strawberries have anti-inflammatory effects that relieve pain. Science has proven that strawberries reduce markers of inflammation (Tucakov, 1984).

2.2. Medicinal Properties of Wild Raspberries and Wild Blackberry

Raspberry is called a female plant because it cures many ailments of female organs. Raspberry leaf tea helps with menstrual cramps and heavy bleeding. It is great for pregnant women because it relieves nausea and pain, helps with childbirth, and encourages the production of milk. Raspberry is an excellent natural remedy for inflammation, lowers the high temperature, strengthens immunity, improves complexion, strengthens hair, and regulates blood sugar levels. The flavonoids in the fruit preserve heart health, strengthen blood vessels, and lower blood pressure. The fiber in raspberry fruits improves the work of the intestines and has a favorable effect on the work of metabolism. Raspberries contain resveratrol (polyphenol) which slows down aging, and a high proportion of ellagic acid, which has an anticancer effect (reduces the risk of cancer). Raspberries also reduce the action of free radicals and protect against cancer of the colon, breast, uterus, esophagus, and prostate. The ketones in raspberries are responsible for controlling blood glucose and helping prevent diabetes. Ketones stimulate fat metabolism, reduce fat deposits and prevent obesity-related diseases. Studies have shown that raspberries, thanks to the anti-inflammatory properties of anthocyanins, successfully fight

arthritis and rheumatism. All these medicinal properties are also related to wild blackberry (Tucakov, 1984).

2.3. Medicinal Properties of Forest Blueberries

Among 40 different types of fruits and vegetables, blueberries are the richest source of antioxidants. Anthocyanins are most responsible for that, which gives this fruit its characteristic blue-black color. In folk medicine, blueberry is a valued medicine for acute and chronic gastrointestinal diseases. Blueberry fruits are a beneficial tool in the prevention and treatment of avitaminosis and hypovitaminosis, as well as in the treatment of non-infectious diarrhea in children. Blueberry successfully eliminates bloating and the appearance of gas in the intestines, which is very important in the treatment of hemorrhoids and intestinal diseases. It cures leukoplakia of the tongue (small inflamed white spots on the tongue) caused by excessive smoking. Numerous studies have shown that blueberry juice is a perfect remedy against anemia, that blueberry slows down the aging process, improves vision function and blood circulation in general, stops the initial stages of cancer development. Fresh blueberry juice removes intestinal parasites, especially earthworms. For hair growth, in folk medicine, it is recommended to rub the head with blueberry tea (leaves). Blueberry leaves are used to prepare a tea drink that is an excellent cardiogenic and diuretic, it is also good for treating the kidneys and bladder, and it is also used as an ingredient in herbal mixtures for the treatment of diabetes. In folk medicine, the healing properties of blueberry leaves stand out to the extent that they are called herbal insulin. They are also

used in disorders of the digestive organs, ie vomiting, diarrhea, stomach cramps, cough (Tucakov, 1984).

3. BIOCHEMICAL ASPECT OF RED FRUIT

The chemical composition of the fruit is complex and depends on several factors, among which, in addition to the species and variety, very important climatic conditions, pedological characteristics of the soil, applied agrotechnical measures, degree of maturity. The chemical composition of the fruit is important both from the point of view of nutrition and from the point of view of technology. Which technological process will be applied, which reactions can be expected during processing, and what kind of product will be obtained, is closely related to the composition of the raw material. The most important components of the chemical composition of fruits are carbohydrates, acids, plant pigments, pectin, minerals, vitamins, proteins, etc.). Knowledge of the content of certain substances in berries such as total phenols, flavonoids, tannins, vitamins, minerals, etc. they enable the human population to get to know all the nutritious and medicinal properties that these fruits offer. Due to the stated characteristics, berries are considered one of the main sources of functional food.

3.1. The Most Important Groups of Phenolic Compounds

Phenolic compounds represent a widespread group of plant metabolites that can be very simple structures, such as phenolic acids or very complex structures, or polycondensed compounds such as condensed tannins. A common feature of phenolic compounds is that they contain an aromatic ring with one or more hydroxyl groups (which can be

methylated or esterified). Phenolic compounds play several key roles in the metabolism of plants and fruits. They are involved in the physiological processes of growth and development and affect various aspects of the fruit before and after harvest. Consumption of certain phenols in food is considered beneficial for human consumption. Epidemiological evidence suggests that foods rich in phenols, derived from fruits, are associated with a lower risk of cancer and coronary heart disease, as well as cataracts and immune dysfunction, and stroke. To date, more than a thousand different natural phenols have been identified, in free form or more often in the form of glycosides.

3.1.1. Flavonoids

More than 5,000 different flavonoids synthesized by plants are known (Popović, 2005). Flavonoids are chemical compounds that are most commonly formed as secondary metabolites in plants. They got their name from the Latin word *flavus* which means yellow. The function of flavonoids in plant tissues is multiple: they are plant pigments, they protect the plant from UV radiation, insects, temperature, and oxidative stress, and they also serve as chemical signaling molecules. They are synthesized in response to increased concentrations of some metals in the soil (e.g. aluminum). Due to their specific structure, flavonoids are potential natural antioxidants. The presence of flavonoids leads to the interruption of free radical reactions, whereby they transfer the hydrogen atom to the radicals and themselves turn into free radicals. The free radicals thus formed are resonantly stabilized and do not have enough energy to initiate a chain reaction with the substrate.

3.1.2. Tannins

Water-soluble plant polyphenols that cause the deposition of proteins from aqueous solutions are called tannins (Popović, 2005). They are divided into two groups, hydrolyzing and condensing. The healing properties of tannins and their use are multiple: they have an effect against bacteria and fungi, they have a hemostatic effect because they narrow blood vessels and create a plug of clotted blood, they alleviate and treat dysentery, they are antidotes to various substances. Tannins are very widespread in the plant world and are found in the cytoplasm of parenchymal cells of various organs. Tannins protect plants from insects and other pests. Perennial herbaceous plants contain the most tannins in the underground organs and in the unripe fruit. During ripening, they are broken down by the enzyme tanase. The precipitate with proteins. Tannins from wild fruits are important as an aid for diarrhea and faster healing of wounds, bites, and the like. For these purposes, blueberries, blackberries and raspberries, quinces, hazelnuts, and various other bitter fruits can be used.

3.1.3. Anthocyanins

Anthocyanins are water-soluble pigments that can appear in various colors (red, purple, or blue) depending on the pH value. In addition to their role to show antioxidant effects and protection from UV radiation, anthocyanins also have a strong protective effect on blood vessels. These substances are powerful protectors against diseases of the cardiovascular system and excessive aging. Anthocyanins can be found in a large number of fruits and vegetables. The most famous are

anthocyanins from grape seeds, berries such as blueberries, blackberries, raspberries, etc. These pigments give the fruit a beautiful red, dark red, or purple color and are a real arsenal of biologically active substances that protect blood vessels from inflammation and loss of elasticity. These positive effects of anthocyanins are most noticeable on the blood vessels of the heart, brain, and kidneys. Anthocyanins have been given an honorable place in the prevention and treatment of some of the most common and deadly diseases of modern society: atherosclerosis, high blood pressure, thrombosis, myocardial infarction, and stroke. Anthocyanins have extremely strong antioxidant power. Their strength is up to 50 times greater than vitamin E. French scientists have discovered that anthocyanins also play an extremely important role in stress. These dyes increase the resistance of cells to stress because they reduce the intensity of the stress reaction. In today's time of constant stress, the level of stress hormones is constantly increasing and that leads to constantly high blood pressure. Regular intake of anthocyanins, either through a diet or dietary supplement, reduces the level of adrenaline and protects us from the effects of stress.

3.1.4. Proanthocyanidins

Proanthocyanidins are a large group of bioflavonoids. These are mostly blue and purple pigments in many plants. Proanthocyanidin extracts show a wide range of pharmacological activity. They raise the level of vitamin C in the body, reduce the fragility and permeability of capillaries, neutralize free radicals and prevent the destruction of

collagen, one of the most important building proteins in the body. They are also thought to help maintain eye function.

3.2. Collection and Preparation of Plant Material

Plant material was collected at different localities in the north of Montenegro in three terms during the vegetation to monitor the accumulation of the tested biomolecules (Table 1). Given the different biology of the examined species, sampling was planned at the beginning, middle, and end of the vegetation period for each species, i.e. in the phase of immature (green) fruit, at the beginning of ripening, and in the phase of fully ripe (consumable) fruit.

Table 1: Location of a Collected Plant Material

Common name	Latin name	Location
Wild raspberry	<i>Rubus idaeus</i> L.	Andrijevica (Sućeška) 42°43' N 19°47' W
Wild blackberry	<i>Rubus caestius</i> L.	Bijelo Polje (Obrov) 43°01' N 19°44' W
Wild strawberry	<i>Fragaria vesca</i> L.	Andrijevica (Sućeška) 42°43' N 19°47' W
Kitnjača strawberry	<i>Fragaria moschata</i> Duch.	Andrijevica (Sućeška) 42°43' N 19°47' W
Forest blueberry	<i>Vaccinium myrtilus</i> L.	Zeletin 42°39' N 19°50' W

At the same time, data were collected on the general characteristics and production of berries in the continental part of Montenegro and the study of methods related to the biochemical analysis of a given fruit. In laboratory conditions, the number of biochemical parameters was determined: the content of total phenols, tannins, flavonoids, anthocyanins, proanthocyanidins.

3.3. Determination of Polyphenolic Compounds in Red Fruits

Based on the processed data from Figure 7, it can be seen that the highest content of total phenols in the fruits of wild raspberries (11.81 mg gallic acid g⁻¹ fw), wild strawberries (11.85 mg gallic acid g⁻¹ fw), Kitnjača strawberries (10.88 mg gallic acid g⁻¹ fw), wild blackberries (13.90 mg gallic acid g⁻¹ fw) and forest blueberries (10.62 mg gallic acid g⁻¹ fw) was recorded in the first phase of ripening.

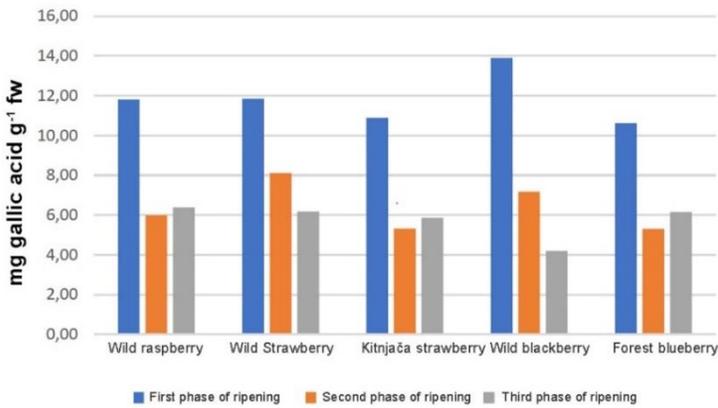


Figure 7: Content of Total Phenolics

The results show that the lowest amount of total phenols in the fruits of wild raspberries (5.98 mg gallic acid g⁻¹ fw), Kitnjača strawberries (5.31 mg gallic acid g⁻¹ fw), and blueberries (5.31 mg gallic acid g⁻¹ fw) observed in the second phase of ripening, while the lowest amount of total phenols in the fruits of wild strawberry (6.18 mg gallic acid g⁻¹ fw) and wild blackberry (10 mg gallic acid g⁻¹ fw) was recorded in the third phase of ripening.

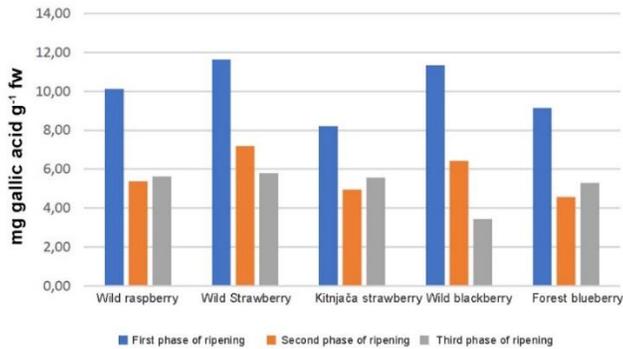


Figure 8: Content of Total Tannins

According to the results from Figure 8, it can be seen that the highest content of total tannins in berries was also recorded in the first phase of maturity, ranging from 8.21 mg of gallic acid g⁻¹ fw to 11.64 mg of gallic acid g⁻¹ fw. In the second phase of ripening, the content of total tannins in the fruits of wild raspberry, Kitnjača strawberry, and forest blueberry was approximately the same, while the lowest amount of total tannins in wild strawberry (5.79 mg gallic acid g⁻¹ fw) and wild blackberry (3.43 mg gallic acid g⁻¹ fw) was also in the third stage of ripening as was the case with the total phenol content.

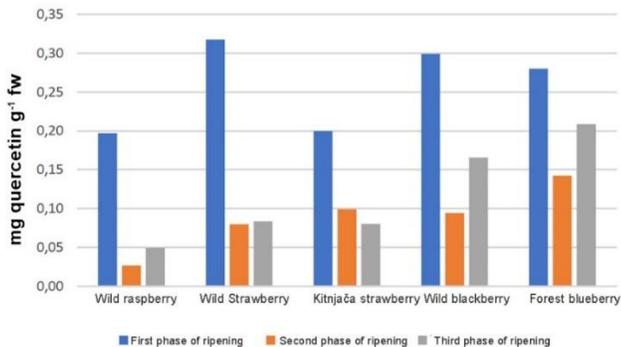


Figure 9: Content of Total Flavonoids

The content of total flavonoids in berries varies and ranges from 0.03 to 0.32 mg of quercetin g⁻¹ fw (Figure 9). The highest content of total flavonoids in all fruit trees was recorded at the beginning of the vegetation, ie in the phase of immature (green) fruit. In wild raspberry and Kitnjača strawberry, the content of total flavonoids in the first ripening phase was identical (0.20 mg quercetin g⁻¹ fw), while in wild strawberry, wild blackberry, and forest blueberry the content of total flavonoids in the first ripening phase was approximately the same. The lowest content of total flavonoids in wild raspberries, wild blackberries and forest blueberries was recorded in the second phase of maturity, while in Kitnjača strawberries the lowest content of total flavonoids was recorded in the third phase of ripening. In the wild strawberry fruit, the content of total flavonoids in the second and third phases was also identical (0.08 mg quercetin g⁻¹ fw).

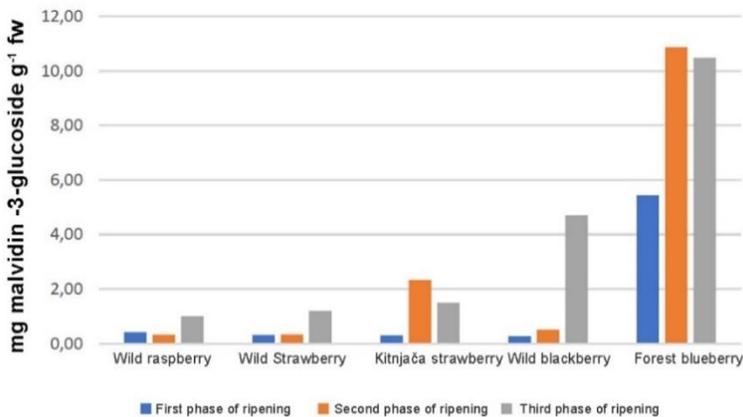


Figure 10: Content of Total Antocyanins

Based on the results from Figure 10, it can be concluded that the highest amount of total anthocyanins is in wild raspberries (1.01 mg malvidin -3-glucoside g^{-1} fw), wild strawberries (1.20 mg malvidin -3-glucoside g^{-1} fw), and wild blackberries (4.71 mg malvidin -3-glucoside g^{-1} fw) were at the end of the vegetation period in the phase of fully ripe (consumable) fruit, while in forest blueberries and Kitnjača strawberries the highest content of total anthocyanins was in the second phase of ripening. In this case, blueberries had a significantly higher content of anthocyanins compared to all other fruit species. All the mentioned fruit species, except raspberries, recorded the lowest content of total anthocyanins in the first phase of maturity.

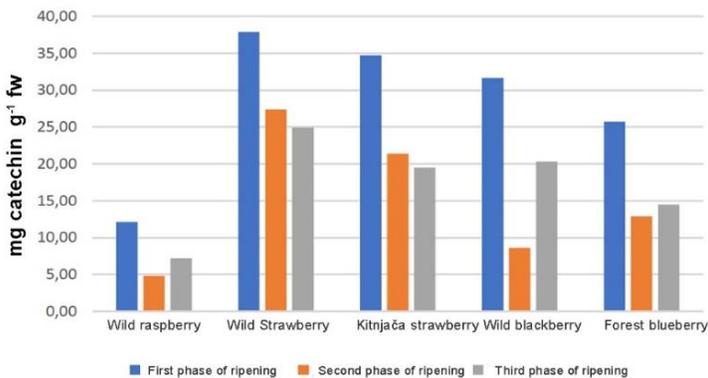


Figure 11: Content of Proanthocyanidins

Examination of the proanthocyanidin content showed that the amount of proanthocyanidin in the samples of the first phase was the highest, while in the samples of the second and third phase it can be noticed that the amount visibly decreased (Figure 11). The highest content of

proanthocyanidins was wild strawberry 37.89 mg catechin g⁻¹ fw, while the lowest value was wild raspberry 4.81 mg catechin g⁻¹ fw.

Figure 12 shows the values of scavenger activity of the extract determined by the DPPH method expressed in %. The highest activity in removing DPPH radicals was shown by wild strawberry 82.17%, while the lowest activity was found in wild raspberry 8.59%.

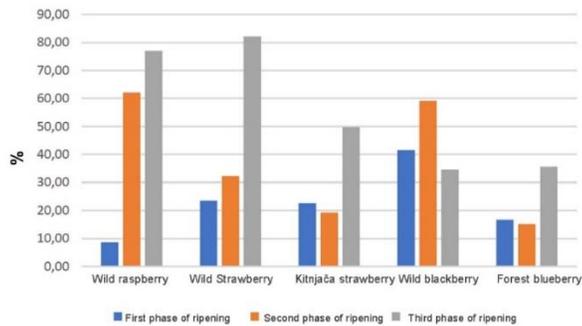


Figure 12: Results of Determination of Scavenger Activity of Extracts by DPPH-Method

Based on the results, it can be seen that the highest values for all fruit species were at the end of the vegetation period, i.e. in the phase of full ripeness, unlike wild blackberries whose highest value of 59.18% was in the middle of vegetation, i.e. in the second phase of ripening.

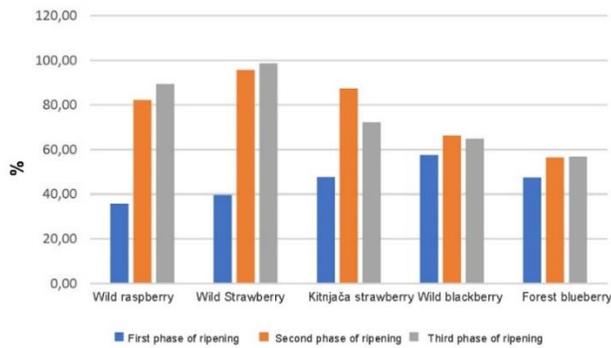


Figure 13: Results of Testing the Ability of Extracts to Neutralize $ABTS^{\cdot+}$ Radicals

Figure 13 shows the ability of the tested extracts to remove $ABTS^{\cdot+}$ radicals. At the beginning of the vegetation period, i.e. in the phase of immature (green fruit), the examined fruit species showed the least activity in removing $ABTS^{\cdot+}$ radicals. The highest value was reached by wild strawberry 98.68% in the phase of full maturity, and then wild raspberry 89.42% in the same phase of ripening. Kitnjača and wild blackberry had the greatest activity in the second phase of ripening, while blueberries had an approximate value in the middle and at the end of the vegetation period.

Bektić et al. (2017) examined the content of total anthocyanins in wild strawberry fruits. The values of total anthocyanins ranged from 0.13 mg / g at the Ilinčica locality to 0.32 mg / g at the Konjuh locality, which is approximately the values obtained in the paper. Huang et al. (2012) investigated the total phenol content for blueberries and it was 9.44 mg gallic acid / g^{-1} fw, while the proanthocyanidin content was 24.38 mg catechin / g^{-1} fw. These values are also approximately equal to the

values obtained in this work. Castrejon et al. (2008) determined the total composition of phenolic compounds in ripe and immature blueberry berries. The analysis showed that the content of total phenols in green, immature berries was 60.76 mg of gallic acid / g⁻¹ fw, and in blue ripe berries 33 mg of gallic acid / g⁻¹ fw. The content of total phenols in blueberry samples collected from different localities of Montenegro is also higher in green, unripe berries. Zheng & Wang (2003) also examined the phenol content in blueberry samples and found a significantly lower value of 4.12 mg of gallic acid / g⁻¹ fw. Giovanelli & Buratti (2009) compared the polyphenol composition and antioxidant capacity of American, high-shrub blueberry (*Vaccinium corymbosum*) and wild blueberry (*Vaccinium myrtillus*) polyphenols. They determined two to three times higher antioxidant capacity in wild species. Wu & Prior (2005) also determined the antioxidant capacity of phenol, flavonoids, and anthocyanins in blueberries, and the values for these substances were 388, 425, and 473 mg of gallic acid / g⁻¹ fw. Yılmaz et al. (2009) examined the content of polyphenolic compounds in berries from the Rosaceae family and reported that the highest content of polyphenols (96 mg / g) is found in pomegranate. This value is much higher than other values published for other berries rich in vitamin C, such as currants (3 - 4 mg / g), blueberries (2.7 - 3.5 mg / g), strawberries (1.6 - 2.9 mg / g) and raspberry (2.7 - 3.0 mg / g). In her doctoral dissertation, Šarić (2016) examined the content of anthocyanins (total and monomeric), total soluble polyphenols, and flavonoids in blueberry and raspberry fruits and presented the results in percentages. According to all the above parameters, blueberry trop

showed higher antioxidant capacity. The content of total soluble polyphenols was about 3 times higher, the content of total flavonoids about 1.7 times higher, while the largest difference was found in terms of the content of total anthocyanins, which were present in blueberry samples about 4.5 times higher than the samples raspberries. In dried blueberry samples, flavonoids accounted for 79.9% of total soluble polyphenols, and 43.2% of total anthocyanins. In the polyphenolic profile of dry extracts examined by Tumbas (2010), it was found that anthocyanins make up 28.7% of the total soluble polyphenols of dried fruit, while the share of flavonoids was 82.2%.

CONCLUSION

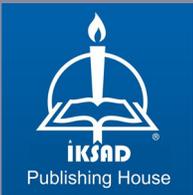
By monitoring and examining the phytochemical parameters of berries such as total phenols, tannins, flavonoids, anthocyanins, proanthocyanidins, and obtaining the results of biochemical analyzes, a clearer picture of them was obtained. The analysis of the results justifies the assumption that more tannins, flavonoids, proanthocyanidins, phenols will accumulate in plant samples in the first phase of maturity, and thus the expectation that the amount of these substances decreases with the growth and development of the fruit is fulfilled. On the other hand, the analysis of anthocyanin results shows that with the growth and development of the fruit, the amount of these substances increases as well as the antioxidant potential. As expected, the obtained results gave a scientific contribution to the study of the chemical composition of the fruits of this species, especially from the aspect of functional food sources. Considering the obtained results, ie the rich chemical

composition of red fruits, it can be concluded that it has a large antioxidant capacity important for preventing the development of various types of diseases. Today's way of life, which includes great mental loads, insufficient amounts of sleep, a monotonous diet, and the consumption of excessive amounts of alcohol, coffee, and cigarettes, greatly contributes to the increase of oxidative stress in our bodies. Therefore, it is recommended to consume red fruits as one of the basic sources of functional food.

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