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# Climate Change, Wildfires and Fir Forests in Greece: Perceptions of Forest Managers

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## Abstract

**Background and Purpose:** The potential impact that climate change may have on fire regime in ecosystems that are not fire-dependent emerges from fires that are nowadays spreading over higher altitudes and northern latitudes. The effects of fire occurrence in high elevation forests of Greece became apparent during the last few years when a number of large forest fires burned a significant number of high altitude fir ecosystems. This paper describes a study that investigated perceptions of wildfire risk to fir ecosystems of Greece in the context of climate change among a sample of Greek forestry experts by conducting personal interviews.

**Materials and Methods:** A total of 63 forest experts answered the developed survey from 43 different forest management units covering all forestry agencies which manage fir forests in the country. The perceived fire risk and management to fir forest ecosystems was assessed by means of a subset of scales previously identified as relevant to climate change and to all fire management aspects, such as fire prevention, fire suppression and post fire treatments.

**Results:** Increased fire risk was rated as a quite important issue in fir forests during the last few years, while the adapting options to climate change are not implemented mainly due to budget and personnel constrains. According to forest experts, fire prevention in fir forests under climate change should be focused on public awareness and fuel management. Nevertheless, the survey results indicate that there is also a need for specific fire prevention measures such as the type of logging activities and other technical measures. The result of this survey highlighted the need for the establishment of a new firefighting organization and for the better coordination of all involved parties during fire suppression.

**Conclusions:** The findings of the current study can be critically important in determining how forest fire management considerations are incorporated into forest management plans and policies under climate change.

**Keywords:** climate change, fir forests, fire management, Greece, survey, wildfires

## INTRODUCTION

Changes in climate, environment and management are altering the world's ecosystems. Forests are of particular importance in this context due to the significant economic, ecological and cultural services they provide. Forests are therefore a public concern, and projecting the development of forests under changing environmental and societal conditions is crucial for developing sustainable forest management strategies [1, 2].

Recent climate models predict that the most likely evolution of Mediterranean basin is towards a hotter and drier climate, with a significant increase in fire hazard and occurrence. Greece is considered a "hot spot", not only because of its high sensitivity to changes in recent decades, such as the processes of rural depopulation, land abandonment and the reduction of traditional forest use, but also for the predicted climate change [3, 4]. According to recent climatic data [5, 6], increased heat waves events, when combined with droughts, can result in large and severe forest fires [7, 8]. Climate projections for 2070–2100 confirm a significant increase of fire potential for Europe, an enlargement of fire prone areas and a lengthening of fire season [9].

Fire statistics show a significant increase in both the number of wildfires and the area burned in Greece. The number of fires doubled and the area burned tripled during the last few years (mainly after 1990) and several reasons have been speculated for this augmentation in wildfire activity, such as the changes in population activities, socioeconomic conditions, land use, fuel accumulation, drought frequency and duration [10]. The increase of the burned area demonstrates that the last few years' wildfires occurred in a more severe mode in terms of fire behavior parameters, such as fire size, fire rate of spread and fireline intensity, thus creating

major difficulties in fire suppression efficiency [11]. Additionally, increased concern has been raised on issues related to the potential impact that climate change may have on the fire regime in ecosystems that are not fire-dependent [12, 13]. The evidence for this emerges from fires that are nowadays spreading over higher altitudes and northern latitudes. In these environments, forest ecosystems are not resilient to fire, since they have not been evolutionary exposed to its frequent action [14]. Dimitrakopoulos *et al.* [15] reported a statistically significant increase in the mean annual number of fires and the burned area in the relatively more humid and colder regions of Greece during the last few years. Additionally, laboratory studies revealed that fire-stricken low elevation forest species do not demonstrate higher flammability traits than the high elevation non fire-stricken species, and that the main factor of increased fire occurrence in high elevation Mediterranean forests could be attributed to climate change [16].

One of the most important forest species in high elevation forests of Greece is fir (*Abies* taxa) Among the fir forests occurring in the Mediterranean Basin, the endemic to Greece *Abies cephalonica* Loudon (Greek fir) can be found at medium and high altitude mountains of the continental Greece and in the islands of Cephalonia, Peloponnesos and Eubea. On the Greek mainland, the hybrid species of *Abies-borisii-regis* Mattf. occur, while at the northern range limit, the hybrid populations mostly resemble *Abies alba* Mill. and grow together with individuals of this species, while at the southern range limit they mostly resemble *Abies cephalonica* [17]. The total forest area for both *Abies cephalonica* and *Abies-borisii-regis* is estimated at almost 200,000 ha [18]. According to the European Forest Types classification scheme [19], these forests are included in category 6.10.6 'Mediterranean and Anatolian

fir forest'. Figure 1 shows the spatial distribution of fir forests in Greece. Overall, fir dominated forests feature a comparatively low fire danger, since they mostly occur in areas with moderate to high rainfall. The high air relative humidity, along with the rather dense closure of fir stands which intercept radiation, results in relatively high moisture contents of surface fuels. This makes fire ignition and spread less severe and intense. However, there is some evidence that, under certain conditions, fir forests could become susceptible to intense fires. A prolonged drought period may be a prerequisite for such fires in fir forests. In contrast to broad-leaved and pine forests, where self-pruning is common, little branch-pruning occurs in fir stands. Thus, there is a substantial ladder fuel from the forest floor to the tree crown, which increases the probability of crowning should a fire occur [20].

The effects of fire occurrence in high elevation forests of Greece became apparent in 2007 when more than 270,000 ha were totally burned and a large number of high altitude forest ecosystems were affected. A large fire on Parnitha Mountain, on the outskirts of the Athens urban area, destroyed approximately 2,180 ha of fir forest. During the same year, a fire in Tayetos Mountain burned 4,500 ha of Greek fir and Black pine forests [21]. Greek fir is vulnerable to fire since it does not produce serotinous cones and does not maintain a canopy seed bank when summer wildfires occur [22, 23].

Several studies which analyze forest and land managers' perspectives regarding the climate change risk in forest ecosystems exist [24-27]. However, knowledge on adaptive forest fire management under climate change is lacking

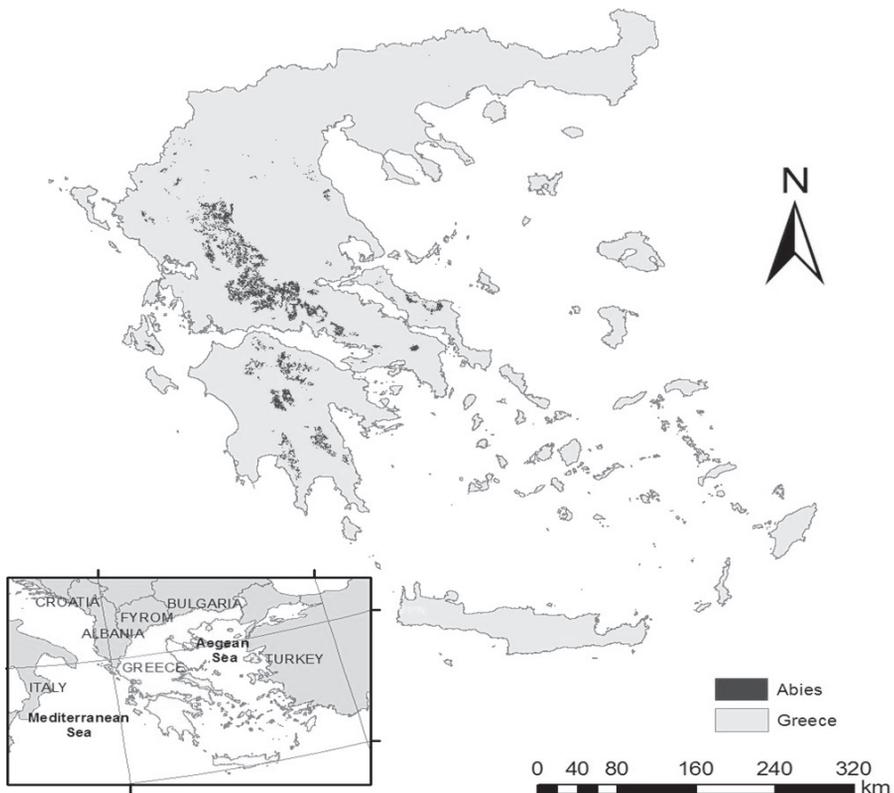


FIGURE 1. Geographical distribution of fir forests in Greece

and/or is limited only to Mediterranean forest ecosystems [28]. Forest managers play a critical role in the adaptive capacity of forests to factors such as climate change and wildfire risk, since they are employed by key forest decision-making agencies. Consequently, their perceptions may be critically important in determining how forest fire management considerations are incorporated into forest management plans and policies under climate change. Documenting the perspectives of forest experts could therefore provide useful insights into the state of knowledge and practice on climate adaptation on fires within fir forest ecosystems [29, 30].

The main objective of the current study is to present the results of a survey regarding the perceptions of forest managers in Greece about fire management needs and adaptation in fir forests under a changing climate.

## MATERIALS AND METHODS

The respondents for this study were Greek forest managers employed in public forest management agencies. Therefore, the respondents represent people with an above-average interest in climate change and an excellent understanding in wildfire risk and its potential implication for fir forest ecosystems. The perceived fire risk and management to fir forest ecosystems was assessed by means of a subset of scales previously identified as relevant to climate change and fire management.

The questionnaire consisted of four parts:

- Part A: Current status, knowledge gaps and needs (8 items)
- Part B: Fire prevention in fir forests under climate change (6 items)
- Part C: Fire suppression in fir forests under climate change (1 item)
- Part D: Post fire restoration and rehabilitation of fir forests under climate change (3 items).

The questionnaire was answered by conducting personal interviews and by visiting all forest authorities which manage fir forests in Greece. Respondents were asked to rate the

importance of each of the adaptation options of forest fire management in fir forests under a changing environment, using a scale from 1 to 5 (1=not important; 2=not so important; 3=important; 4=very important; 5=most important). The duration of the interviews varied from 30' to 1 h due to the flexibility of the survey and the effort made to deepen the understanding of personal views. In total, 63 forest experts answered the questionnaire from 43 different forest management units. The mean with its standard deviation (SD) and the coefficient of variation (CV) were calculated and presented.

## RESULTS AND DISCUSSION

### Current Status, Knowledge Gaps and Needs

In the beginning, the respondents were asked questions aimed at establishing their general attitudes and beliefs about climate change (Table 1). The responses to the attitudinal questions revealed that most of the respondents agreed that climate change which has already affected or may affect fir forests in the near future, is already happening and that climate change is a serious or a very serious problem. The respondents were also asked to report how well-informed they were about climate change. They claimed to be either moderately or well-informed about these topics (mean: 3.08, SD: 0.93, CV: 30.2%). Additionally, most of the respondents believe that the funding research regarding climate change and the guidelines for adaptation to fir forests are very important components for managing these ecosystems under a regional climate change.

To understand why the adaptation planning to climate change in fir forests is not taking place more widely, respondents were asked to rank the most important factors preventing them from planning the adaptation. Overall, budget and personnel constraints were the two most common answers as well as the two options with the highest rankings (Table 2a). Additionally, lack of information is also considered quite

**TABLE 1.** General perspectives about climate change and fir forests

Questions (Ratings 1-5)	Mean	Standard Deviation	Coefficient of Variation (%)
a) Do you believe that climate change has affected or may affect in the near future the fir forests in your area?	3.68	1.02	27.7
b) How important do you consider the adaptation of forest management in fir forests after having taken account of climate change?	3.95	0.84	21.3
c) How well informed you feel are about climate change in fir forests?	3.08	0.93	30.2
d) How important are the regulations of the General Secretariat of Forests to provide issues for adapting fir management in relation to climate change?	4.08	0.91	22.3
e) In your opinion, how important is the research on climate change in fir forests to be financed by national and EU funds?	4.46	0.79	17.7

important (mean: 2.73, SD: 1.20, CV: 43.9%). To assess the perceptions of local vulnerability to climate change, the respondents were asked to rate the importance of five potential impacts of climate change on the fir forests managed by their office. The intent of these questions was to understand how managers were viewing climate change and fir forests in the context of their particular region. Five of the potential consequences that had been offered were: fire risk increase, fir decline and dieback, infestations by pathogens, insects' attacks and the decrease of timber production (Table 2b). The most important effect of climate change in fir forests was considered to be fir decline and dieback (mean: 4.15, SD: 0.91, CV: 21.9%) followed by infestations by pathogens and insects attack. This could be expected since the decline of Greek fir as a consequence of climate change in terms of drought has often been reported throughout Greece during the last three decades [31]. Fire risk and the decrease of timber production were perceived as the least moderately possible and believed to have moderate possibility of happening as a result of climate change in fir forests of their region. One of the main goals of this research was to identify the most important measures which have to be taken related to climate change adaptation of

fir forests on the ground. The respondents were asked to rank the most important management strategies or plans to deal with the potential impacts from climate change in fir forests. The survey offered eight potential adaptation measures (Table 2c). In their opinion, immediate logging of infected trees (sanitary logging) was by far the most important adaptation measure. The limitation of grazing, the application of selective logging and the thinning, as well as the changes in species composition, were also rated as important. Sanitary logging (the removal and disposal of infected trees) is a common practice implemented at areas suffering from epidemic bark beetle or fungi outbreaks. However, during logging activities, the creation of irregular stand structure and gaps in the forest should be avoided, as sanitary logging can even cause alterations in stand structural complexity, community composition and species populations [32]. The limitation of grazing both by wild and domestic mammals is considered to be necessary for the protection of regeneration and soil resources [33]. Grazing has a negative impact on the understory as well as on the regeneration of species, as it is considered to be an important regulatory factor especially following disturbances, such as forest fire. Thus, the fencing of areas that are under regeneration

**TABLE 2.** Perspectives of climate change as risk to fir forests

Perception (Ratings:1-5)	Mean	Standard Deviation	Coefficient of Variation (%)
<i>a) Which are the most important factors that prevent you from planning for adaptation in fir forests to climate change?</i>			
1) Budget constrains	4.29	0.85	19.8
2) Personnel constrains	4.28	0.84	19.6
3) Lack of information and knowledge	2.73	1.20	43.9
4) Not high priority in my responsibility area	2.13	1.19	55.8
<i>b) Based on your knowledge, which are the most important impacts of climate change affecting fir forests in your responsibility area?</i>			
1) Fire risk increase	2.61	1.13	43.3
2) Fir decline and dieback	4.15	0.91	21.9
3) Infestations by pathogens	2.98	1.05	35.2
4) Insects attack	2.86	1.15	40.2
5) Decreased timber production	2.57	1.22	47.4
<i>c) What are the most important forest management measures need to be taken to adapt fir forests to climate change?</i>			
1) Immediate logging of infected trees (sanitary logging)	4.35	0.90	20.7
2) Changes in species composition by introducing tolerant in climate change species	3.16	1.40	44.3
3) Irrigation of specific fir stands when necessary	1.88	1.17	62.2
4) Application of selective logging and thinning	3.31	1.19	35.9
5) Enhancement of regeneration (either natural regeneration or by seeding/planting)	2.95	1.34	45.4
6) Extension of rotation period	2.47	1.10	44.5
7) Thinning targeting to better balance of water in fir stands	2.62	1.20	45.8
8) Limitation of grazing	3.47	1.19	34.3

process and the adjustment and rationalization of grazing by domestic mammals or control of wild animals' populations are needed. The application of selective logging and thinning was important as recommended by the respondents. It has been found that they can reduce both inter- and intraspecific competition for water, light and nutrients and thus render species less vulnerable to the attacks of pathogens [34]. Moreover, water balance within the forest ecosystem is restored, and negative impacts from droughts and forest fire are mitigated [35].

### Fire Prevention in Fir Forests under Climate Change

Tables 3 and 4 present a series of questions that intend to elicit insights about general and

specific adaptation to climate change measures for the prevention of forest fires in fir forests. Most of the responders indicated public awareness raising, fuel management and the strength of fire suppression in personnel and infrastructure as the most important general adaptation measures to climate change in fir forests. However, measures such as the update of fire weather index in the present climate conditions and the application of different silvicultural treatments (logging) in fir forests are also considered quite important (Table 3).

In order to gain a better insight into fire prevention adaptation measures in fir forests, the respondents were asked a series of more specific questions (Table 4). Information spots on TV and radio, the education of students in schools,

**TABLE 3.** Perspectives of climate change and fire prevention to fir forests

Perception (Ratings:1-5)	Mean	Standard Deviation	Coefficient of Variation (%)
<i>What in your opinion are the most important general adaptation measures for the prevention of forest fires in fir forests in your area under climate change?</i>			
1) Strengthening fire suppression in personnel and infrastructure	3.98	1.11	27.8
2) Raising public awareness	4.19	0.94	22.4
3) Fuel management	4.09	0.96	23.4
4) Application of different logging techniques	2.81	1.18	41.9
5) Update of the fire weather and danger system	2.98	1.19	39.9
6) Technical and legislative measures	2.73	1.12	41.1

signs and information within the fir forests and the distribution of informative material for the visitors have been rated from important to very important specific fire prevention measures in terms of raising public awareness with ranking means varying from 3.30 to 4.60. Fire prevention programs in Greece should be addressed to public awareness, since the 97% of the fire causes in the country are attributed to human activities such as arsons and negligence [10]. Forest managers strongly believe that the local population and the tourists should be educated about how a warmer climate may increase fire frequency, become aware of the threats of wildfires to the security and health of their families, as well as to private property and public infrastructures, and to learn how to reduce or mitigate these potential threats.

The respondents did not rate fuel management very important for reducing fire risk in the context of climate change in fir forests. This is mainly due to the fact that in most areas in Greece, fir forests are not managed intensively and another reason lies in the fact that fir forests are usually located within NATURA 2000 areas, where a number of restrictions on management practices in forest areas exist [36]. To assess the perceptions of adapting fuel management in fir forest to climate change, the respondents were asked to rate the importance of nine fuel management treatments on the fir forests managed by their office. According to the respondents, the options of thinning, pruning and slash removal after loggings were by far the

most important fuel management adaptation measures. Understorey removal, favoring the mixed stands with broadleaved species and clearing the dry grasses in fir forest stands were also rated as quite important by the responders. Thinning and pruning in conifer stands can substantially reduce fire potential, since they modify stand structure and prevent the spread of surface fire and crown fire activity [37], as well as increase the resistance to drought and insect attack [38]. On the contrary, fuel management strategies such as prescribed burning and controlled grazing were rated as less important although there are a lot of studies which highlight the importance of these two fuel management strategies in reducing fire potential. Prescribed burning has been widely used to reduce surface fuels and, thus, decrease fire hazard and fire severity [39]. In Europe, prescribed burning which has been carried out in western Mediterranean (Portugal, Spain) and which has also been proved as a fast and cheap method for reducing dead and live biomass, from an ecological perspective, surrogates the natural impact of low to medium intensity fires [40]. The respondents ranked prescribed burning as a less important fuel management strategy due to the fact that is not allowed in Greece, and, consequently, there is no such field experience. Moreover, they stated their concerns about the potential damage that might occur if such fires escaped, which seemed to far outweigh any benefit they might offer. A rational fuel management in fir forests under a changing climate is essential for combining both surface

(understory removal, controlled grazing etc.) and canopy fuel treatments (thinning, pruning). The combination of these fuel treatments must be followed by the removal of residues from logging and thinning. Otherwise, the contribution of the canopy fuel to the surface fuelbed could easily lead to crown ignition. The need of raising public awareness and the applying of fuel management techniques were also considered the most important climate change adaptive fire prevention measures by the respondents in a similar work, which referred to Mediterranean low elevation and fire stricken forests [28]. The authors stated different priorities in adaptive fire management options among several countries in Mediterranean basin. Nevertheless, their survey indicated that there is a need for an integrated fire management. The respondents also rated as important the application of selection cuttings and irregular shelterwood cuttings for preventing severe fire occurrence in fir forests. They justified their opinion by stating that these types of logging focus on water balance improvement and at drought effects mitigation and, sequentially, they would strengthen fir forests resistance against wildfires.

The respondents were also asked to rank the most important fire detection measures that should be adapted for fire prevention strategies in fir forests. Although a lot of new technologies (remote sensing, thermal sensors, and fire weather systems) exist, they ranked as the most important measures the traditional ones, such as the surface patrols in the forests and the fire observation towers. The application of new technology fire detection systems in Greece is limited, and operated mainly in local level, while robust evaluation is lacking. Recent works seem promising [41], but a long term evaluation is needed in order to make them widely applicable in an operational basis.

In Greece, technical and legislative measures are very important in fire prevention due to the strong influence of socioeconomic factors and changes which occurred recently and affected fire occurrence in the country [10]. The respondents were asked to rank the most important technical and legislative strategies or plans to deal with

fire prevention in fir forests under climate change. The survey offered eleven potential adaptation measures (Table 4e). According to the respondents, the options of the maintenance and expansion of forest road network and the creation of water trunks were by far the most important adaptation measures. The development of fire management plans, preventive actions in electric power poles and networks of firefighting hydrants were also rated as important. Greece has a long tradition in fire suppression practices and all the above mentioned measures are considered important in fire prevention mainly in facilitating fire suppression tactics for reducing initial attack time. Fir forests are mainly located in mountainous and remote areas, and most of the responders stated that technical measures and the improvement of the infrastructure within and around the forest stands will substantially improve fire management under the expected climate change. Other adaptive measures such as the improvement of brake materials on railways, the improvement of the machines which use internal combustion engines and the creation of safety smoking devices for use in bee-hives were rated less important. This can be attributed to the different existing agricultural and social conditions among the various fir ecosystems in the country.

### **Fire Suppression in Fir Forests under Climate Change**

Table 5 presents the responds of the forest managers' perspectives and fire suppression in fir forests under climate change. To understand the low effectiveness of fire suppression forces in Greece, the respondents were asked to rank the most important factors which should be taken into consideration for a successful firefighting in fir forests. Overall, the establishment of a new firefighting organization and the improvement of coordination among all involved parties during fire suppression were the two most common answers as well as the two options with the highest rankings (Table 5). Additionally, the improvement of firefighters' and volunteers' training was reported as an important factor (mean: 3.80, SD: 1.10, CV: 29.2%). The

**TABLE 4.** Perspectives of specific prevention measures for adaptation of fir forests to fires under climate change

Perception (Ratings:1-5)	Mean	Standard deviation	Coefficient of Variation (%)
<i>a) What in your opinion are the most important adaptation measures for the information and awareness of forest fires in fir forests in your area under climate change?</i>			
1) Informative Spots on TV and radio	3.94	0.96	24.3
2) Education of students in the schools	4.60	0.75	16.3
3) Signs and information within the forests	3.53	1.20	33.9
4) Distribution of informative material to visitors	3.30	1.19	36.1
<i>b) What in your opinion are the most important adaptation measures for fuel management in fir forests in your area under climate change?</i>			
1) Pruning	3.59	1.29	35.9
2) Thinning	3.77	1.15	30.5
3) Understory removal	3.20	1.23	38.4
4) Fuelbreaks	2.61	1.40	53.6
5) Controlled grazing	2.87	1.22	42.5
6) Prescribed burning	1.92	1.08	56.2
7) Favoring of mixed stands with broadleaved species	3.11	1.35	43.4
8) Slash removal	3.44	1.17	34.1
9) Clearing of dry grasses to prevent fuel accumulation	3.09	1.43	46.2
<i>c) What in your opinion is the most important way of implementing logging in fir forests in your area to prevent forest fires under climate change?</i>			
1) Clear-cuttings	1.12	0.55	49.1
2) Strip shelterwood cuttings	2.04	0.99	48.5
3) Irregular shelterwood cuttings	3.65	1.09	29.8
4) Selection cuttings	4.28	0.97	22.6
<i>d) What in your opinion are the most important fire detection measures for the prevention of forest fires in fir forests in your area under a climate change?</i>			
1) Surface patrols in the forests	4.50	0.79	17.5
2) Fire detection from the air	3.06	1.20	39.2
3) Fire towers	4.09	1.10	26.8
4) Fire weather and danger systems	3.04	1.30	42.7
5) Systems and networks of thermal detection sensors	3.06	1.35	44.1
<i>e) What, in your opinion, are the most important technical and legislative measures to prevent fires in fir forests in your area under climate change?</i>			
1) Development of fire management plans	4.04	1.05	25.9
2) Maintenance and expansion of forest road network	4.42	0.81	18.3
3) Creation of water tanks	4.27	0.72	16.8
4) Networks of firefighting hydrants	3.79	1.19	31.3
5) Building heliports near fir forests	2.60	1.31	50.3
6) Improvement of legislation regarding land use and property	3.33	1.19	35.7
7) Improvement of brake materials on railways	1.90	1.01	53.1
8) Improvement of machines that use internal combustion engines (e.g., chainsaw)	2.22	1.02	45.9
9) Create safety smoking devices for use in bee-hives	2.46	1.05	42.6
10) Rules and regulations for citizens safety in fir forests during fire season	3.61	1.17	32.4
11) Preventive actions in electric power poles (insulated wire, pruning trees touching the wires, cleaning around transformers)	4.03	1.18	29.2

establishment of a new firefighting organization has received the highest ranking of importance among the responders (mean: 4.47, SD: 1.01, CV: 22.5%). This is not a surprising finding. In 1998, the Greek government decided to transfer the responsibility of forest firefighting from the Forest Service to the Fire Service with no provision for cooperation between the personnel of the Forest and the Fire Services at all levels. Over the next years, Greece faced large number of devastating fire events (1998, 2000, 2007 and 2009) and that decision is still in dispute in terms of its effectiveness by the forest managers, since firefighting officers did not have any experience on forest fire incidents in the past. However, at the same time, forest managers believe that the transfer of firefighting responsibility again to Forest Service is less important (mean: 2.74, SD: 1.76, CV: 64.2%). Moreover, the standard deviation and the coefficient of variation for this question presented high values, suggesting greater disagreement among respondents. Currently, as time passes since the deprivation of forest fire responsibility from the forest service, the collective knowledge and mostly the experience of its personnel on forest fires, is being lost. Furthermore, approximately one fourth of its personnel as well as many pieces of equipment (vehicles, radios etc.) were moved to the Fire Service without any replacement. This is negative both in regard to better firefighting efficiency in the future, as well as in regard to use of backfire technique during fire suppression (mean 2.38, SD: 1.15, CV: 48.3%). In Greece, during fire events, a number of organizations (Fire Service, the General Secretariat for Civil Protection, the Police, the Air Force, the Army, municipalities and other local authorities) are involved without having clear duties and responsibilities. This fact made the fire suppression system very complex and ineffective [42]. The respondents claimed that the improvement of coordination among all involved parties during fire suppression is very important for suppressing fires in fir forests under climate change (mean: 4.44, SD: 0.83, CV: 18.6%).

Although, a lot of different fire suppression schemes exist among the Mediterranean countries, forest experts and managers recognize

the improvement of firefighting coordination as the most important adaptation measure in forest fire management under climate change [28]. Fire suppression organization in Mediterranean basin has failed to mitigate extreme fire seasons (e.g., Portugal 2003, 2005; Greece and Italy 2007), despite its increased funding, a large number of permanent and seasonal personnel and the infrastructure in terms of ground and aerial means in the last few years. For that reason, forest managers strongly highlighted that fire prevention activities are the most important component of forest fire management under climate change compared to fire suppression. This is also in agreement with a similar study in the area [43].

### **Post Fire Restoration and the Rehabilitation of Fir Forests under Climate Change**

Table 6 summarizes the responses to the questions about perceived post fire management to fir forests under climate change. The respondents perceived the protection of fir natural regeneration (mean: 4.36, SD: 0.92, CV: 21.1%) and the protection of soils (mean: 4.31, SD: 1.06, CV: 24.5%) as having the most significant importance (Table 6.a). The degree to which the respondents felt that post fire seeding and planting would be a good choice to climate change in fir forests exceeded the midpoint value (mean 3.25, SD: 1.24, CV: 38.1%). The ratings for the degree to which the respondents felt that post fire logging of standing dead trees is an important post fire rehabilitation measure in fir forests under climate change were near the midpoint (mean: 2.81, SD: 1.25, CV: 44.4%). Post fire logging poses a large debate among fire scientists and managers worldwide. After stand-replacing wildfires, there is often considerable conflict when post-fire logging is proposed. Harvesting fire-killed trees immediately after wildfire can provide economic benefits to local communities and may reduce risks of insect and disease outbreaks that can kill additional trees [44]. However, there is concern that the ecological costs of post-fire logging may outweigh the economic benefits [45].

**TABLE 5.** Perspectives of climate change and fire suppression to fir forests

Perception (Ratings:1-5)	Mean	Standard Deviation	Coefficient of Variation (%)
<i>What, in your opinion, are the most important measures for fire suppression in the fir forests of your area under climate change?</i>			
1) Transfer of firefighting responsibility to forest service	2.74	1.76	64.2
2) Establishment of a new firefighting organization	4.47	1.01	22.5
3) Use of backfire during fire suppression operations	2.38	1.15	48.3
4) Improvement in training of firefighters and volunteers	3.80	1.11	29.2
5) Improvement in coordination of all involved parties during fire suppression	4.44	0.83	18.6

**TABLE 6.** Perspectives of post fire management measures for adaptation of fir forests under climate change

Perception (Ratings:1-5)	Mean	Standard Deviation	Coefficient of Variation (%)
<i>a) What in your opinion are the most important post fire rehabilitation measures in fir forests in your area under climate change?</i>			
1) Protection of soil resources	4.31	1.06	24.5
2) Protection of natural regeneration	4.36	0.92	21.1
3) Logging of standing dead trees	2.81	1.25	44.4
4) Seeding/planting	3.25	1.24	38.1
<i>b) What in your opinion are the most important post fire soil protection measures in fir forests in your area under climate change?</i>			
1) Contour-felled logs	4.01	1.11	27.6
2) Contour branch barriers	3.95	1.08	27.3
3) Log check dams	3.77	1.16	30.7
4) Concreted dams	1.82	0.96	52.7
<i>c) What, in your opinion, are the most important post fire regeneration measures in fir forests in your area under climate change?</i>			
1) Prohibition of grazing within the burned areas	4.60	0.81	17.6
2) Declaring the reforestation of all burned areas	4.57	0.91	19.9
3) Accurate burned area mapping	4.22	1.18	27.9
4) Leaving the standing dead trees	2.14	1.11	51.8
5) Facilitation of fir regeneration by introducing other species for favoring its natural regeneration (creation of shading conditions)	3.65	1.15	31.5

The respondents were also asked to rank the most important factors which should be taken into consideration for successful post fire soil protection in fir forests. Overall, the establishment of contour-felled logs, contour branch barriers and the log check dams were the two most common answers as well as the two options with the highest rankings (Table 6b). On the contrary, the establishment of concreted dams was re-

ported as a less important factor (mean: 1.82, SD: 0.86, CV: 52.7%) mainly due to ecological restrictions. Effectiveness assessment of various site rehabilitation treatments to reduce post fire soil erosion is critical in forest ecosystems. Based on their experience the responders suggested the contour-felled logs and contour branch barriers as the most important ones. However, there are studies which state that contour-felled

logs are effective in trapping sediments on steep slopes [46] and for small rain events [47], while they are ineffective in other slopes and for large storms [48]. To assess the perceptions of adapting post fire regeneration of fir forest to climate change, the respondents were asked to rate the importance of five post fire regeneration treatments on the fir forests managed by their office (Table 6c). The respondents suggested prohibition of grazing within the burned areas, declaring the reforestation of all burned areas and accurate burnt areas mapping as the most important measures. The degree to which the respondents felt that post fire facilitation of fir regeneration by introducing other species for favoring its natural regeneration would be a good choice to climate change in fir forests exceeded the midpoint value (mean 3.65, SD: 1.15, CV: 31.5%). The latter choice has been justified by the fact that fir species are shade-demanding with a low growth rate during its early stages, making its post fire establishment difficult, even artificially. Moreover, fir species are obligate seeders, while the seeds produced ripen in autumn [23] thus, after a fire event; there are no mature seeds to ensure regeneration. This fact could lead to an increased risk of non-potential reestablishment of the burned forest due to regeneration failure, and burned forests are likely to turn into scrublands and/or grasslands. Therefore, their natural post-fire recovery is limited, and strongly dependent on seed dispersal from neighboring unburned individuals or patches [14]. More recent results also showed that early post fire regeneration succession in burned fir forests of Greece is characterized by species adapted to wildfires such as shrubs and herbaceous [49]. Furthermore, the respondents rated as less important to leave the standing burnt trees in order to facilitate the natural regeneration of fir seedlings (mean: 2.14, SD: 1.11, CV: 51.8%). Most of the respondents stated that as standing dead trees would start to decompose; they may become more fractured and flammable, increasing the duration of flaming and smoldering combustion, and increasing tree mortality and soil heating in potential future fire events.

## CONCLUSIONS

The perceptions of forester managers in Greece were investigated on the issues of climate change, wildfires and adaptive measures facing the increased risk of fir forests. The survey consisted of a broad set of questions covering all aspects of fire management such as fire prevention, fire suppression and post fire treatments, and it had been conducted in all forest units which manage fir forests in the country. Increased fire risk was rated as a quite important issue in fir forests during the last years, while adapting options to climate change are not implemented mainly due to budget and personnel constrains. According to forest experts, fire prevention in fir forests under climate change should be focused on public awareness and fuel management. Nevertheless, the survey results indicate that there is also a need for specific fire prevention measures such as the type of logging activities and other technical measures. The result of this survey highlighted the need for the establishment of a new firefighting organization and for the better coordination of all involved parties during fire suppression. The main reason for such responds is likely to be the pitfalls and problems which the current fire suppression system in the country shows. An improved communication between science and management through the funding research has also been rated as critical for supporting forest managers in their response to climate change, especially in post fire management of fir forests

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# Assessment of Forest Damage in Croatia using Landsat-8 OLI Images

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## Abstract

**Background and Purpose:** Rapid assessments of forest damage caused by natural disasters such as ice-break, wind, flooding, hurricane, or forest fires are necessary for mitigation and forest management. Forest damage directly impacts carbon uptake and biogeochemical cycles, and thus, has an impact on climate change. It intensifies erosion and flooding, and influences socio-economic well-being of population. Quantification of forest cover change represents a challenge for the scientific community as damaged areas are often in the mountainous and remote regions. Forested area in the western Croatia was considerably damaged by ice-breaking and flooding in 2014. Satellite remote sensing technology has opened up new possibilities for detecting and quantifying forest damage. Several remote sensing tools are available for rapid assessment of forest damage. These include aerial photographic interpretation, and airborne and satellite imagery. This study evaluates the capability of Landsat-8 optical data and a vegetation index for mapping forest damage in Croatia that occurred during the winter of 2014.

**Materials and Methods:** The change detection analysis in this study was based on the Normalized Difference Vegetation Index (NDVI) difference approach, where pre- and post- event Landsat-8 images were employed in the ENVI image change workflow. The validation was done by comparing the satellite-generated change detection map with the ground truth data based on field observations and spatial data of forest management units and plans.

**Results:** The overall damage assessment from this study suggests that the total damaged area covers 45,265.32 ha of forest. It is 19.20% less than estimated by Vuletić *et al.* [3] who found that 56,021.86 ha of forest were affected. Most damage was observed in the mixed, broadleaf and coniferous forest. The change errors of commission and omission were calculated to be 35.73% and 31.60%, respectively.

**Conclusions:** Landsat-8 optical bands are reliable when detecting the changes based on the NDVI difference approach. The advantage of Landsat-8 data is its availability to acquire data and detect

changes within a few days after an event. The data are publicly available and free of charge. The spatial resolution of 30 m is fine enough for a rapid assessment of forest damage. Merging different optical sensors (e.g. Landsat and Sentinel-2), or, considering active and/or thermal remote sensing satellite imagery would be necessary for monitoring damaged areas during winter time.

**Keywords:** ice break, floods, forest, remote sensing, Landsat-8

## INTRODUCTION

Every year we observe forest damages caused by natural disasters such as floods, tornados, ice-breaks, windstorm events, forest fires and hurricanes. Due to climate changes, in recent years natural disasters appear to be increasing, which calls for rapid assessment and monitoring of damage and conditions. Change detection studies are important to aid human response to forest cover change. It is important to collect the information about the processes that cause changes as well as patterns and extent of the changes [1]. The regular assessment is necessary for updating forest cover maps and the management of natural resources.

Forest damage directly affects forest ecosystem, as well as other natural processes and socio-economic state and well-being of human population. Assessment of forest damage is important for estimating forest carbon uptake as well as understanding biogeochemical cycles, climate change, soil erosion, and watershed processes. Forests allow for multiple resource benefits that include livestock grazing, wildlife habitats, as well as tourism and recreation. The preservation of forests helps conserve natural habitats and global biodiversity of flora and fauna, while providing other benefits for the public. Recently, because of increased public awareness of natural resource policy, public concerns have shifted toward sustainable forest management and long-term preservation of public lands including urban forests. Urban forest management is receiving a special attention for the reasons of increased health and economic vitalities to many communities. Burban and Anderson [2] emphasized three phases of emergency action for a natural disaster mitigation: 1) preparation - monitoring

the severe weather systems, developing a disaster response plan and allocating responsibilities to the community members; 2) response – immediate activity during and after the disaster such as tree damage clean-up and identifying different methods of better and safe migration of people through the damaged area; 3) recovery - attempts to restore conditions including tree planting and care, and protection of young trees and young forest ecosystem.

Quantification of cover change represents a challenge for management and inventories of forests as the damaged areas are often vast and located in remote mountainous regions [3]. While physical access to the affected areas and ground assessments may be complex, remote-sensing techniques enable a fast regional-scale damage assessment. This technology provides some of the most accurate means of measuring the extent of damaged forest [4-7]. In addition to the spatial dimension, multi-temporal satellite data allow for quantifying the forest cover changes patterns within a spatio-temporal framework for better forest management and policy decision-making.

As summarize by Chehata *et al.* [8], the medium or high spatial resolution sensors are commonly used to detect defoliation [9], clear-cut detection [10-11], or deforestation [12, 13]. Landsat TM and Modis have been applied in the studies related to windfall damage [14, 15] using the pixel based approach. Chehata *et al.* [8] used the object based change detection in windstorm damaged forest using high-resolution multispectral Formosat-2 images. Multispectral images are commonly used to assess the damage in two different approaches 1) based on vegetation indices, 2) using image classification methods. In the former, the trend between vegetation indices, before and after

the event, are commonly compared in a per-pixel approach. The later includes classification of pre- and post-event maps and the differences are based on the changes in land cover classes.

The relationship between the Normalized Difference Vegetation Index (NDVI) and proportion of damaged areas per pixel [16], or, between image texture and the percentage of crown loss, are commonly explored approaches [17, 18]. Traditional pixel-based supervised classification using supervised object-oriented classification and image differencing of vegetation indices are equally used in the forest change detection studies [19, 20].

Desirable indices include numerous Vegetation Indices (VIs) such as the Normalized Difference Vegetation Index (NDVI), fraction of Photosynthetically Active Radiation (fPAR), and Leaf Area Index (LAI). Vegetation indices are computed across all pixels without prior assumption of biome type, land cover condition or soil type [21].

NDVI is a function of near infrared (NIR) and red (R) spectral range and is computed using the following equation [21]:

$$\text{NDVI} = (\text{NIR} - \text{R}) / (\text{NIR} + \text{R})$$

NDVI ranges from -1 (bare land) to 1 (high vegetation cover). Healthy vegetation has low reflectance in the red spectral region due to high absorption of light by chlorophyll and other pigment, and has high reflectance in the NIR due to the internal reflectance by the structural properties of the mesophyll spongy tissue of green leaf. The NDVI is a measure of leaf greenest and the photosynthetic capability of leaves [22]. Any abrupt change in NDVI suggests changes in vegetation canopies and thus, NDVI can be an adequate measure of forest change. Although NDVI can change due to different environmental factors such as soil moisture and the weather, this index is commonly used for the forest damage detection due to non-systematic variation as described by Huete and Liu [23] and Liu and Huete [11].

This study evaluates the capability of Landsat-8 and NDVI derived from this sensor

for mapping forest damage caused by extreme weather events in western Croatia during the winter of 2014.

Landsat-8 is an American Earth observation satellite launched on February 11, 2013 that began normal operations on May 30, 2013. Landsat-8 carries two instruments: the operational land imager (OLI), which includes a deep blue band and a shortwave infrared band for cirrus detection in addition to the existing visible and infrared bands, and thermal infrared sensor (TIRS), which provides two thermal bands. Both sensors provide improved signal-to-noise (SNR) radiometric performance quantized over a 12-bit dynamic range. Landsat-8 data products are consistent with all standard Level-1 data products as explained on the USGS web site [24], where also all details about processing levels of all Landsat data products could be found. The Level 1T data products are terrain corrected datasets with spatial resolution of 30 m for OLI multispectral bands. The Landsat-8 satellite images the entire Earth every 16 days. The data are georeferenced and processed to the Universal Transverse Mercator (UTM) map projection using the World Geodetic System (WGS) 84 datum. The Level 1G data product provide systematic and geometric accuracy, which is derived from data collected by the sensor and spacecraft. The Level 1T provides systematic radiometric and geometric accuracy by incorporating ground control points and applying a Digital Elevation Model (DEM) for topographic accuracy.

The forest damage was observed over five Croatian counties between January 31<sup>st</sup> and February 6<sup>th</sup> 2014 [3]. The damaged resulted from freezing rain and flooding. Freezing rain (ice-breaks) damaged forest stands in the Primorsko-Goranska County. Extreme rain events resulted in excessive flooding in the Zagrebačka County and Sisačko-Moslavačka County. To a lesser extent, the damage was observed in Karlovačka County and Ličko-Senjska County. A state of natural disaster was proclaimed in all five counties. To apply to the European Union (EU) Solidarity Fund, Vuletić *et al.* [3] presented their field methodology for

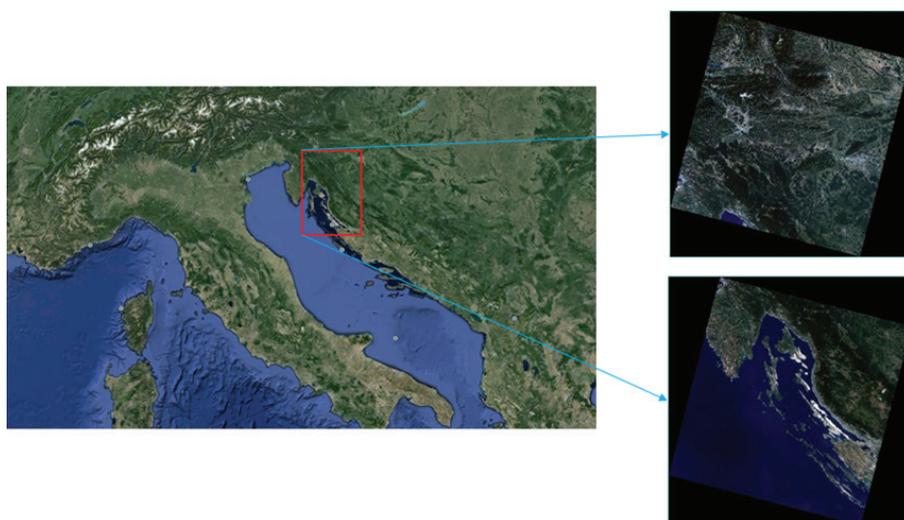
rapid damage assessment and quick recovery of the damaged forested areas. The damage cost assessment included damage of forest and forest ecosystem, forest roads and skid-trails, the cost of recovery, afforestation and post-afforestation protection, as well as the cost of emergency interventions during the event and damage risk prevention.

As stated by Vuletić *et al.* [3], there is no methodology for fast and reliable assessment of forest damage in Croatia. Thus, we decide to explore remote sensing technology and assess the damage using widely available multi-spectral remote sensing technology.

## MATERIALS AND METHODS

The study area was chosen based on the paper of Vuletić *et al.* [3]. The area consisted of five Croatian Counties affected by ice-breaks and flood events in the winter of 2014. The impact severity on the forest organizational units (Forest Administrations - FA) in each county which are managed by Croatian Forests Ltd. was different (Figure 1).

The method used in this study was based on Landsat-8 Level 1T images (paths/rows 190/28 and 290/29) [24]. Data from summer 2013 (August 2013) and 2014 (July 2014) were radiometrically and atmospherically corrected using FLAASH, a module embedded in ENVI software (Figure 2). FLAASH corrects wavelengths in the visible through near-infrared and shortwave infrared regions, up to  $3\ \mu\text{m}$  [25]. The module created a cloud map classification image used during FLAASH processing to refine the calculations. Mid-Latitude Summer (MLS) atmospheric model was used to run the corrections. Several clouds observed on the images were masked and excluded from the calculations. Two Landsat-8 tiles from each year were mosaicked to cover the area of interest (Figure 1). The histogram equalization was applied to avoid any differences in radiometric scheme of different images. Instantaneous NDVI was calculated for each image and the simple image NDVI difference approach was used to detect the forest damage. The images were chosen in the peak of growing seasons when any changes in NDVI are most prominent. Using the ENVI image change flow approach



**FIGURE 1.** Landsat-8 images for the region of interest ( $45^{\circ}33'41.83''$  N and  $14^{\circ}59'47.32''$  E) acquired during the summer 2014 (path/row 190/28 and 190/29). Source: Google Earth and USGS Landsat.

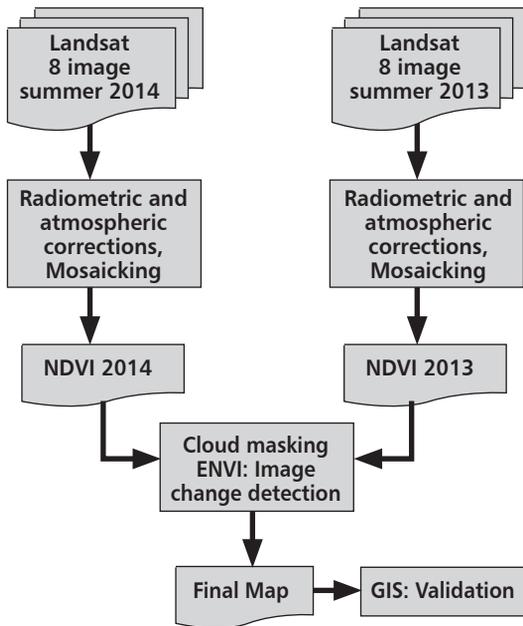


FIGURE 2. Major steps used in the analysis

the NDVI difference image was generated. The default threshold was applied to generate the NDVI difference image between 2013 and 2014. The change detection areas were delineated based on the NDVI default value of zero for change-no change areas. Kernel size of 20 and aggregation minimum size of 100 was used in the ENVI process. The change class image, change class vector, change class statistics and difference image were imported to ArcGIS for further analysis.

ArcGIS was used to compare the findings with the ground truth data collected in 2014 and reported by Vuletić *et al.* [3]. The ground truth polygons were based on the damage intensity of forest stands in accordance with the Methodology for Damage Assessment Caused by Natural disasters, Croatia. The forest units were separated in broadleaf and coniferous forests although many units consisted of mixed forest represented by both broadleaf and coniferous stands. Each forest type was assigned a grade based on the damage intensity and was classified as destroyed or damaged at different levels, using percentages.

The trees were considered damaged when their upper third of the crown were damaged more than 50%. Broken and uprooted trees, and trees with majority of the crown broken were considered as totally damaged (destroyed) trees [3]. The *damaged* forest considered areas where less than 70% of trees were damaged. The *destroyed* forest considered forest stands where damaged trees were estimated to be higher than 70% [3].

The land use map was provided by Corine Land Cover Croatia (CLC Croatia) [26], which was then used to compare the NDVI difference maps and the ground truth polygons for broadleaf and coniferous forest.

## RESULTS AND DISCUSSION

Our results suggest that the area affected by the natural disaster in the western Croatia during the winter of 2014 occupies a total area of 45,265.32 ha (Figures 3, 4 and 5). It is 19.20% less than estimated by Vuletić *et al.* [3] who found that 56,021.86 ha of forest were affected.

Furthermore, Vuletić *et al.* [3] suggested that 19,245.79 ha of forest were seriously damaged, while 9,808.22 ha were totally destroyed. In our findings, based on the NDVI differences and the natural breaks generated in ArcGIS, the

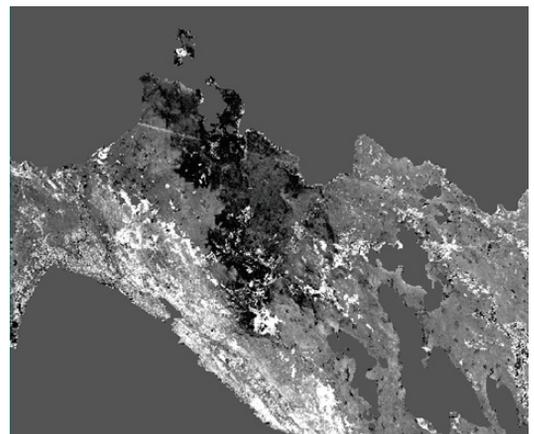


FIGURE 3. NDVI difference map (Dark color represents the damaged area)

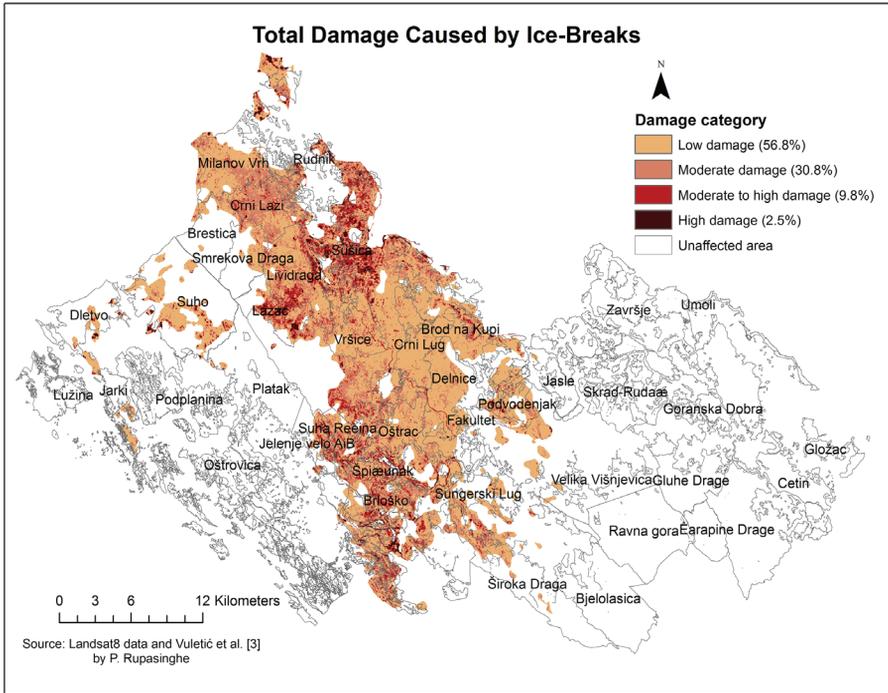


FIGURE 4. Total damaged area generated by Landsat-8 using the NDVI difference approach and classification using ArcGIS natural breaks

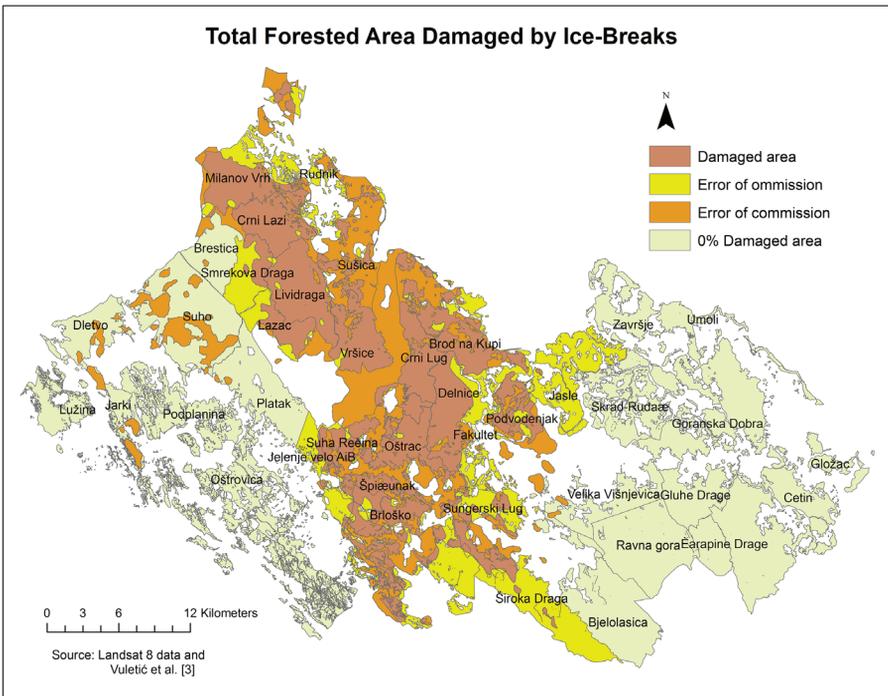


FIGURE 5. Comparison of damage between the remote sensing approach and field method

classification results show that high damaged area occupies 1,151.91 ha, moderate-to-high area occupies 4,448.43 ha, moderately damaged area occupies 13,940.10 ha, and low damaged area consists of 25,724.79 ha (Figure 4). In this approach, the class breaks are identified that best group similar values and that maximize the differences between classes. Differently than for ground truth findings, where the damage variability within each county is not considered, the resulting map considers high variability and shows different levels of damages regardless of administrative boundaries.

The errors of commission and omission associated with change are calculated to be 35.73% and 31.60%, respectively (Figure 5). The errors are observed generally at the edges of the damaged areas, except for a pathway that is running throughout the area. The satellite generated map classified this area as damaged, whereas the same area is excluded in the ground truth maps. Based on the provided land cover map, we have observed that the pathway consists of mixed and broadleaf forest.

As shown in Figure 4, we categorized the levels of damage intensity on the NDVI difference classes in this study. Overall, our study suggests that minimum of 16.24% of broadleaf forest, 24.32% coniferous, and 54.83% of mixed forests were damaged or destroyed by the ice-breaks in 2014.

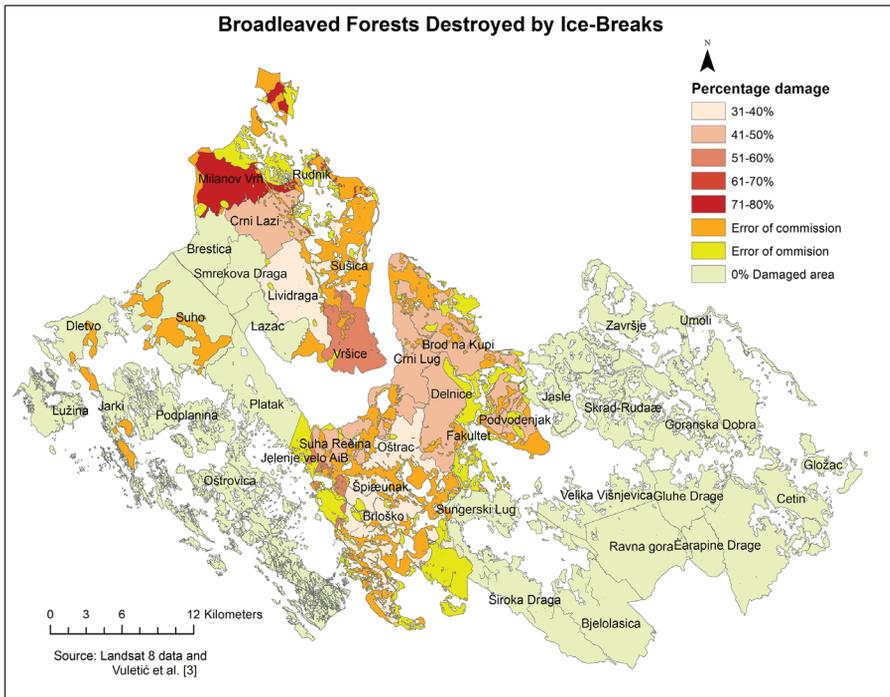
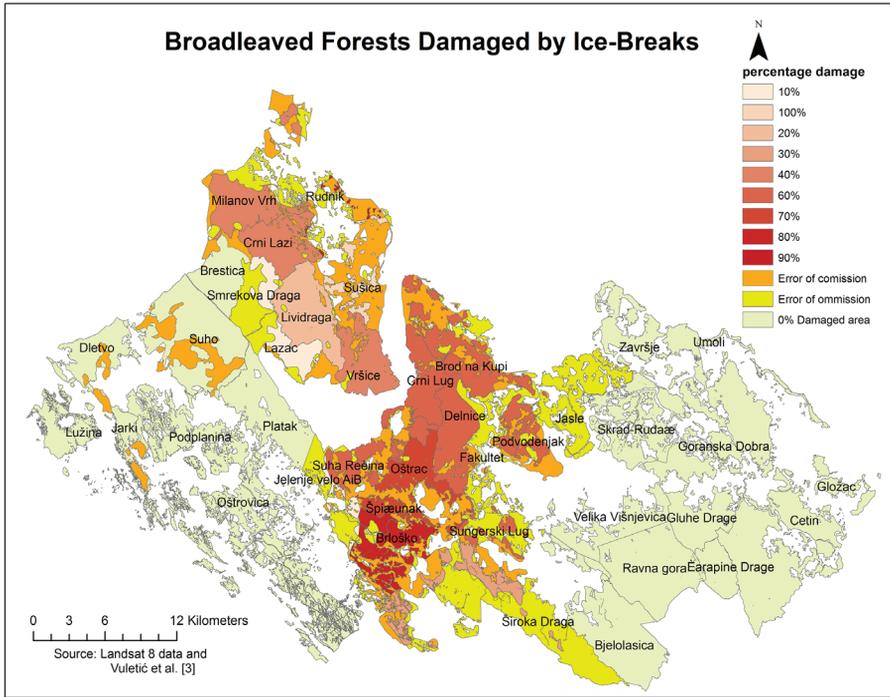
For the purpose of practicality, we draped the polygons with different levels of damage intensity generated by Vuletić *et al.* [3] over the map generated in this study (Figure 6). We generated maps for different levels of damage over broadleaf and coniferous forests. Both the broadleaf and coniferous forests show somewhat higher errors of omission for damaged areas than for the destroyed areas. In other words, more agreements can be seen for destroyed areas suggesting that satellite-generated maps are more accurate in some high severity areas. This is reasonable to expect as satellite imagery is more sensitive to larger NDVI differences. In addition, visibility of the damage during the summer period may become somewhat unnoticed when low damaged

broadleaf trees receive new leaves, which may occur as early as 10-14 days following the event. However, Ciesla *et al.* [27], suggest that experience has shown that ice storm damage incurred during winter can still be resolved and classified on leaf-on color infrared (CIR) aerial photos taken at a scale of 1:8,000 during the summer leaf-on period following the event.

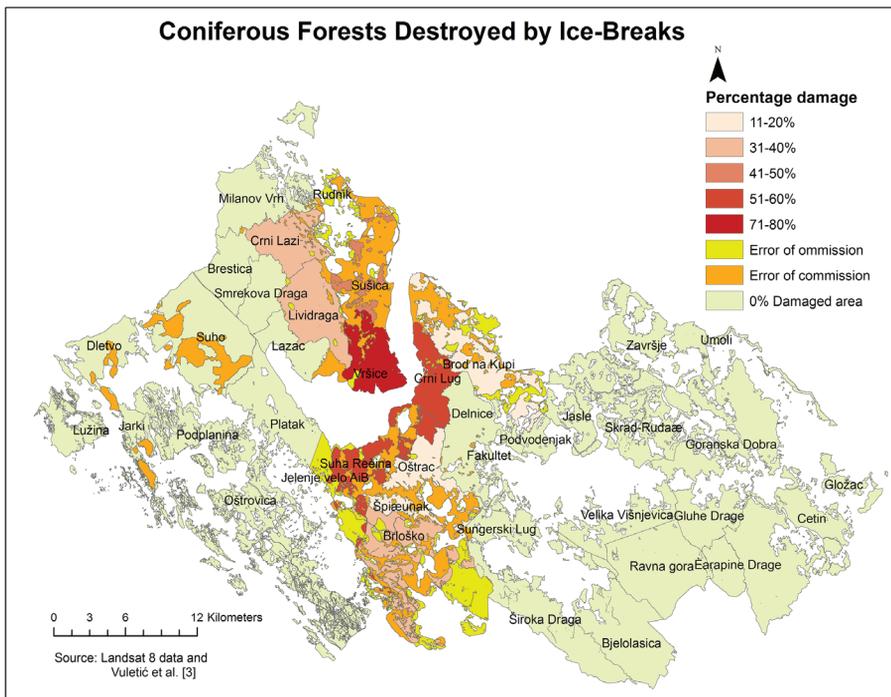
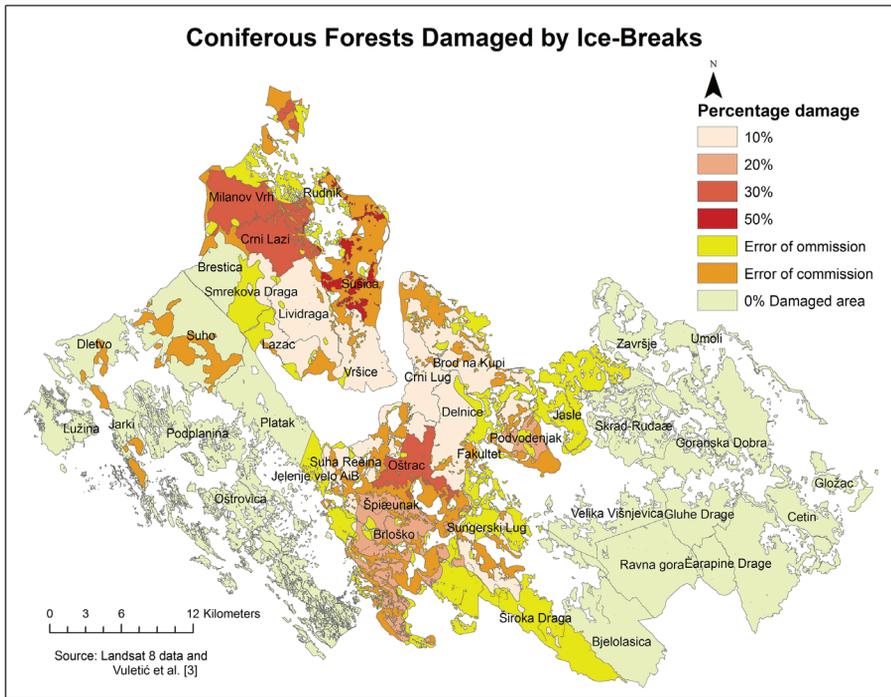
To develop a robust assessment of change detection it is important to pay attention to quantifying the change omission errors. Also, in a robust assessment, the area of 5,524.51 ha may be included in the calculations due to cloud cover.

Landsat-8 is widely available imagery, free of cost and can be downloaded in a few days after the data are collected. The spatial resolution of Landsat-8 images of 30 m is fine enough for the initial assessment of the damage under a clear sky. Our further analysis will involve thermal bands and also temporal bands throughout the winter time (weather permitted) to examine the impact of weather/seasons, flooded water, soil moisture, and surface temperature on the delineation of the damaged areas. For instance, more water and colder temperature result in longer retention time of ice in the forest. Remote sensing images acquired during winter time together with meteorological ancillary data could provide addition information about behavior of the damaged areas during the winter time. This can considerably aid the preparedness plan after the ice melt. However, remote sensing data acquisition using optical sensors during the winter time stays a challenge. Besides the weather factor, the 16 day revisit cycle of Landsat can be another obstacle especially in the winter time when the weather is cloudy. Merging different optical sensors (e.g. Landsat and Sentinel-2) and to increase temporal data acquisition, or, considering active and/or thermal remote sensing satellite imagery would be necessary for monitoring damaged areas during winter time.

Although we believe that some uncertainties in our results may come from the NDVI threshold values, cloud masking, and spatial



**FIGURE 6.** The comparison of the damaged and destroyed areas for broadleaf and coniferous forests. Note: We excluded the area in the middle of the maps where the damage is not considered by the ground truth polygons. Also, small areas in the top left corner of the maps are excluded due to clouds.



**FIGURE 6.** The comparison of the damaged and destroyed areas for broadleaf and coniferous forests. Note: We excluded the area in the middle of the maps where the damage is not considered by the ground truth polygons. Also, small areas in the top left corner of the maps are excluded due to clouds - continuation.

resolution of the sensor, one should consider that the ground truth observations can be challenging and biased as the area is remote in some instances and the estimation of the percentage damage is often subjective. To explore the impact of digital elevation model (DEM) embedded in Landsat-8 datasets we attempted to incorporate the ASTER DEM dataset in the calculations; however, the differences were insignificant.

## CONCLUSION

This study explores a commonly used approach for estimating forest damage caused by ice-breaks, using Landsat-8 datasets, available

to the public. Landsat-8 is expected to be advantageous over the previous Landsat sensors as it has better radiometric resolution and more available bands. In this study, the NDVI difference approach was used to delineate the damage. Two Landsat-8 OLI images were used in the analysis, one for summer 2013 (pre-event dataset) and summer 2014 (post-event dataset). The resulting map shows that the damaged area is 19.20% less than estimated by Vuletić *et al.* [3]. The error of omission is 31.60% and error of commission is 35.73%. The results indicate that proposed index NDVI approach performs sufficiently well in detecting the forest changes. However, the time series analysis including different seasons is needed to refine the findings.

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# The Potential Use of Indigobush (*Amorpha fruticosa* L.) as Natural Resource of Biologically Active Compounds

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## Abstract

**Background and Purpose:** Recent research indicates that a weed like Indigobush (*Amorpha fruticosa* L.) gives great opportunities for its commercialization through a rich spectrum of its beneficial biological features with possible use in the forestry and biotechnology field. Therefore, in this study we wanted to explore some of potential application of Indigobush extract, as a source of biologically active compounds, for animal cell culturing as well as green corrosion inhibitors.

**Materials and Methods:** The effect of ethanol extract of Indigobush seeds was studied on human tumor cell lines (HeLa and MCF-7) and cell viability was determined by WST-1 method after 72 hours of treatment with 6 different extract concentrations (0.5-10 mg·mL<sup>-1</sup>). The inhibition effect of Indigobush seeds extract on the corrosion of aluminum in 0.5 M hydrochloric acid solution was investigated by potentiodynamic polarization and electrochemical impedance spectroscopy (EIS) methods.

**Results:** Results showed that the addition of Indigobush extract had a stimulatory effect on MCF-7 cells growth at the concentrations >1 mg·mL<sup>-1</sup> while the same effect on HeLa cells was observed only

at the highest concentration of Indigobush extract (10 mg·mL<sup>-1</sup>). The stimulatory effect of Indigobush extract on cell viability was more pronounced when the cells were grown in a medium with 5% FBS compared to 10% FBS (v/v). Indigobush extract did not show cytotoxic effect on MCF-7 and HeLa cells. Electrochemical studies showed that with increasing extract concentrations (2.5-15 mg·mL<sup>-1</sup>) the values of corrosion current densities decrease, while the polarization resistance values increase. The maximum inhibition efficiency of Indigobush extract is reached at concentration of 15 mg·mL<sup>-1</sup> (82.9%).

**Conclusions:** The Indigobush ethanol extract has no cytotoxic effect on human tumor cell lines MCF-7 and HeLa. Results confirmed that extract originated from Indigobush has the potential to utilize for the mammalian cell culture media formulation by replacing the animal serum. Furthermore, data indicates that Indigobush extract has potential as green alternative to existing synthetic corrosion inhibitors.

**Keywords:** Indigobush, biologically active compounds, *in vitro* cell growth, green corrosion inhibitors

## INTRODUCTION

Indigobush (*Amorpha fruticosa* L.) belongs to the genus of deciduous shrubs and semi-shrubs (*Amorpha* L.) from the *Fabaceae* family. The range of its natural habitat is from southern Canada to Mexico. It was introduced to Europe in the first half of 18<sup>th</sup> century. In Croatia it is present since the beginning of 20<sup>th</sup> century in the valleys of the rivers Sava, Drava and Kupa in the lowland oak forests. According to the recent investigation Indigobush is the most common in Posavina region [1]. It is an upright shrub which can reach 3 meters in height and spread to twice that in width. The leaves are pinnately compound. The violet flowers that appear in June-July are in upright narrow racemes that can be clustered or solitary. The fruits are tiny warty pods, each containing one seed. It tolerates poor site conditions, prefers neutral, low-acid and low-alkaline soils. It can grow in semi-shade or no shade and requires moist soil, so it can be found along riversides and streams from where its seed spread by water, especially floods. Because of its quick and rich growth it rapidly conquers forest habitats as weed species, and is often a limiting factor for forest regeneration. It forms a dense shrub level in the shade of the old oak and ash trees and competes with young plants for water and nutrients [2]. Because of that Indigobush is considered as an undesirable species in forestry, but recent research indicates that it is

a plant of potentially great significance from economic aspect. It can be used for making blue dye, insecticides, repellents, perfumes, margarines and spices. Dense bushes make good cover for the animals and its twigs can be used for making flower arrangements and baskets. The great value of Indigobush is in its oil which can be used as biofuel and, if refined, as edible oil. The apiarist worship Indigobush as a rich honey producing crop [3]. With its root system preserve the soil from erosion as dense bushes planted in lines are making excellent protection against wind. In the USA, Indigobush is cultivated as decorative bush. Indigobush biomass research and its potentially bioenergetics value are extremely interested for the economy [1]. Indigobush seed is extremely viable and doesn't show any indications of germination loss, neither the viability loss during the storage. The results of germination tests of fresh seed imply at the phenomenon of seed dormancy due to the tightness of the seed shell [4].

The plants and their extracts have long been recognized to provide a potential source of chemical compounds or more commonly products known as phytochemicals with potent biological activity. Although leaves, roots, flowers, whole plants and stems were examined for useful phytochemicals in many studies, few reports refer to seeds as source for phytochemicals. Yet, a large number of chemical compounds are present in seeds or

seed coats, including alkaloids, lectins and phenolic compounds [5]. Also, Indigobush as a promising reservoir for biologically active compounds has been recognized and studied in recent years. Published studies indicate the presence of various phenolic compounds in the Indigobush extracts. Three different isoflavones and five rotenoides were purified from Indigobush. The Indigobush bean gland contains about 3.5% of oil which is composed of various terpenes. The Indigobush seed is composed of fatty acids including linoleic, oleic, palmitic and stearic acid [6-8]. In 1943 glycoside amorphin, which is applied as a drug for nerve and heart disease, was isolated from Indigobush fruits [1]. According to Zheleva-Dimitrova [9], Indigobush extract shows antioxidant activity and could be useful in therapy of free radical pathologies and neurodegenerative disorders. Despite the fact it contains so many useful substances, Indigobush is mainly used for erosion control and to restore wasteland.

In this study, we wanted to explore some of potential application of Indigobush extract, as a source of biologically active compounds, for animal cell culturing as well as green corrosion inhibitors. Effects of ethanol extract of Indigobush on the viability of human tumor cell lines (HeLa and MCF-7) as well as possibility of partial substitution of animal serum with extract of Indigobush were studied. Also, the inhibition effect of Indigobush extract on the corrosion of aluminum in 0.5 M hydrochloric acid solution was investigated by potentiodynamic polarization and electrochemical impedance spectroscopy (EIS) methods.

## MATERIALS AND METHODS

### Indigobush (*Amorpha fruticosa* L.) Ethanol Extract Preparation

Seeds were collected in natural stand of Indigobush on the area of Forest office Sunja, Forest administration Sisak in 2012. The seeds were ground to fine powder and extracted with 300 mL of 96% ethanol at the reflux

temperature for 2 hours. The crude extract (0.05 g·mL<sup>-1</sup>) was then filtered through filter paper. For determination of biological activity of extract, aliquot was taken, sterile filtered (0.22 μm) and stored at -20°C before use.

### Cell Lines and Culture Conditions

Human tumor cell lines MCF-7 (breast adenocarcinoma; ATCC: HTB-22) and HeLa (cervical carcinoma; ATCC: CCL-2), purchased from American Type Culture Collection (ATCC) were cultured in 25 cm<sup>2</sup> T-flasks in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, UK) supplemented with 5 or 10% (v/v) fetal bovine serum (FBS, Gibco, UK) and maintained in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C.

### Cytotoxicity Assay

The effect of Indigobush extract on human tumor cells proliferation and viability was examined by the WST-1 assay (Roche, Germany) which is a modification of the classical MTT test [10]. MCF-7 and HeLa cells from the exponential growth phase were trypsinized and plated out in 96-well plates at initial concentration of 5x10<sup>4</sup> cells mL<sup>-1</sup> (100 μL cell suspension), allowed to attach for 24 h. After that, MCF-7 and HeLa cells were exposed to sterile Indigobush ethanol extract for 72 h. The range of applied nominal concentrations (0.5- 10 mg·mL<sup>-1</sup>) was obtained by adding different volumes (1-20 μL/well) of Indigobush ethanol extract (0.05 g·mL<sup>-1</sup>). Since ethanol was used for Indigobush seed extraction, 96% ethanol was added to the control. Morphology, general condition and number of the cells were assessed using Trypan blue exclusion method [11]. Following exposure, 5 μL of tetrazolium salt WST-1 was added to each well and cells were incubated for further 3-4 h at 37°C. The absorbance was measured at 450 nm on the microplate reader (Tecan, Switzerland). The experiments were performed three times with six parallels for each extract concentration. Cell viability was presented as percentage of control cells according to expression:  
 Cell viability (%) = [(mean value A450 (sample) / mean value A450 (control))] x 100

### Evaluation of Anticorrosion Properties

Corrosion tests were performed using coupons prepared from 99.85% pure aluminum. A specific volume of prepared extract was taken and added directly to 0.5 M HCl solution to prepare the desired concentrations in range from 2.5-15 mg mL<sup>-1</sup>.

Electrochemical measurements were carried out in a conventional three electrode cylindrical glass cell using Potentiostat type VersaSTAT 3 (Princeton Applied Research), controlled by a personal computer. A saturated calomel electrode (SCE) and graphite electrode were used as reference and auxiliary electrodes, respectively. The working electrode was cut from aluminum and had a surface of 1 cm<sup>2</sup>. Before measurements the working electrode was abraded with emery paper to a 1200 metallographic finish, rinsed with distilled water and acetone. The all electrochemical measurements were performed in the test solution after reaching the open-circuit potential (E<sub>ocp</sub>). Potentiodynamic polarization studies were performed at scan rate of 0.5 mV·s<sup>-1</sup> in the potential range from ±150 mV with respect to the E<sub>ocp</sub>. All reported potentials refer to SCE. Electrochemical impedance spectroscopy (EIS) measurements were performed in the frequency range from 100 kHz to 10 mHz with an AC voltage amplitude perturbation of 5 mV. Inhibition efficiency (η<sub>pp</sub>(%)) is calculated using the equation:

$$\eta(\%) = \frac{I_{corr} - I'_{corr}}{I_{corr}} \times 100$$

where I<sub>corr</sub> and I'<sub>corr</sub> are the corrosion current densities in the absence and presence of inhibitor [12]. While the inhibition efficiency obtained from EIS measurements (η<sub>EIS</sub>(%)) is calculated using the equation:

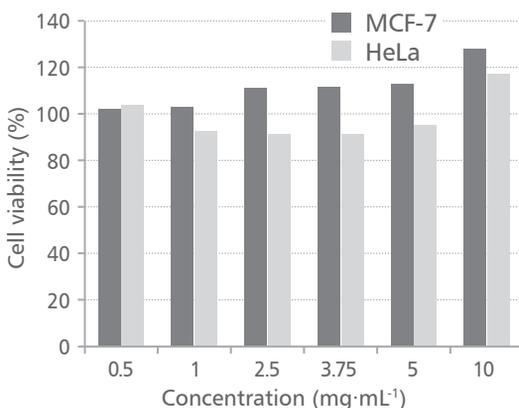
$$\eta(\%) = \frac{R'_{ct} - R_{ct}}{R_{ct}} \times 100$$

where R'<sub>ct</sub> and R<sub>ct</sub> are charge transfer resistance with and without inhibitor, respectively [12].

### RESULTS AND DISCUSSION

#### Cytotoxic Effects of Indigobush Extract in MCF-7 and HeLa Cells

To evaluate cytotoxicity of Indigobush ethanol extract on human tumor cell lines MCF-7 and HeLa following 72 h-exposure we used WST-1 cell proliferation assay. The effect of different extract concentrations on both human tumor cell lines, expressed as a percentage of control, are summarized and shown in Figure 1.

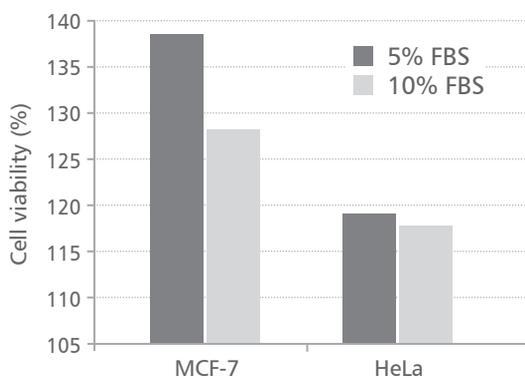


**FIGURE 1.** Effect of Indigobush ethanol extract on MCF-7 and HeLa cells after 72 h of exposure using WST-1 assay

Results showed that the addition of Indigobush extract had stimulatory effect on MCF-7 cells growth at the extract concentrations higher than 1 mg·mL<sup>-1</sup>. There was no stimulatory effect on HeLa cells, except at the highest concentration of Indigobush extract (10 mg·mL<sup>-1</sup>). Similarly, the positive effect on the growth of human T-cells (approximately +15%) was observed with the addition of 0.5 g·L<sup>-1</sup> of selected Indigobush fruit extracts compared to the control [8]. In the same study, the methanol fruit extract of Indigobush showed relatively low cytotoxicity of 19% at a maximum concentration of 1 g·L<sup>-1</sup> in normal human lung cell line (HEL299). Despite relatively high tested concentration of the extract (10 mg·mL<sup>-1</sup>), in our study, cytotoxic effect on both human tumor cell lines was not observed.

Since the positive effect of Indigobush extract on MCF-7 and HeLa cells growth was observed, we were interested in whether the effect will be visible when cells were cultured at reduced volume of serum (5%). Therefore, we established experiment where the cells were plated in medium with 5% and 10% of FBS. After 24 h cells were treated with  $10 \text{ mg}\cdot\text{mL}^{-1}$  of Indigobush extract since that concentration caused the most pronounced growth stimulation of MCF-7 and HeLa cells. The cell viability was determined by WST-1 assay after 72 h of exposure. The results are shown in Figure 2.

Results showed that the stimulatory effect of Indigobush extract on cell viability is more pronounced when the cells were grown in a



**FIGURE 2.** Effect of Indigobush extract ( $10 \text{ mg}\cdot\text{mL}^{-1}$ ) at various volume fraction of serum (5% and 10% FBS)

medium with 5% FBS compared to 10% FBS (v/v). This difference is more significant for MCF-7 than HeLa cells. Also, the stimulatory effect of Indigobush ethanol extract on cell viability is stronger on MCF-7 cells compared to HeLa cells, which may indicate the specificity of action with respect to the cell type. Obtained results provide useful information for the utilization of Indigobush extract as medium additive or as a substitute for serum since various plant components have been proposed to replace serum in mammalian cell cultures [13]. However, more extensive research will be required to determine the qualitative and quantitative composition of the Indigobush extract and

its mechanism of action, as crude extract and individual fractions or pure components. Also, it would be interesting to prepare a hydrolyzate of the plant and to examine its effect on different cell lines, since such data are not available in the literature and contribute to changing attitudes towards Indigobush as harmful and useless plant species.

### Inhibition Effect of Indigobush Extract on Aluminum Corrosion

Aluminum is widely used in many industries, such as, the production of heat exchangers, filters, various containers, printing plates etc. and often comes into contact with different aggressive media that can cause significant corrosion. The addition of corrosion inhibitors in aggressive media is one of the frequently used methods for corrosion protection. Nowadays, a new class of corrosion inhibitors with low toxicity and good efficiency are plant extracts, therefore the exploration of natural products of plant origin as an inexpensive and green corrosion inhibitors is an essential field of study [14-16]. One of the goals of this research was to investigate the effects of Indigobush extract as complex system on corrosion of aluminum in 0.5 M HCl solution. Corrosion parameters were performed by using electrochemical measurements (potentiodynamic polarization and electrochemical impedance spectroscopy) (Table 1 and Figure 3).

In acidic solutions the main anodic reaction is the dissolution of aluminum in the form of  $\text{Al}^{3+}$  aqueous complexes and their passage from the metal surface into the solution, while the main cathodic reaction is a reduction of hydrogen ions and evolution of hydrogen gas [17]. Therefore, the inhibitor can influence on either the anodic or the cathodic reaction, or both.

Potentiodynamic polarization curves obtained for aluminum in 0.5 M HCl solution in the absence and presence of various concentrations of Indigobush extract at  $25^\circ\text{C}$  are shown in Figure 3a. From this figure is evident that addition of Indigobush extract in HCl solution causes remarkable decrease in current densities

**TABLE 1.** Electrochemical parameters obtained for aluminum in 0.5 M HCl solution in absence and presence of Indigobush extract at 25°C

c (mg·L <sup>-1</sup> )	E <sub>corr</sub> (mV <sub>SCE</sub> )	I <sub>corr</sub> (mA·cm <sup>-2</sup> )	η <sub>pp</sub> (%)	R <sub>ct</sub> (Ω·cm <sup>2</sup> )	CPE (μF·cm <sup>-2</sup> )	η <sub>EIS</sub> (%)
0	-861	0.662	-	59.1	69.0	-
2.5	-908	0.398	39.9	106.3	37.6	44.4
5.0	-893	0.245	63.0	153.4	26.1	61.5
7.5	-905	0.173	73.9	212.0	18.8	72.1
10.0	-914	0.132	80.1	231.4	17.2	74.5
15.0	-938	0.113	82.9	273.2	14.6	78.4

c - nominal concentrations of Indigobush ethanol extract; E<sub>corr</sub> - electrochemical corrosion potential; I<sub>corr</sub> - corrosion current densities in the absence of inhibitor; η<sub>pp</sub> - inhibition efficiency; R<sub>ct</sub> - charge transfer resistance without inhibitor; CPE - constant phase element; η<sub>EIS</sub> - inhibition efficiency obtained from EIS measurements

and shifts the corrosion potentials in cathodic direction.

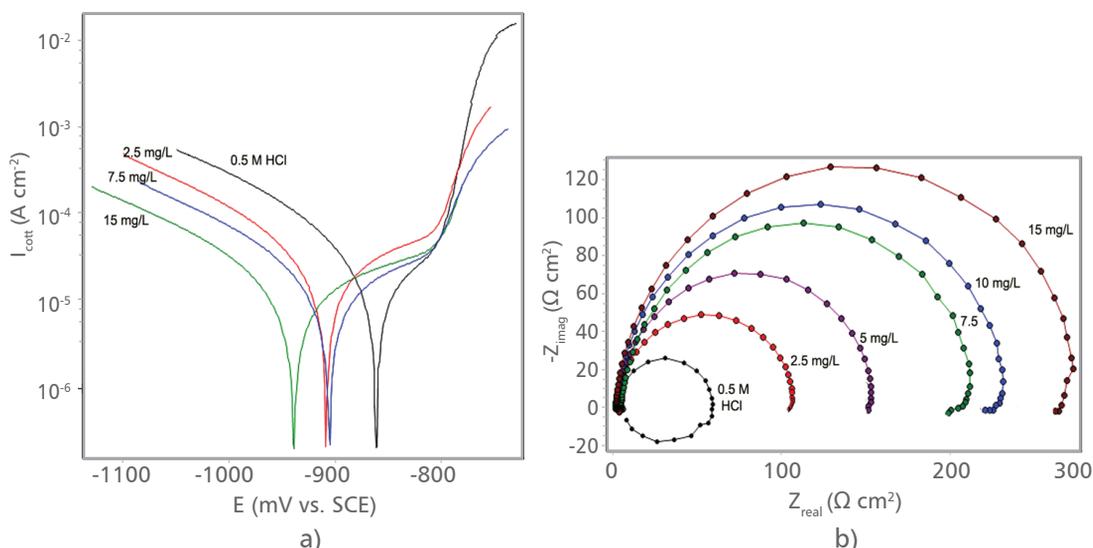
Inhibition efficiency (η<sub>p</sub> (%)) values increase with increasing extract concentration and reach a maximum value of 82.9% for addition of 15 mg·mL<sup>-1</sup> Indigobush extract.

The effects of different concentrations of Indigobush extract on the impedance behaviour of aluminum in 0.5 M HCl solution have been obtained and results are given as Nyquist plots in Figure 3b. By addition of inhibitor to the

HCl solution, the diameter of the capacitive loop enlarges, this means that the charge transfer resistance, R<sub>ct</sub> values increase and an improvement in corrosion resistance of aluminum in acid solution is achieved.

The calculated EIS parameters for aluminum in 0.5 M HCl solution containing different concentrations of Indigobush extract are also presented in Table 1.

Inspection of these parameters reveals that the charge transfer resistance, R<sub>ct</sub> values



**FIGURE 3.** Potentiodynamic polarization curves (a) and Nyquist plots (b) obtained for aluminum in 0.5 M HCl solution in the absence and presence of Indigobush extract at 25°C

increase, while CPE (constant phase element) decrease with the increasing concentration of Indigobush extract. The decrease of CPE values can result from an increase in the thickness of the electrical double layer and suggests the active molecule adsorption on aluminum surface. This also confirms that compact film of inhibitor is formed on the aluminum surface, which acting as a physical barrier for preventing the attack of the aggressive hydrochloric acid solution. This could be mainly attributed to the physical adsorption of active molecules present in Indigobush extract and adsorption process is possible because active molecules largely exist in the protonated form in acid solution. These protonated species can be adsorbed on the cathodic site of the aluminum decreasing the hydrogen evolution reaction.

Calculated inhibition efficiency values ( $\eta_{EIS}$  (%)) reach a maximum value in presence of 15 mg L<sup>-1</sup> Indigobush extract (78.4%) and they are quite similar with efficiency values obtained by potentiodynamic polarization method.

Electrochemical results revealed that Indigobush extract is a good eco-friendly inhibitor for the corrosion control of aluminum in 0.5 M hydrochloric acid.

Inhibition efficiency values increases with increasing Indigobush extract concentration, reaching a maximum value at concentration of

15 mg·mL<sup>-1</sup> (82.9%). Indigobush extract can be used as renewable and easily available inhibitor for aluminum corrosion.

## CONCLUSIONS

These results confirmed that plant extracts originated from invasive plants like Indigobush can be used directly to develop new and effective classes of natural chemicals with potential application in various green technologies. This study will contribute to changing attitudes towards Indigobush as harmful and useless plant species.

Furthermore, results obtained in this study showed that Indigobush extract has the potential to utilize for the mammalian cell culture media formulation by replacing the animal serum and as green alternative to existing synthetic corrosion inhibitors. However, more extensive research will be required to determine the qualitative and quantitative composition of the Indigobush extract.

Within these challenges, screening native plants in general is useful for revealing antimicrobial, antioxidant activity and antitumor activity that may lead to the development of new products for use as nutritional and pharmaceutical agents.

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# Decision-Making Tool for Cost-Efficient and Environmentally Friendly Wood Mobilisation

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## Abstract

**Background and Purpose:** With development of forest management technologies, the efficiency of wood production was significantly improved, and thus the impact on forests has changed as well. The article presents a practical decision-making tool for selection of most suitable harvesting system, considering given terrain as well as expected soil conditions on harvesting sites. The decision-making tool should support cost-efficient and environmentally friendly mobilisation of wood.

**Materials and Methods:** The presented decision-making tool is based on ground bearing capacities (relevant environmental parameter) and nominal ground pressure (harvesting system characteristics). Soil and terrain (slope) characteristics were taken into account for selection of the most suitable harvesting system. Three-step methodological approach was suggested, where soil and terrain conditions were defined in first step, while harvesting system were described using wood process charts ("functiogramms") in second step. In final step ecological and technological requirements were matched.

**Results:** To exemplify the three-step methodology, a decision-making tool was prepared for the three selected harvesting systems. The proposed harvesting systems differ in technological, ecological and economic aspects, but each is limited by at least one of the aspect.

**Conclusions:** The decision-making tool in combination with the presented wood process charts ("functiogramms") can simplify and facilitate forest production planning, although it can also be used in case of unforeseen event e.g. changing of soil moisture, machinery failure and insufficient current capacities. Considering the envisaged quantities and types of forest wooden assortments, it is possible to use the decision-making tool for a basic selection of most appropriate harvesting systems. The main idea behind the suggested three step methodological approach is that forest workers can prepare individual decision-making matrix (based on ecological and technological parameters such as technical or economical limitations of harvesting machines).

**Keywords:** decision support model, soil-machinery interaction, terrain conditions, wood process charts, forest operations

## INTRODUCTION

Over the last few decades, the exploitation of new technological solution in the field of round wood production has increased. This has resulted in a massive effort towards forest mechanization. Harvesters, processors and forwarders have become widespread in all industrialized countries not only in Slovenia, but also in other parts of the South-east European (SEE) region, far beyond the borders of the Nordic countries where they were first developed and thoroughly studied [1-4]. In general, perception about modern harvesting systems is related with high productivity and cost efficiency on the one hand and with heavy machinery causing severe ecological implications to the stand (i.e. wounding of trees and devaluation of wood quality) and soil (e.g. soil compaction, erosion, mixing of soil horizons, etc.) on the other hand [5]. Due to the vast amount and range of available harvesting systems, operators (foresters) often select less suitable harvesting solutions for the particular stand/site, with negative ecological and environmental consequences [6, 7]. Thus, enhances a negative general opinion about modern harvesting systems.

Magagnotti *et al.* [2] investigated the impact of conventional motor-manual and fully-mechanised thinning on Mediterranean pine plantations and concluded that properly applied fully-mechanised harvesting does not cause heavier soil impacts than traditional motor-manual harvesting; in addition, it results in much lower stand damage. Magagnotti *et al.* [3] also performed a study on salvage harvesting after forest wind-throw, using a versatile fully mechanised system; the results showed moderate soil compaction and unlikely adverse effects on advanced regeneration.

Selection of harvesting systems can depend on different preconditions which can be divided in three major groups: (i) terrain properties, (ii) extent of environmental impacts and (iii) production costs. In Slovenia the appropriate harvesting system for a particular site is determined in the Forest management

plans [8]. The key element in harvesting system selection is the forest entrepreneur, where rationality and competitiveness are the leading guidelines. The organisation of work is related to the company size (which performs forest operations) and its technological equipment. After a site inspection, a trained and experienced employee selects harvesting system, based on current working conditions and available machinery. However, when selecting the proper harvesting system for particular forest site, both economic and ecological aspects should be considered [9].

Numerous studies on nominal ground pressure of wheels and machines on forest soils were carried out in the past. Despite extensive professional work the systems for predicting deformations and their physiological consequences have remained relatively unreliable [5].

In Germany, minimum standards are determined for soil protection; i.e. in the Forestry Act, Nature Conservation Act and Soil Conservation Act [10]. Principles of appropriate forest management practices in the German Forest Act determine declaratory requirement of sustainable, careful and proper forest management: (i) maintenance of soil fertility, (ii) conservation of natural soil functions, (iii) soil and stand-friendly forest management, considering also the natural habitats of animal and plant species, (iv) demand limited and nature-friendly forest exploitation, considering also the landscape and forest function [10].

According to Owende *et al.* [11], the environmental impacts of mechanised harvesting operations depend on several factors such as: (i) site type, suitability of machinery to respective terrain, (ii) harvesting system, (iii) layout of the trails, and (iv) time of year during which the operations are carried out (weather conditions).

In order to meet the ecological requirements, decision tools were developed to facilitate eco-efficient harvesting techniques [7, 11, 12]. The decision tool presented by Owende *et al.* [11] is simple to use and consists of ground specifications (e.g. ground bearing

capacity, ground roughness and slope) and machinery parameters (e.g. nominal ground pressure). The decision-making tool, however, does not consider the impact of soil moisture/terrain properties. Gröll [12] and Erler and Gröll [13] presented a site-focused decision-making model, where the decision-making process is supported by classifying the natural value (producing capacity) of the soil, site trafficability and technical suitability of the wood harvesting procedure. The outcome was a combined ecological and technical evaluation of the procedure. The tool presented by Gröll and Erler is detailed, but on the other hand more complex and somehow difficult to implement under other conditions (i.e. in other environments and countries). Approaches using a multi-criteria decision support tool based on Multi Criteria Analysis (MCA) as presented Kühmaier and Stampfer [14] are also promising, although used to estimate a proper energy wood supply chain.

The terrain properties and soil-machine interactions [7, 11], as well as stand-machine interactions (damages to roots and remaining trees) were widely studied in the past years, with the data suitable to be used for the preparation of reliable decision-making tools. The main objective of this study was to suggest a new practical decision-making tool for selection of most suitable harvesting systems, considering terrain steepness, ground bearing capacity and soil moisture. The decision-making tool should support cost-efficient and environmentally friendly mobilisation of wood and should be easily adapted to different conditions.

## MATERIALS AND METHODS

For a successful selection of suitable harvesting systems the suggested decision-making tool (DMT) includes relevant parameters such as soil and terrain characteristics and basic machine properties. The DMT variables were quantified based on studies carried out by other research groups, e.g. by Owende *et al.* [11].

The presented tool is bidirectional; it can be used to determine the proper harvesting system for individual sites, or vice-versa.

During the harvesting system selection process, given terrain and soil conditions should be considered. Soil damages during off-road machine operations are mostly influenced by soil properties and conditions in the moment when under load and by nominal ground pressure of machinery [6, 7]. The preparation/adaptation of DMT to specific site conditions and harvesting systems is supposed to be carried out in three consecutive phases: (i) estimation of soil and terrain conditions, (ii) prediction of suitable harvesting systems and depiction in wood process charts ("functiogramms"), (iii) finalization of decision matrix (Figure 1).

### Soil Characteristics

The strength of soils were classified based on their ground bearing capacities (GBC); i.e. capacity to support the load pressing on it. In forestry, the soil bearing capacity is usually considered as the maximum allowable wheel contact pressure. Soil bearing capacity, shear strength and penetration resistance are dependent on soil moisture, dry density and particle size distribution. However, development of universal models explaining relationships among mentioned parameters seems difficult, because both frictional and electro-chemical forces are involved in the formation of the soil strength [15]. The following soil type classes (from soft to hard) (Table 1) were adopted according to Owende *et al.* [11]: (i) very soft soil (GBC  $\leq 40$  kPa), (ii) soft soil (GBC from 40 to 60 kPa), (iii) average soil (GBC from 60 to 80 kPa) and (iv) strong soil (GBC  $\geq 80$  kPa).

It should be considered that GBC, in particular, is greatly reduced when soil is saturated with water.

One of the main criteria in selection of machinery to corresponding site condition is the wheel/soil contact pressure. It is a simplification of the vertical stress, where loaded tyre or track imposes on the soil [11].

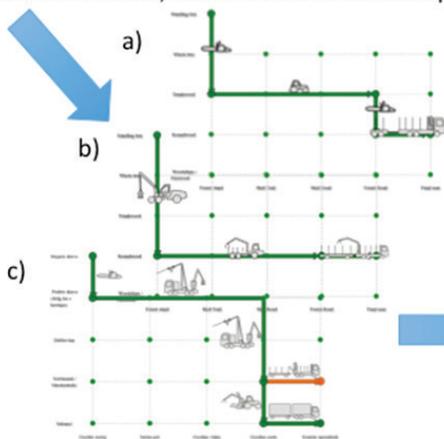
**a) First step:**

- Define soil and terrain classes.

		Terrain steepness		
		up to 35%	35% - 60%	above 60 %
Soil strength classes	Softest			
	to			
	to			
	Hardest			

**b) Second step:**

- Define harvesting systems considering variety of site conditions, as well as economical aspects.



**c) Final step:** Complete the decision matrix with harvesting systems (defined during the second step) matching the ecological requirements (site conditions) with economical and technological requirements.

Soil strength	up to 35%	35% - 60%	above 60 %
Very soft [≤40 kPa]	c**		
Soft soils [40-60kPa]	a	c	c
Average soil [60-80kPa]	a,b	b*,c	c
Strong soil [≥80kPa]	a,b	b,c	c

FIGURE 1. Process of building decision-making matrix

TABLE 1. Different soil strength classes

Soil strength classes	Soil description		Basic soil types for Croatia	Basic soil types for Slovenia
References	Hyvärinen and Ahokas [16]	Ragot [17]	Poršinsky <i>et al.</i> [6]	Urbančič <i>et al.</i> [18]
Very soft soil	Peatland (open), Snow (virgin)		Wet peats	Peat
Soft soil	Snow (old, -10)	Alluvial soils, Peatland (open)	Wet gleys and peaty soils	Deep siliceous soil
Average soil	Peatland (wooded)		Soft mineral or iron-pan soils	Deep carbonate soil
Strong soil	Moraine, Gravel, Sand, Clay, Snow (compressed, -10C)	Gravel, Sand, Clay, Snow (hardpacked, -10C)	Dry sands and gravels, Firm mineral soils	Shallow soil

Nominal ground pressure (NGP) is widely used, easy to assess mobility variable [15], although it has the disadvantage of neglecting the influence of tyre deformation. Influence on forest floor/soil is mainly related to the wheel/soil interaction, thus nominal ground pressure

(NGP) is less destructive on soils with higher ground bearing capacity than those with lower [15].

NGP is calculated as the ratio between the wheel load and dimension of tyres [11]:

- For wheeled machines:

$$NGP_{wheels} = \frac{W}{r \times b}$$

where NGP is the nominal ground pressure in kPa, W is wheel load in kN, r is wheel radius in m, and b is tyre width in m.

- For tracked machines:

$$NGP_{tracks} = \frac{W}{b} (125+L)$$

where W is track load in kN, L is the length between the wheel centres in m, and b is track width in m.

### Terrain Characteristics

Nominal ground pressure on the axle depends on distribution of the mass on the vehicle, which is different on flat ground than on great slopes [19]. The research carried out by Marenče [19, 20] demonstrates the principles of weight transfer to the rear axis during upward hauling and transfer of loads to the front axis during downward hauling. Technical characteristics of chosen machinery are thus of vital importance when selecting harvesting systems; weight distribution on the axis greatly depends on slope of the terrain. E.g. during downward hauling the forwarder with one axis in front and two rear axis represents a specific problem since the downhill axis are under higher load (bearing two thirds of the load).

The DMT should enable the selection of harvesting system, most appropriate for particular types terrain, soil conditions at selected working/harvesting site. In order to meet specific conditions in the first step, a matrix containing terrain and soil conditions needs to be configured as exemplified in Figure 1a.

For the purpose of this article we suggested three terrain slope classes: (i) up to 30%, (ii) 31%-60% and (iii) above 60%. The selected classes were proposed based on limitation of different technologies.

### Harvesting Systems

During the second step, available and commonly used harvesting system in the region should be evaluated (Figure 1b). In the past, different approaches to visualise the harvesting

systems have been developed and used [21-25]. For describing of working processes and harvesting systems, Kuratorium für Waldarbeit und Forsttechnik e.V. KWF (2015) [21] presented a wood process charts ("functiogramms") that symbolizes cutting, hauling and transport in the process from the standing tree in the stand to the final product either on the forest road or at the end consumer. The idea of harvesting system visualisation has been developed and utilized by Eler and Weiß [26] and Eler and Dög [27].

To exemplify the three-step methodology, a decision-making matrix was prepared for the three selected harvesting systems: (i) chain saw - tractor, (ii) harvester - forwarder, and (iii) chainsaw - cable yarder.

Chain saw - tractor (Figure 2) is a traditional combination of motor-manual felling with chainsaw and haulage with forestry tractor. Wood cutter fells a tree and immediately afterwards follows the delimiting operation. Cutting is followed by stem wood extraction along the skid-road with forestry tractor using forest winch. The cross cutting of stems to assortments is foreseen at the storage by the side of forest road and then transported to end-user by truck and trailer.

Harvester - Forwarder (Figure 3) addresses machines for fully-mechanized harvesting. Cutting and assortment production take place along skid trail and is carried out by harvester. Cutting is followed by haulage of assortments with forwarder. After harvesting is completed, transport to end-user is foreseen.

Chainsaw - cable yarder (Figure 4) is a modern way of forest production with the possibility of the whole-tree yarding by cable line, where chainsaw operator fells a tree. The hauling from stand to forest road is done by the cable yarder, where delimiting and cross-cutting is performed by harvesting processor. After the completed yarding the transport of round wood is envisaged.

Wood process charts ("functiogramms") can be also used as a starting point for cost evaluation and estimation of environmental or ecological impacts. The Slovenian Forestry Institute developed WoodChainManager (WCM)

## Costs of selected processes

Machine	Fixed costs in € / year	Fixed costs in € / hour	Variable cost of fuels and lubricants	Variable costs of maintenance in € / hour	Total cost of supply chain [€ / h]
Chainsaw (4 kW)	148.00€	1.35€	1.29€	1.20€	3.83€
Safety gear and cutting equipment	111.00€	0.56€	0€	0.42€	0.98€
4WD agricultural tractor (95-125 kW)	7668.33€	15.34€	18.67€	4.28€	38.28€
Cabine protection (complete)	2293.33€	4.59€	0€	1.28€	5.87€
Wheel chains for mud (28-32")	143.33€	1.79€	0€	0.50€	2.29€
Two drum winch (8 t)	974.67€	3.48€	0€	2.72€	6.20€
Radio remote control	172.00€	1.27€	0€	0.48€	1.75€
Chainsaw (4 kW)	148.00€	1.35€	1.29€	1.20€	3.83€
Semi-truck with trailer and crane for roundwood (300 kW)	29600.00€	29.60€	50.91€	14.80€	95.31€

Fuel prices used for calculation: Diesel: 1.2190 €, Petrol: 1.3230 €

Total cost of supply chain [€/ h]: 158.34 €

## Visualization of supply chain

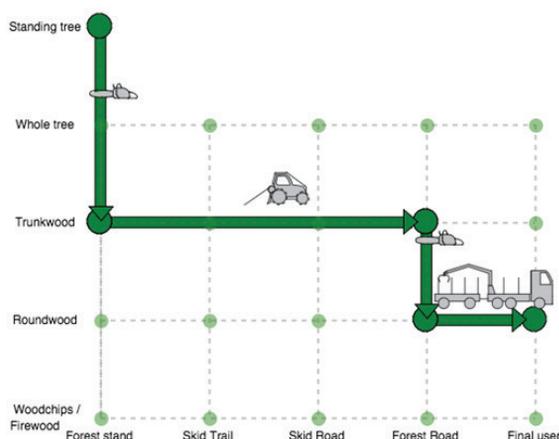


FIGURE 2. Wood process chart ("funciogrogram") of motor-manual cutting and hauling with tractor harvesting system

[28] an internet tool for calculation of machinery costs [29], and visualisation of harvesting systems. Machinery costs are calculated per scheduled machine hour, where scheduled time is the time during which equipment is scheduled to do productive work [30]. Final selection of harvesting system depends on costs and productivity of selected machinery, especially in cases where soil conditions as well as terrain enable more than one option. Comparison of machinery costs along different

harvesting systems facilitate the selection of suitable harvesting systems.

### Evaluation of Technological, Ecological and Economic Aspects of Selected Harvesting Systems – Finalization of DMT

In the third phase, the ecological (soil and slope conditions) and technological requirements (NGP, safe slope angle, etc.) should be matched (Figure 1c).

The main characteristics of above presented harvestingsystems(Figure2,3,4)arelistedinTable 2, however is should be considered that these are only three from widely differing harvesting systems that can be found in the Mediterranean and SEE regions. Different systems should be analysed and included in DMT in order to match it with the accessible technology and existing environmental conditions. When proposing different harvesting systems, the evaluation by technological, ecological and economic aspects should be considered. The presented harvesting systems are limited by several constraints. For example, driving with tractor is only possible on slopes that do not exceed 35% (very steep terrains are out of limits). On the other site, fully-mechanized harvesting system is limited

with ecological aspects due to off-road driving on skid trails, their NGP exceed GBC and therefore cause damages on forest soils. Skid trails are defined as alignment in the forest, in which harvester and forwarder are moving. Cable crane yarding is limited neither with technological nor ecological aspects, but rather with economic aspects. Due to high cost of production in order to be economically efficient it is only appropriate for steep terrains with higher volumes of harvestable timber.

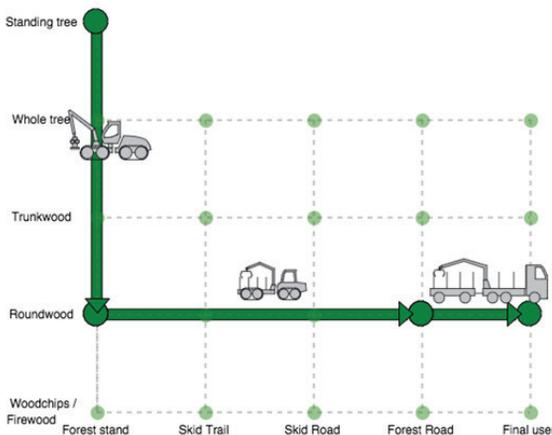
The presented parameters (Table 2) are the main input data to fill in the decision-matrix. Technological and ecological aspects of harvesting machines are available in their technical description. Costs are calculated using the WCM tool [28].

### Costs of selected processes

Machine	Fixed costs in € / year	Fixed costs in € / hour	Variable cost of fuels and lubricants	Variable costs of maintenance in € / hour	Total cost of supply chain [€ / h]
Harvester (170 kW)	56616.67€	62.91€	29.69€	39.50€	132.10€
Forwarder (140 kW)	48000.00€	48.00€	23.76€	30.00€	101.76€
Semi-truck with trailer and crane for roundwood (300 kW)	29600.00€	29.60€	50.91€	14.80€	95.31€
Fuel prices used for calculation: Diesel: 1.2190 €, Petrol: 1.3230 €					

**Total cost of supply chain [€ / h]: 329.16 €**

### Visualization of supply chain



**FIGURE 3.** Wood process chart ("functiogramm") of fully-mechanized harvesting system

**TABLE 2.** Selected aspects important for DMT

Harvesting system		Ecological aspect		Technology aspect		Economical aspect	
Harvesting system	Machinery	Gross mass (kN)	NGP (kPa)	Max. slope (%)	Engine power (kW)	Cost (€·h <sup>-1</sup> )	Productivity (m <sup>3</sup> ·8h <sup>-1</sup> )
a) Chain saw - tractor	Chainsaw	NR	NR	-	4	3.78	15
	Tractor with winch	65	117	<35	110	41.89	25
	Harvester (12t)	132	54	<60	140	118.73	70
b) Harvester - Forwarder	Forwarder (12t)	230	55 (front tyres), 100 (back tyres)	<60	140	101.33	60
	Chainsaw	NR	NR	-	4	3.78	15
c) Chainsaw - cable yarder	Cable crane mounted on truck with processor head	NR	NR	-	300	413.99	80
	Chipper powered by agricultural tractor	NR	NR	<30	110	112.05	80

NR - not relevant

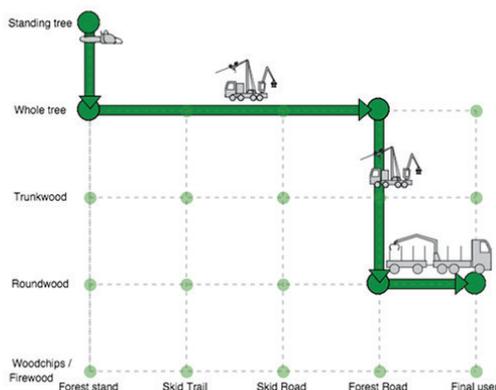
### Costs of selected processes

Machine	Fixed costs in € / year	Fixed costs in € / hour	Variable cost of fuels and lubricants	Variable costs of maintenance in € / hour	Total cost of supply chain [€ / h]
Chainsaw (4 kW)	148.00€	1.35€	1.29€	1.20€	3.83€
Safety gear and cutting equipment	111.00€	0.56€	0€	0.42€	0.98€
Cable crane mounted on truck with processor head	83200.00€	104.00€	50.91€	260.00€	414.91€
Semi-truck with trailer and crane for roundwood (300 kW)	29600.00€	29.80€	50.91€	14.80€	95.31€

Fuel prices used for calculation: Diesel: 1.2190 €, Petrol: 1.3230 €

Total cost of supply chain [€/ h]: 515.02 €

### Visualization of supply chain



**FIGURE 4.** Wood process chart ("functiogramm") of cable crane yarding harvesting system

## RESULTS

### Preparation of the Decision-Making Matrix

The three above presented harvesting systems, i.e. motor manual harvesting system (Figure 2), Harwarder – Forwarder harvesting system (Figure 3) and cable crane yarding harvesting system (Figure 4) were inserted into the matrix (Figure 5), following the requirements and limitations described above. For example, the traditional motor-manual harvesting (Figure 2) has technological limitation as described above and the only ecological limitation that needs to be considered are very soft soils with low GBC. In case of fully mechanized harvesting (Figure 3), ecological requirements are the limiting factor, thus measures to lower site impact should be considered for example for sites with low ground bearing capacity (e.g. using of the boogie tracks, carrying only half loads, etc.). The unconventional cable crane yarding (Figure 4) is in accordance with

ecological and technological requirements and suitable for all proposed site conditions, but due to economical limitation (high production cost) applicable only where no other harvesting system can be applied or at high productive working sites.

### Example of Decision-Making Matrix Developed for Slovenian Conditions

The above presented DMT is differing from the one developed for Slovenia in two aspects. In the Slovenian matrix the ordinate axis defines different soil type and therefore two moisture categories (damp and dry soils) where introduced. Since in the above presented DMT soil types where replaced by GBC classes the soil moisture parameter is redundant. A decision-matrix developed for Slovenian conditions is containing more than 12 different technological solutions (Figure 6). The matrix includes harvesting systems from cable crane yarding (1), combinations of motor-manual harvesting (2-4), different combinations of fully mechanized

Soil strenght	up to 35%	35% - 60%	above 60 %
Very soft [ $\leq 40$ kPa]	c**		
Soft soils [40-60kPa]	a	c	c
Average soil [60-80kPa]	a,b	b*,c	c
Strong soil [ $\geq 80$ kPa]	a,b	b,c	c

#### Remarks:

a stand for motor-manual cutting and hauling with tractor harvesting system (Figure 2)

b stand for fully-mechanized harvesting system (Figure 3)

c stand for cable crane yarding harvesting system (Figure 4)

\* stand for using additional equipment (i.e. using fitted boogie tracks on harvester or forwarder)

\*\* stand for additional cost consideration

**FIGURE 5.** Decision-making matrix

	up to 35%		35% - 60%		above 60 %	
Peat	6	6				
Deep siliceous soil	2, 3	2, 3, 5.B, 5.F	1, 2, 3	1, 2, 3	1	1
Deep carbonate soil	2, 3, 5.B, 5.F	2, 3, 4, 5.A, 5.B, 5.C, 5.D, 5.E, 5.F, 5.G	1, 2, 3	1, 2, 3, 5.B*, 5.F*	1	1
Shallow soil	2, 3, 4, 5.A, 5.B, 5.C, 5.D, 5.E, 5.F, 5.G	2, 3, 4, 5.A, 5.B, 5.C, 5.D, 5.E, 5.F, 5.G	2, 3, 4, 5.B, 5.C, 5.D, 5.F	2, 3, 4, 5.A, 5.B, 5.C, 5.D, 5.F	1	1
	damp soil	dry soil	damp soil	dry soil	damp soil	dry soil

**Legend:**

1-6 stands for harvesting systems from cable crane yarding (1), combinations of motor-manual harvesting (2-4), different combinations of mechanized harvesting (5) to helicopter yarding (6). Harvesting systems cannot be directly matched to the systems described in figure 5 as they are focusing onto a woodchip production.

5.A represent fully mechanized harvesting systems with large machines (according to FHP 2010).

5.B represent fully mechanized harvesting systems with large machines (according to FHP 2010) with fitted caterpillar to its boggie axles.

5.C represent fully mechanized harvesting systems with medium-sized machines (according to FHP 2010).

5.D represent fully mechanized harvesting systems with small machines (according to FHP 2010).

5.E represent fully mechanized harvesting systems with smallest machines (according to FHP 2010).

5.F represent partly mechanized harvesting systems where felling is partly done using chainsaw.

5.G represent fully mechanized harvesting systems where forwarder is equipped with chipper (and chips container).

\* Technologies suitable only on slopes from 35% - 40%

**FIGURE 6.** Decision-making tool for the selection of suitable harvesting system in Slovenian conditions

harvesting (5) to helicopter yarding (6), although not common in Slovenia, applicable in extreme conditions. The methodology and DMT were developed within a national project with the main aim to provide forest operators and forest managers with a simple planning tool. The final project report includes a detailed description of all harvesting system in order to facilitate the decision process of forest operators [31].

**DISCUSSION**

With the changes due to increased fully-mechanized harvesting the efficiency of forest production have changed. Together with them, the impacts of these technologies on

forests have changed as well. The expensive modern machines require suitable training of not only machinists but executive and technical personnel [32] as well. In the case of mechanized cutting, the machinist’s suitable qualification is of exceptional importance from the aspect of potential damages caused to forest stands [33]. Presented decision-making tool could be used as a part of regular training for forest operators.

The decision-making tool is applicable for planning of forest production on concrete working sites (harvesting sites). Primarily, the decision-making should simplify the analysis/ evaluation of forest operations, although it can also be advantageously used during the execution of work, when working conditions change:

- changed humidity conditions at working site,
- machine has broken down and has to be replaced with a new one,
- to fill the capacities, another/unforeseen machine is used.

The decision-making tool supports the planning of harvesting operations. Since there is no uniform decision making matrix for all different types of soils or different harvesting systems, DMT can be prepared individually and with region-specific technology.

Considering the predicted quantities and types of forest wooden assortments, it is possible to use the DMT for a basic selection of most appropriate harvesting systems. It is designed in such a way that a new harvesting system can be added at any time.

## CONCLUSIONS

The decision-making tool for evaluating harvesting systems was developed on the basis of foreign experience and own studies

concerning the green woodchips supply chain carried out in a national research project. The presented methodology serves as a guideline for preparation of region specific DMT for selection of most appropriate harvesting systems in the phase of silvicultural planning as well as in the phase of forest operations planning or operational work management. The developed tools should be checked in practice and supplemented in the sense of practical utility. Economic aspects of different harvesting system can be easily estimated using WoodChainManager, an internet tool that is based on FAO methodology for machinery cost calculation and also enables visualisation of harvesting system.

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# The Importance of CIR Aerial Imagery in Inventory, Monitoring and Predicting Forest Condition

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## Abstract

**Background and Purpose:** The main goal of this paper was to highlight the importance of colour infrared (CIR) aerial photographs for efficient inventory, monitoring and predicting the health status of forests in changed site conditions. CIR aerial photographs from two aerial surveys conducted in 1989 and 2008 were used to identify and analyze the damage in lowland pedunculated oak (*Quercus robur* L.) forests during each period, as well as to obtain a dieback trend in the observed period.

**Material and Methods:** The research was conducted in lowland pedunculated oak forests of Josip Kozarac management unit. CIR aerial photographs (1989) of the research area were taken with a classical camera, while aerial images in 2008 were taken with a digital camera and then converted from digital to analogue form (contact copy - photograph) in order to perform photointerpretation with a SOKKIA MS27 Carl ZEISS Jena mirror stereoscope, magnified by 8x. The health status of particular trees (crowns) was assessed by means of photointerpretation keys in a stereomodel over a systematic 100x100 m sample grid on both 1989 and 2008 aerial photographs. The degree of damage of 4 individual trees was assessed at every grid point in the surveying strips covering the surveyed area. Damage indicators were calculated and thematic maps were constructed on the basis of the interpretation of data for all the grid points.

**Results:** For the research area a damage index (IO) of 68.36% for oak was determined by photointerpreting individual trees (2008); in other words, this percentage of pedunculate oak trees in the surveyed area was found to be in the damage degree of 2.1 and more. Of 68.36% trees classified in the damage degree of 2.1 or more, mean damage (SO<sub>1</sub>) amounted to 52.16% and could be classified in the damage degree of 2.2. In 1989, the mean damage index (IO) for pedunculate oak was 48.00%, and pedunculate oak trees with mean damage degree of 2.1 or more (SO<sub>1</sub>) amounted to 36.03%. The comparison of photointerpretation results from 1989 and 2008 shows that the damage index (IO) for pedunculate oak increased by more than 20%, whereas mean damage (SO) rose by almost 15%. An increase of more than 15% in mean damage of considerably damaged pedunculate oak trees can also be observed.

**Conclusions:** The obtained results provide not only the current forest condition but also indicate the condition in the field and the purposefulness of aerial surveying, since aerial photographs allow an insight into the condition in the field in a short time period. This in turn enables the efficient application of measures to mitigate the consequences of dieback.

**Keywords:** CIR aerial photographs, photointerpretation, crown damage, monitoring, pedunculate oak (*Quercus robur* L.), narrow-leaved ash (*Fraxinus angustifolia* Vahl.)

## INTRODUCTION

A good quality monitoring of forest damage is based on the systematic collection of data on forests in a particular area and at a particular time. In Croatia, monitoring has been effectively performed by observation in the field since 1987, and by interpretation of colour infrared (CIR) aerial photographs since 1988. Pedunculate oak (*Quercus robur* L.) forests have for a relatively long time been subjected to changes that fundamentally affect their stability, which is directly reflected on their productivity. According to the results of field assessment of forest damage in Croatia (a method prescribed by ICP Forests), considerable damage of pedunculate oak is gradually increasing, making pedunculate oak the most endangered broadleaved species in Croatia [1, 2].

Tree dieback is a complex process that involves a large number of site, stand and biotic factors [3-8]. The negative consequences are mitigated by monitoring the condition of pedunculate oak trees and by assessing crown damage [9].

Research to date [10-14] show that in terms of accuracy the method of forest damage assessment by photointerpreting CIR aerial images is equal to field method of damage assessment; however, it is much more efficient in terms of work efficiency and objectivity.

According to Kalafadžić and Kušan [15, 16], damage inventories employing aerial photography is based on the assumption that there are explicit differences in the way of mapping between healthy and damaged trees. These differences are conditioned by changes in the spectral system of the reflected solar radiation and by a changed crown shape.

In CIR aerial photographs, forest damage is inventoried by assessing the degree of damage of particular trees (crowns) seen in the aerial photographs. The method of mapping particular degrees is established with a carefully established photointerpretation key [12].

In Croatia, pilot aerial surveys using CIR films [17, 18] were followed by the application of CIR aerial photographs to inventory damage over larger areas since 1988 [15, 16, 19]. Forest damage inventory was used to investigate both the impact of some biotic and abiotic factors on stand damage [20] and the reliability of damage assessment in CIR aerial photographs [10]. Remote sensing methods allow for simple monitoring of changes in the forest condition and identifying new hot spots of tree dieback or other damage. This is particularly important in today's changed and disturbed ecological conditions as it makes it possible to predict efficient measures for alleviating dieback consequences [21, 22]. Research to date has shown that more attention should be paid to trees damaged by over 25%, because such trees cannot recover any more; in other words, it is assumed that such trees will die before the end of rotation [4].

Initial aerial survey using CIR film were performed in 1989 over lowland forests of pedunculate oak of Josip Kozarac management unit for the purpose of detecting damage degrees. Since this survey showed all the advantages of photointerpreting CIR aerial photographs for forest damage assessment, the same area was again surveyed 19 years later to monitor the forest condition.

The main goal of this paper was to highlight the importance of CIR aerial photographs for efficient inventory, monitoring and predicting the health status of forests in changed site

conditions. For that purpose, CIR aerial photographs from two aerial surveys conducted in 1989 and 2008 were used to identify and analyze the damage in lowland pedunculated oak (*Quercus robur* L.) forests during each period, as well as to obtain a dieback trend in the observed period.

## MATERIAL AND METHODS

As already mentioned, the objects of this research were lowland pedunculated oak forests of Josip Kozarac management unit (MU) (Figure 1).

Josip Kozarac MU has an area about 5500 ha, and it is fairly representative of the lowland forests with pedunculate oak and narrow-leaved ash as the main tree species. This area is located about 5 km south of the Zagreb-Belgrade motorway, near the villages of Kraljevena Velika and Lipovljani, and about

15 km south east of Kutina. The hydrological conditions of these forest ecosystems depend on the River Sava, which is about 5 km south of the facility and its left bank tributaries. The relief is typically lowland, gently rolling, with marked microelevations and micro-depressions, and with the height above sea level being 91-105 m. The age of the forest stands is 105 -150 years.

The area of Josip Kozarac MU was firstly surveyed with CIR film (Kodak Aerochrome 2443) in 1989 using a Zeiss Jena LMK 305/23 camera ( $f=305.18$  mm), providing analogue CIR aerial photographs in the format 23x23 cm and with spatial resolution of 50 cm. The average scale of was 1:6100. Ninety compartments were partially or fully captured in a total of 41 aerial photographs with forward overlaps of 60% and side overlaps of 20-30% collected in 5 strips. The second aerial survey of the same area was conducted in 2008 with a Microsoft UltraCamX digital large-format aerial camera



FIGURE 1. The location of the study area - Josip Kozarac management unit

( $f=100.5$  mm). A digital camera was used for the first time for inventory forest damage in Croatian forestry, providing digital CIR aerial images of high spatial resolution (ground sampling distance - GSD). In total, 122 CIR digital aerial images of 10 cm GSD with forward overlaps of 60% and side overlaps of 36% were taken in 12 strips (Figure 2).

According to Figure 2, strips 8527, 8528 and 8531 (aerial survey of 2008) fully coincide with strips 537, 538 and 539 (aerial survey of 1989). Since the aerial survey of strips from 2008 covered the entire area of the management unit, the two remaining strips (535 and 536) from the earlier surveying operation are also comparable with the results of photointerpretation.

For the needs of this research, the images from 2008 were converted from digital to analogue form (contact copy - photograph)

in order to perform photointerpretation with a SOKKIA MS27 Carl ZEISS Jena mirror stereoscope, magnified by 8x. The photographs from 1989 were also photointerpreted by means of a mirror stereoscope, but on the table for illumination since the recordings were the original films.

Firstly a radial triangulation of aerial photographs was conducted. Prior to photointerpretation, a quality photointerpretation key needs to be established. The photointerpretation keys of the main tree species of Josip Kozarac management unit, pedunculate oak (*Quercus robur* L.) and narrow-leaved ash (*Fraxinus angustifolia* Vahl), have already been established within previous studies [10, 22] and were used for the purpose of this research. It is important to emphasize that the field data for photointerpretation keys

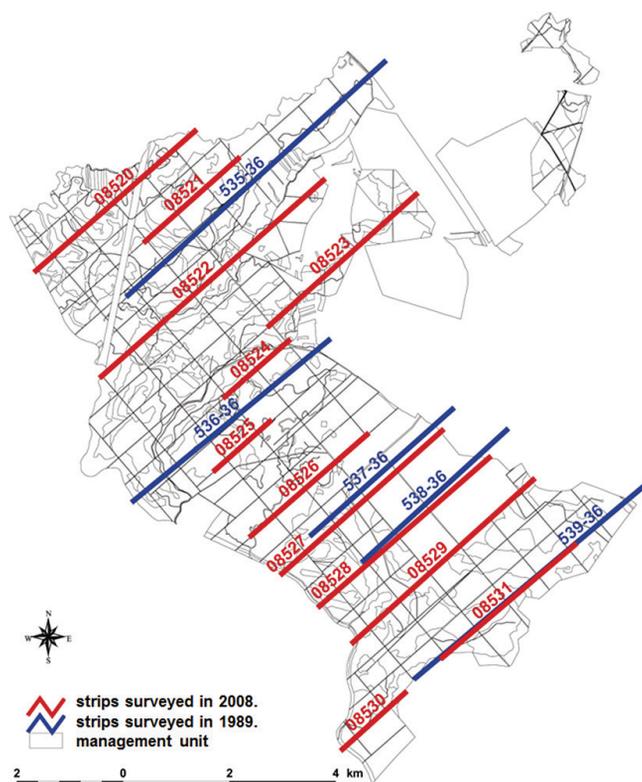


FIGURE 2. Strips surveyed in 1989 and 2008 in Josip Kozarac management unit

were collected in the same time (vegetation period of 1989 and 2008) as aerial surveys. The trees for which it was assumed that they could easily be detected in aerial photographs were sampled. An attempt was made to achieve it in a sample of a sufficient number of trees in every damage degree. To each selected tree in the field an adequate damage class was assigned. In order to be found in aerial photographs, the position of each tree in the field was outlined in relation to recognizable environmental topographic details. The positions of the trees sampled during 2008 were recorded using Global Positioning System receiver. In addition, especially characteristic trees or group of trees were photographed and described. In this way, most of the sampled trees were identified in the aerial photographs. Finally, the photointerpretation keys were defined on the basis of an idea of how a particular tree species or damage degree are reflected on CIR aerial photographs.

Using previously established photointerpretation keys, the health condition of individual trees (crowns) was assessed in the stereo-model in a systematic 100x100 m sample grid on both 1989 and 2008 aerial photographs. At each grid point, damage degree was assessed to four individual trees (crowns) closest to the grid point in the upper left and right, and bottom left and right corner. In this way, the damage degree of 4 individual trees (crowns) was assessed on every grid point according to the scale shown in Table 1.

**TABLE 1.** Damage degree scale

Damage degree (%)	Damage percentage (%)
0	0-10
1	11-25
2.1	26-40
2.2	41-60
3.1	61-80
3.2	81-100
4	Snags

The data for all the grid points were interpreted and damage indicators were calculated according to the following equations [16]:

- Damage (O):

$$O = \frac{\sum f_{(1-4)}}{\sum f_{(0-4)}} \cdot 100 \quad (\%)$$

- Mean damage (SO):

$$SO = \frac{\sum f_i \cdot x_i}{\sum f_i} \quad (\%)$$

- Damage index (IO):

$$IO = \frac{\sum f_{(2-4)}}{\sum f_{(0-4)}} \cdot 100 \quad (\%)$$

- Mean damage of significantly damaged trees (SO<sub>1</sub>):

$$SO_1 = \frac{\sum f_{(2-4)} \cdot x_{(2-4)}}{\sum f_{(2-4)}} \quad (\%)$$

where:

- $f_i$  - number of trees in  $i$  - damage stage,
- $x_i$  -  $i$  - stage interval center in the damage stage scale for single trees (0=5%; 1=17.5%; 2.1=32.5%; 2.2=50%; 3.1=70%; 3.2=90%; 4=100%).

A more detailed description of damage indicators can be found in Kalafadžić and Kušan [16] and Pernar *et al.* [14].

In accordance with the acquired results, thematic maps were constructed using ArcGIS 9.2 software in a scale of 1:5000 with spatial distribution of mean damage (SO) and damage index (IO) for the main tree species, and overall for the management units (compartments/subcompartments) in individual recording strips.

## RESULTS AND DISCUSSION

Damage indicators for the main tree species (pedunculate oak and narrow-leaved ash) and overall for the management unit were calculated on the basis of the results of CIR aerial photograph interpretation from two surveying periods (Table 2).

A comparison of significant oak damage (IO) from two surveying periods shows that oak damage was higher in 2008, i.e. it rose by about 20% (Table 2, Figure 3). Mean damage of significantly damaged trees also increased (36.03% in 2008, 52.16% in 2008). This is also confirmed by the results of field assessment of forest damage in Croatia (a method prescribed by ICP Forests), stating that significant damage of pedunculate oak has been gradually increasing since 2002, thus making pedunculate oak the most endangered broadleaves species in Croatia [1, 2].

As for ash (Table 2, Figure 4), mean damage increased only slightly (in 1989, SO = 28.87%; in 2008, SO = 30.59%). Significant damage was even lower than in the earlier surveying period (in 1989, IO = 58.40%; in 2008, IO = 56.74%). This coincides with ash weevil outbreaks recorded in the area in 1989.

This is also illustrated by mean damage of significantly damaged trees (SO<sub>1</sub>), which in the case of ash is on a slight rise, unlike mean damage of significantly damaged pedunculate oaks, which rose by about 16%. Consequently, a slightly higher damage percentage in ash is attributed to pest attacks [23], whereas in the case of oak, damage shows continuous rise: unlike ash, the crown structure in oak is also disturbed. If the amount of assimilation organs is decreased, the intensity of radiation reflected from these organs also decreases, forcing other parts of the tree - shoots, branches and stem,

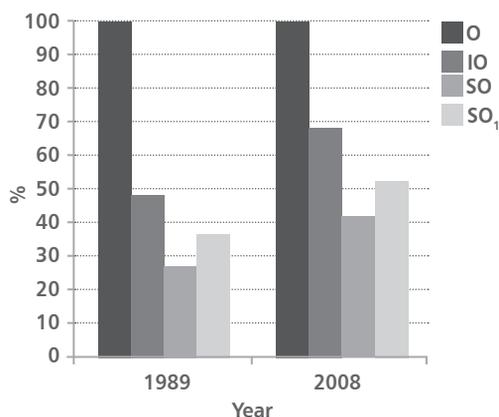


FIGURE 3. Comparison of pedunculate oak damage indicators for two surveying periods

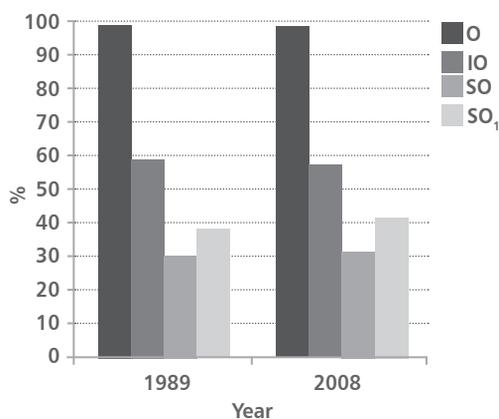


FIGURE 4. Comparison of narrow-leaved ash damage indicators for two surveying periods

TABLE 2. Damage indicators for Josip Kozarac management unit (aerial survey in 1989 and 2008)

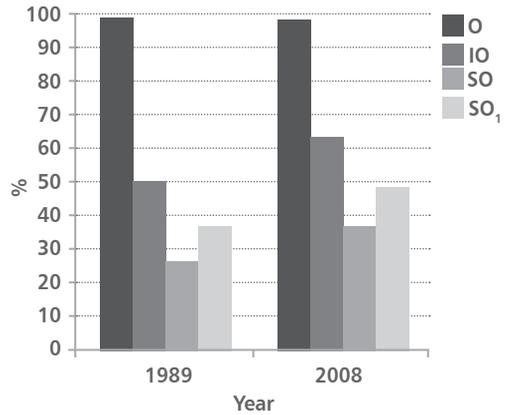
Damage indicators	Tree species				Total	
	Pedunculate oak		Narrow-leaved ash		1989	2008
	Year					
1989	2008	1989	2008	1989	2008	
O	99.83	99.26	98.37	97.92	98.89	98.77
IO	48.00	68.36	58.40	56.74	50.70	64.12
SO	26.37	41.10	28.87	30.59	27.02	37.26
SO <sub>1</sub>	36.03	52.16	37.50	41.02	36.64	48.56

O - damage; IO - damage index; SO - mean damage; SO<sub>1</sub> - mean damage of significantly damaged trees

to participate in the reflection process [24]. This results in decreased intensity of red colour, which in turn affects the assessment of crown damage.

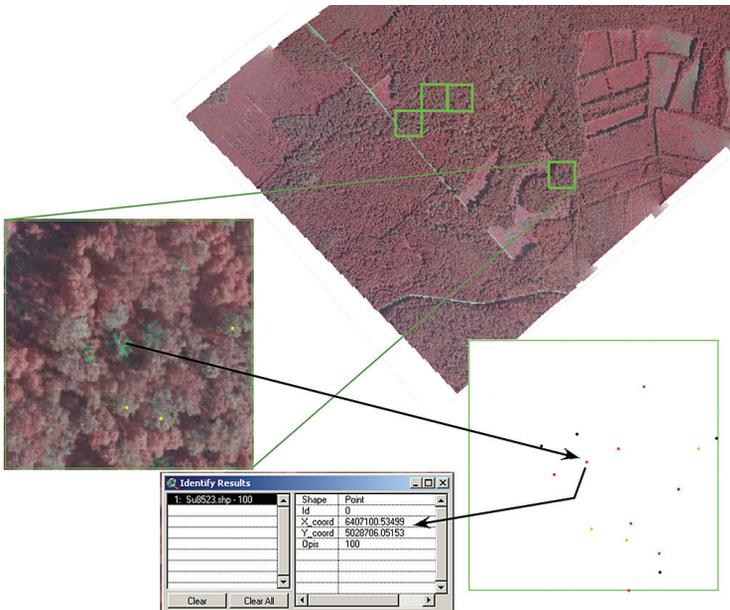
In terms of mean damage (total) in 2008 - SO = 37.26%, it can be seen that it is higher in relation to 1989 - SO = 27.02% (Table 2, Figure 5). Significant damage also increased. In 1989, 50.70% of the trees were damaged above 25%, while in 2008, 64.12% of the trees were damaged above 25%.

The obtained results of damage detection show distinct oak damage in the surveyed area. Research to date indicates that more attention should be paid to significantly damaged trees, since such trees are hard to recover, or in other words, it is assumed that they will die before the end of rotation. An increasing proportion of sanitary fellings in the annual cuts forces us to pay particular attention to the health status of forests and the amount of unplanned felling: therefore, we also analyzed the delineation selection of the compartments/subcompartments in which photointerpretation of CIR aerial images showed that oak damage exceeded 60%, or in which more than 50% of the trees were significantly damaged (IO),



**FIGURE 5.** Comparison of damage indicators overall for all the interpreted species from two surveying periods

according to the quantity of sanitary fellings. Infrared digital orthophoto (1:5000) was used to inventory (map) more severely damaged trees and snags, whose marking provided spatial distribution in the surveyed area (Figure 6). According to the results, the highest number of trees marked for sanitary fellings were found precisely in the compartments in which photointerpretation revealed the highest



**FIGURE 6.** Excerpt from a surveying stripe (2008) with mapped trees in 1 ha samples (snags are marked red, and trees in damage degrees 3.1 and 3.2 are marked yellow and purple)

damage degrees (2b, 3a, 3b) (Figure 7).

Identifying and monitoring the spatial distribution of damaged trees and snags is one of the priorities of sustainable management [19]. Therefore, timely location of stands of poorer health is necessary so that appropriate measures can be applied to maintain their vitality and productivity at an optimal level. This is enabled by interpretation of CIR aerial photographs. The achieved results provide not only the current forest condition, but also indicate the condition in the field and the purposefulness of aerial surveying. Aerial photographs allow an insight into the field condition in a short time period and enable efficient prediction of measures to alleviate dieback consequences.

## CONCLUSIONS

The results obtained by photointerpreting CIR images from two periods for the area of Josip Kozarac management unit relate to identifying and analyzing damage in pedunculate forests, as

well as obtaining data on the spatial distribution of damage. They serve as a basis for the study of characteristics that affect the health condition of pedunculate oak forests.

A comparison of the results of photo-interpretation for the period of the past 19 years shows that, in terms of all the interpreted species (overall), mean damage rose by about 10% in relation to 1989. In contrast, the damage index is higher by about 14%, i.e., 19 years ago there were 50.70% trees in the damage degree of 2.1 and more, whereas in 2008, there were 64.12% trees in this degree. In terms of oak damage,  $SO = 41.10\%$  in 2008, and  $SO = 26.37\%$  in 1989. This means that mean damage for pedunculate oak rose by about 15% over a period of 19 years.

The acquired results provide an insight into the condition of pedunculate oak in the surveyed area and can serve as a basis for future planning of this area in changed site conditions. They can also be used to predict future development and assess the probability and pace of dieback.

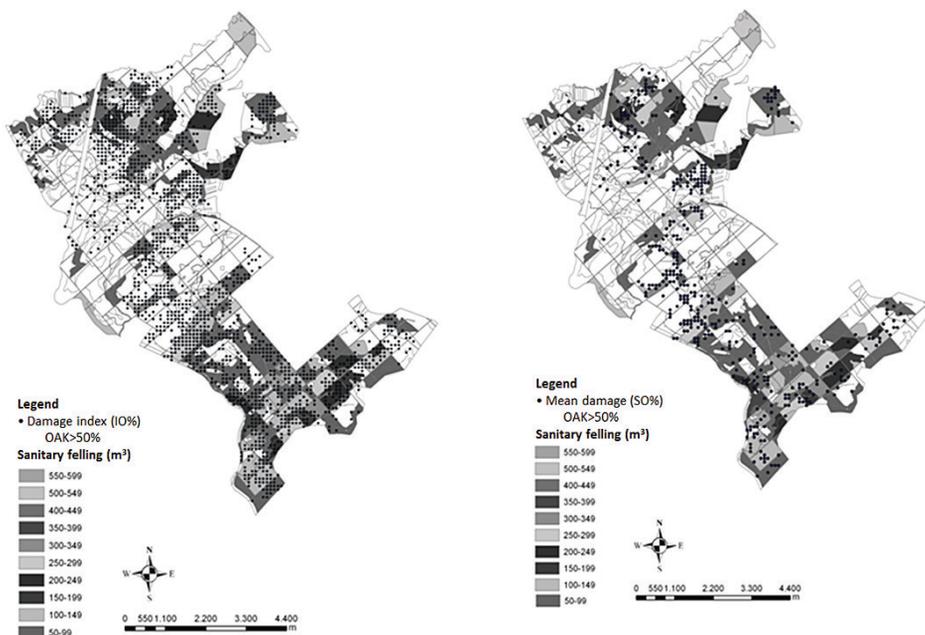


FIGURE 7. Spatial distribution of damaged trees and snags and sanitary fellings

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# Black Locust (*Robinia pseudoacacia* L.) Root Cuttings: Diversity and Identity Revealed by SSR Genotyping: A Case Study

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## Abstract

**Background and Purpose:** Black locust (*Robinia pseudoacacia* L.) is a valuable species native to North America and today widely planted throughout the world for biomass production. In Hungary, where *Robinia* has great importance in the forest management, the clones have been selected for plantations on good, medium and poor quality sites. To conserve the identity, superior clones are vegetatively propagated by root cuttings. At times the collection of root cuttings can cause uncertainty for clonal identity because of the overlap of roots from neighboring plants. This can occur especially when the repository is damaged from severe environmental accidents and the planting layout has been lost. The aim of this study has been to verify by molecular markers the diversity or identity of black locust clones by root cuttings harvested in a damaged trial.

**Materials and Methods:** Root cuttings of 91 clones belonging to five cultivars were collected in a trial severely damaged by storms and flooding periods. The obtained plantlets were analyzed with nine microsatellite (SSR) markers and the genetic identity/diversity within and among the plants was tested using the software GenAIEx version 6.

**Results:** Multilocus genotypes (MLG) and the Paetkau's assignation test (1985) revealed genetic variability among the samples: the analyzed plantlets were grouped in four classes instead of the five expected. In addition, 6 unique genotypes have been detected.

**Conclusions:** This study remarks problems that may arise during the harvest of *Robinia*'s root cuttings, especially when the planting layout has been confused. Molecular analyses can be successfully used to control the germplasm before its sale as guaranty for nurseries, farmers and stakeholders.

**Keywords:** Black locust (*Robinia pseudoacacia* L.), vegetative propagation, molecular markers, microsatellites, genotyping

## INTRODUCTION

Black locust (*Robinia pseudoacacia* L.) is a nitrogen-fixing leguminous tree, widely spread over the temperate regions of the world because of its rapid growth and adaptability to environmental stress such as low-fertility soils [1]. The species, native to North America, was the first forest tree species introduced and acclimated to Europe in the 17th century where, at present, it can be considered naturalized [2]. Although in some European countries *Robinia* is still considered alien and invasive, in other regions it is appreciated for arboriculture [3, 4] as a tree species which is fast growing and multipurpose for various production (fuel and quality wood, fodder, poles, honey) [5-7].

In Hungary, black locust covers approximately 24% of the forest area and provides about 19% of the annual timber output [8]. Since 1960s, many cultivars have been selected and certified [9, 10] for quality wood production and exported to many countries for arboriculture plantations. Hungary possesses one of the most abundant black locust germplasm repositories in the world. The clonal cultivations aim not only to improve the timber quality and apiary purport, but also to produce biomass, to establish short rotation plantations, to mitigate the effect of global climate change and to contrast soil erosion [8].

Black locust genotypes can be multiplied by seed and by root cuttings. Seed propagation is easy and cheap, but seed-raised plants often present great genetic variation unsuitable for cultivars [11]. Propagation from root cuttings is suitable for the reproduction of individuals and cultivars because superior traits can be preserved in the clones. However, the production of clones of *Robinia* by root cuttings demands utmost care and attention

because the root system forms a complex grid with frequent overlapping from nearby clones. A sampling error could cause the propagation of an unselected genotype; this is an important concern where the conservation of gain by clonal selection is necessary. Recently, 12 new Hungarian clones of black locust have been identified for superior form, wood quality for industry and for their ability to tolerate different environmental conditions [8]. These clones, commonly regenerated by root cuttings, can be sold to farmers for short rotation forestry in poor and difficult soils. The source of clones is conserved in plots at the Hungarian Forest Research Institute, (Kecskemét, HU) and is available to satisfy the increasing requirements of international forest markets.

During the last ten years, Hungary has been affected by difficult environmental conditions (<http://www.eurometeo.com>). The Kecskemét repository has been strongly affected by strong storms and snow, soil flooding and summer dryness. Several plots have been seriously damaged and many trees have been eradicated. In these conditions trial management was difficult, expensive, and the reservoir of clones was abandoned for 6 years. Recently, an increasing request of selected certified clones from international countries has been received, and the repository of Kecskemét has been considered again for collection of germplasm. The damage caused by adverse past weather conditions appeared so serious that the planting layout was beyond recognition. Thus, the identification and the collection of root cuttings from a required genotype was particularly uncertain.

Molecular markers are tools commonly employed for fingerprinting analysis of humans, animals and plants. Several biochemical (Isozymes) and molecular markers (RAPDs, ISSR)

have been applied to black locust for population genetic studies, genome mapping and marker-assisted selection in a broad sense [12-16]. Microsatellites, also known as Simple Sequence Repeats (SSRs), are neutral co-dominant markers, widely dispersed in eukaryotic genomes and highly variable. SSRs are specific sequences of DNA nucleotides which contain tandem repeats of 2-6 base pairs. The length of the repeated unit is the same for the majority of the repeats within an individual SSR locus, but the number of repeats for a specific locus may differ, resulting in alleles of varying length. A vast amount of data emerging for SSR markers across organisms makes microsatellite analysis a widely accepted tool for identifying individuals, clones, cultivars. SSRs have been developed and applied in black locust to differentiate genets, ramets, and provenances [17-21].

This study was carried out to investigate involuntary putative mistakes during the collection of root cuttings in a damaged clonal repository. Contextually, the research aimed to verify the regeneration efficiency of roots collected in a troubled plantation. We have applied nine SSR molecular markers to test the identity/diversity of black locust plantlets generated from root cuttings collected at the foot of clones belonging to 5 cultivars, respecting the planting layout valid before the storms.

## MATERIALS AND METHODS

Root cuttings of five black locust cultivars, suitable for plantation in low-fertility soils, were chosen for molecular investigation. Clones were located and collected in the experimental field trial of the Hungarian Forest Research Institute, Kecskemét City, Kiskunság region, Hungary (Lat: 46°54'22"N; Long: 19°41'28"E; elevation 120 m a.s.l.). Until the beginning of the 19th century, the region presented large pastures, then abandoned because of the overgrazing. Currently, concentrated reforestation and the planting of fruit and vines have been installed to stabilize

the sandy soil. The characteristic weather in the Kiskunság region is continental warm, dry, sometimes extreme, but the amount of sunlight makes it possible to grow agricultural crops. The warmest month in Kecskemét is July (mean T 20.9°C), the coldest is January (-1.9°C); early spring and late autumn frosts are frequent. During the last ten years serious storms affected the territory and the repository was damaged: many plants fell, roots of the neighboring sprouted, and the mixing of the original plantation plots occurred. Clones belonging to specific cultivars are therefore difficult to be identified, while the collection of material certified for farmers and nurseries is not secure.

Ninety one black locust's root cuttings of five cultivars were sent to CNR-IBAF, Porano (Italy) in November 2012 (Table 1). They were immediately planted in numbered pots (one root per pot) containing  $\frac{3}{4}$  garden soil and  $\frac{1}{4}$  sand; the pots were placed in greenhouse at 25°C, 70% humidity and natural photoperiod for three months. At the beginning of June 2013 the pots have been transferred outdoors. In July 2013, young leaves were collected from each sprouted plantlet and stored at -80°C until the genetic analysis was conducted. The list of the clones with the identification codes and the number of root cuttings per each clone are reported in Table 1.

Originally the clones were distributed in blocks 6 m × 3 m; each block corresponding to one cultivar. Actually, the spatial distribution of clones is uncertain because of the above described complex situation of the experimental field. Thus the collection of root cuttings followed the original schema of plantation, and they were sampled where the mother plants were sited. Because of this incertitude, we have considered the obtained plantlets as one artificial population (group) putatively divided into 5 small sub-groups.

Genomic DNA was extracted and purified using the DNeasy96 Plant Kit (QIAGEN) according to the manufacturer's instructions and stored at -20°C. The DNA in the samples is brought to a working concentration of

**TABLE 1.** A list of the analyzed clones (each group of clones corresponds to one cultivar), identification codes and the number of root cuttings per each clone. To obtain plantlets each root cutting was planted in different pots (1 root-1 pot). The pots were labeled with the ID code of clone followed by the number of root (eg OS01, OS02,....OS20). The sprouted plantlets conserved this own labeling.

Clone name	Identification code (ID)	Number of root cuttings per clone
Oszlopos (PV 233A1)	OS	20
Bácska (KH 56 A 2/5)	BA	21
Homoki (MB 17D 3/4 )	HO	16
Szálás (PV 35 B2)	SZA	18
Vacsi (PV 201 E 2/1)	VA	16

5 ng· $\mu\text{L}^{-1}$ . Nine unlinked microsatellite loci (Rops02, Rops04, Rops05, Rops08, Rops09, Rops10, Rops15, Rops16, Rops18 [18, 19] are used to characterize the samples (Table 2). Polymerase chain reaction (PCR) is performed using 15 ng of DNA template, 10 mM Tris-HCl (pH=8.0), 50 mM KCl, 1.5 mM MgCl<sub>2</sub> reaction buffer, 200  $\mu\text{M}$  dNTP (each), 0.008  $\mu\text{g}$  BSA, 0.2U of Taq polymerase (Roche Applied Science), 0.5  $\mu\text{mol}$  fluorescently labelled M13 forward primer [NED-M13(-21) primer NED-5'-TGAAAACGACGGCCAGT-3' [22, 23], 0.5  $\mu\text{mol}$  reverse primer, 0.1  $\mu\text{mol}$  M13-tailed forward primer in a total volume of 10  $\mu\text{L}$ . The reactions were performed in a GENEamp 9700 Thermocycler according to the following procedure: an initial denaturation at 94°C for 5 min, followed by 10 cycles of touchdown PCR, each consisting of denaturing at 94°C for 30 sec, 90 sec at 63°C decreasing by 1°C per cycle, elongation at 72°C for 30 sec; followed by 20 cycles at 94°C for 30 sec, 90 sec at the optimum annealing temperature for each primer pair, and 30 sec at 72°C and a final extension step at 72°C for 10 min. PCR amplification fragments were resolved by capillary electrophoresis with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). A 500 bp internal-lane size standard (Gene Scan TM -500 ROX, Applied Biosystems) was used. The resulting data were collected using Gene Scan Analysis version 3.7 software and genotype profiles were assigned with GenTyper version 3.7 NT software (Applied Biosystems).

The identity/diversity within and among sub-groups was tested using the software GenAlEx version 6. [24]. Descriptive statistics including allele frequencies, the total number of the observed alleles ( $N_a$ ), the effective number of alleles ( $N_e$ ) [25, 26] expected heterozygosity ( $H_e$ ) and Shannon information index ( $I$ ) were computed for each sub-group. The Shannon diversity index is widely used in ecology but less in clonal diversity because it is subject to sampling variance [27]. The index  $I$ , calculated on a single locus basis ( $I = \sum p_i \cdot \ln p_i$ , where  $\ln$  is the natural logarithm and  $p_i$  is the frequency of the  $i^{\text{th}}$  allele), provides a measure of allelic and genetic diversity. The number of private alleles for a single sub-group and the number of common alleles ( $f > 5\%$ ) within each sub-group were counted. For each sub-group, the frequency of private alleles at each locus and the number of private genotypes were determined. Genotype data were used to assess the number of different multilocus genotypes in the group of 27 individuals: the genotype probability has been calculated for each multilocus genotype considering the frequency of each allele in the genotype ( $p_i$ ) and the number of heterozygous loci ( $h$ ) (Genotype probability = product( $p_i^2$ )<sup>h</sup>). To verify the affiliation of the genotypes to the five candidate sub-groups, the frequency based assignment test of Paetkau [28, 29] was performed. This approach removes the individual being assigned (leave one out procedure), computes the allelic frequencies in all candidate groups, calculates the likelihoods

**TABLE 2.** A list of the 9 nuclear microsatellite loci [18, 19] applied for the analysis of 27 black locust plantlets sprouted from the root cuttings. For each locus the sequence, the repeat length, the annealing temperature, the resulted size range and the number of alleles are reported.

Reference	Locus	Repeat	Primer sequence (5'-3')*	Ta (°C)	Size range (bp)	No. of alleles
Lian and Hogetsu, 2002 [18]	Rops02	(AC) <sub>13</sub> (AT) <sub>4</sub>	<u>TGTA AACGACGGCCAGT</u> CAGA ACTGTGGAGAATAATTCT ACCGCGCCATCTGTTAGITTTGTTGC	60	122-234	8
Lian and Hogetsu, 2002 [18]	Rops04	(AC) <sub>10</sub>	<u>TGTA AACGACGGCCAGT</u> GTCTA ATTTCACTTTTCTCACGAGGGACA CCACCRAAATTCTACC	56	123-127	3
Lian and Hogetsu, 2002 [18]	Rops05	(AC) <sub>2</sub> GC(AC) <sub>7</sub>	<u>TGTA AACGACGGCCAGT</u> TGGTG ATTAAGTCGCAAGGTGTTGTGAC TTGTACGTAAGTC	56	133-166	7
Lian and Hogetsu, 2002 [18]	Rops08	(CA) <sub>3</sub> TA(CA) <sub>3</sub>	<u>TGTA AACGACGGCCAGT</u> TTCTG AGGAAGGGTTCCGTGGGTTAAAG CAACAGGCACATGG	56	209-221	4
Lian and Hogetsu, 2002 [18]	Rops09	(TA) <sub>6</sub> A <sub>4</sub> (TA) <sub>2</sub> (TG)	<u>TGTA AACGACGGCCAGT</u> CTCCA GGTCACTCGATTGAGGTTTCTCATT TGATACGACCCC	56	86-148	8
Lian and Hogetsu, 2002 [18]	Rops10	T <sub>12</sub> AAT <sub>4</sub>	<u>TGTA AACGACGGCCAGT</u> AAC TTT TTCCGTATAGGGGTCCAGTTT TACA CTTGGTCAAACC	56	195-200	4
Lian <i>et al.</i> , 2004 [19]	Rops15	(CT) <sub>20</sub>	<u>TGTA AACGACGGCCAGT</u> CCCATT TTCAGAATCCATATATTGGTCATCC TTGTTTTGGACAATC	54	125-233	8
Lian <i>et al.</i> , 2004 [19]	Rops16	(CT) <sub>13</sub>	<u>TGTA AACGACGGCCAGT</u> AACCCT AAAAGCCTCGTTATCTGGCATT TTTT GGAAGACACC	56	216-234	7
Lian <i>et al.</i> , 2004 [19]	Rops18	(AC) <sub>3</sub>	<u>TGTA AACGACGGCCAGT</u> AGATAA GATCAAGTGCAAGAGTGTAAAGTAAA TCCTCGAGGGAACAATAC	54	159-237	4

of the individual's multilocus genotypes occurring in each group (the independence of loci), and assigns the individual to the group with the highest log-likelihood (for instance, the group showing the least negative log-likelihood value). The statistical threshold was calculated simulating 1,000 genotypes by the novel Monte Carlo resampling method [29]. We converted likelihoods from negative to positive values by multiplying by -1, thus the samples were assigned to the group with the smallest value. To estimate the probability that two unrelated individuals drawn from the same group would have the same multilocus genotype, the Identity (PI) and Exclusion Probability (PExc) were also calculated.

PI ( $PI = 2(\sum pi^2) - \sum pi$ , where  $pi$  is the frequency of the  $i^{th}$  allele) represents the probability of two individuals sharing the same multilocus genotype by chance (rather than because they are clones) and is calculated for each of the five sub-groups for individual loci and for all loci together. PExc accounts for the average mismatching probability for any genotype. Because our group of samples is artificially constructed, we have computed PExc assuming the absence of both parents for each individual [30]. PI and PExc are also indicators of the statistical power of the loci we use and provide the minimum number of loci necessary for reliable genetic tagging. Finally, the Software NTSYS pc version 2.1 [31] was used to calculate

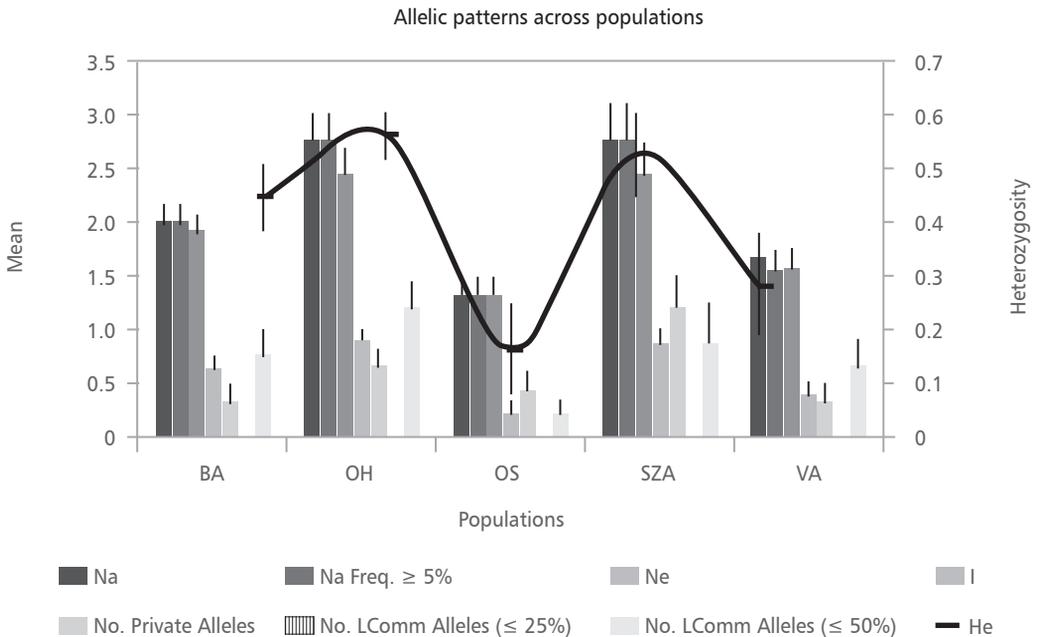
the standard biased genetic distance among 27 individuals according to Nei's algorithm [32]. Considering the small size of our sample, we chose Nei's biased estimation [33]. The cluster analysis has been also carried by the software NTSYS.

## RESULTS

Not all root cuttings in the pots give rise to plants by the end of July, thus the number of obtained plantlets was not the same for each clone. Of 91 root cuttings, only 27 plantlets were obtained, demonstrating that the sampled tissues had a low sprouting percentage (30%). The clone BA was the most recalcitrant as only two plantlets were obtained from the 21 transplanted root cuttings (9.5% sprouting).

From 16 root cuttings of the clone VA we obtained 15 plants (94.0% sprouting); among the five tested clones VA showed the highest percent sprouting. Sprouting percentages of the other three clones were low: 25.0% for HO (4 plants), 16.6% for SZA (3 plants) and 15.7% for OS (3 plants). Over five clones, nine microsatellite loci showed a different number of alleles (with different frequencies) ranging from 3 (primer Rops04) to 8 (primers Rops02, Rops09 and Rops15) with a size range from 86 bp to 237 bp (Table 2).

The indices of genetic diversity (Figure 1 and Table 3) demonstrated variable levels of heterozygosity among the sub-groups: the lowest value ( $H_e=0.167$ ) for the sub-group OS, the highest for OH ( $H_e=0.559$ ). In agreement,  $N_e$  ranged from 1.333 observed for OH to 2.438 for the sub-group SZA which also presented



**FIGURE 1.** Mean values of the gene diversity indices accounted across five *Robinia pseudoacacia* tested sub-groups.

Na - number of alleles; Na Freq.  $\geq 5\%$  - number of different alleles with a frequency  $\geq 5\%$ ; Ne - number of effective alleles,  $N_e=1/(\sum p_i^2)$ ; I - Shannon's Information Index,  $I=-1 \cdot \sum (p_i \cdot \ln(p_i))$ ; No. Private Alleles - number of alleles unique to a single sub-group; No. LComm Alleles ( $\leq 25\%$ ) - No. of locally common alleles (Freq.  $\geq 5\%$ ) found in 25% or fewer sub-groups; No. LComm Alleles ( $\leq 50\%$ ) - No. of locally common alleles (Freq.  $\geq 5\%$ ) found in 50% or fewer sub-groups; He - expected heterozygosity,  $H_e=1-\sum p_i^2$

**TABLE 3.** The mean of gene diversity indices detected across the five *R. pseudoacacia* sub-groups (in brackets Standard Error (SE) values)

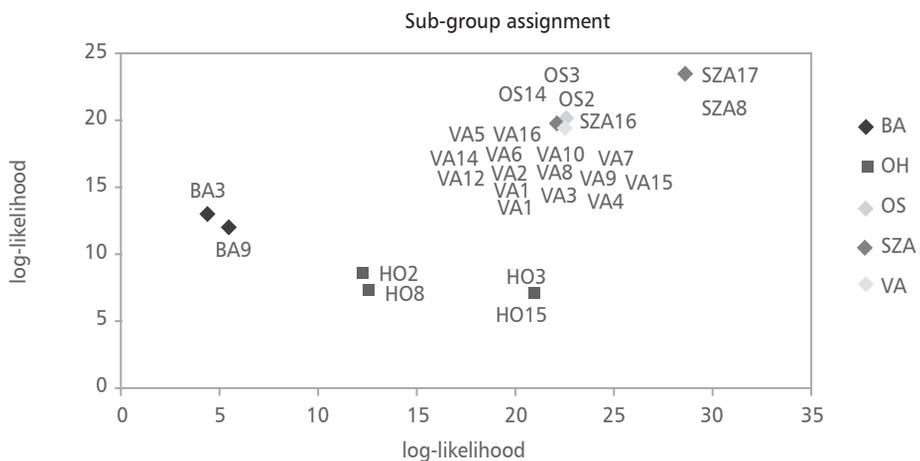
Sub-group	Na	Na Freq. $\geq 5\%$	Ne	I	No. Private Alleles ( $\leq 50\%$ )	No. Common Alleles ( $\leq 50\%$ )	He
*BA	2.000 (0.167)	2.000 (0.167)	1.919 (0.146)	0.640 (0.091)	0.333 (0.167)	0.778 (0.222)	0.444 (0.059)
*HO	2.778 (0.222)	2.778 (0.222)	2.444 (0.245)	0.918 (0.090)	0.667 (0.167)	1.222 (0.222)	0.559 (0.042)
*OS	1.333 (0.167)	1.333 (0.167)	1.333 (0.167)	0.231 (0.116)	0.444 (0.176)	0.222 (0.147)	0.167 (0.083)
*SZA	2.778 (0.324)	2.778 (0.324)	2.438 (0.292)	0.880 (0.138)	1.222 (0.278)	0.889 (0.351)	0.525 (0.075)
VA	1.667 (0.236)	1.556 (0.176)	1.570 (0.181)	0.399 (0.127)	0.333 (0.167)	0.667 (0.236)	0.281 (0.089)

\*sample size less than 5 plants

Na - No. of alleles; Na Freq.  $\geq 5\%$  - No. of different alleles with a frequency  $\geq 5\%$ ; Ne - No. of effective alleles,  $Ne = 1 / (\sum \pi_i^2)$ ; I - Shannon's Information Index,  $I = -1 \cdot \sum (\pi_i \cdot \ln(\pi_i))$ ; No. Private Alleles ( $\leq 50\%$ ) = No. of alleles unique to a single population; No. Comm Alleles ( $\leq 50\%$ ) - No. of locally common alleles (Freq.  $\geq 5\%$ ) found in 50% or fewer sub-groups; He - expected heterozygosity.  $He = 1 - \sum \pi_i^2$

the highest number of alleles (Na=2.778). Although some private alleles were observed for all five sub-groups, SZA showed the highest number (11 private alleles) (Table 4) with a mean frequency of 1.222 (Table 3). The highest number of alleles in common among the sub-groups was detected for OH (frequency=1.222) (Table 3, Figure 1). Sub-groups OH and SZA

showed high values of the Shannon Information index (0.918 and 0.880, respectively). However, we should remark that all scored values of genetic diversity in this study should be treated with caution since the sample size for all sub-groups, except VA, is less than 5 plants. By considering our samples as components of one artificial group, on the basis of the allele



**FIGURE 2.** Frequency based test of assignment [28] to assign the genotypes to clusters: an individual is assigned to the group for which it has the highest likelihood. The graph is obtained by plotting the values reported in Table 5.

frequencies, the frequency of an expected genotype at each locus was calculated across loci and log-transformed and converted to a positive value to provide the log likelihood value (Table 5). The Paetkau's frequency method of assignment revealed 96% of samples assigned to the original sub-groups; only sample SZA16 (4%) was assigned to sub-group VA. In order to have a graphical representation of the

relationship among the genotypes, the results of the assignment test are plotted in Figure 2. Samples OS3, OS14, OS2 and SZA16 occupy a position close to the sub-group of VA samples. Increasing the number of loci, the probability that two unrelated individuals would share the same genotype (PI) decreases (Figure 3). Four loci (Rops02+Rops04+Rops05+Rops08) are sufficient to exclude a sample from sub-groups BA, OH and SZA (PI values  $3 \times 10^{-2}$ ;  $2.7 \times 10^{-2}$ ;  $5.4 \times 10^{-3}$ , respectively) but not enough to differentiate between sub-groups OS and VA. A multilocus genotype with seven loci (Rops02+Rops04+Rops05+Rops08+Rops09+Rops10+Rops15) could be used to differentiate a member among all sub-groups. The probability of exclusion (P3Exc) was calculated assuming that the samples had no parent pairs in common, results already appreciable by the combination of 5 loci (R.02+R.04+R.05+R.08+R.09). For sub-groups SZA and OH, additional loci did not remarkably increase the probability of exclusion (Figure 4). These results were in agreement with the assignment tests. Putative new sub-groups (or maybe clones) based on repeated multilocus genotypes (MLG) are detected for the 27 analyzed plantlets (Table 6). Despite our initial hypothesis of five clones, four new sub-groups were detected and labeled with different letters. The most consistent cluster included all fourteen VA samples except one (frequency of VA MLG on the total samples=51%). The other sub-groups were obtained by grouping samples OS3, OS2 and OS14 (frequency 11.1%), OH3 with OH15 (frequency 7.4%) and SZA8 with SZA17 (frequency 7.4%). In addition, six unique outsider genotypes were detected, each representing 3.7% of the total (Table 6, Figure 5).

UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram obtained from the matrix of genetic distance [32] calculated for the 27 multilocus genotypes and 9 SSR loci (Figure 6) revealed two main clusters: the first including genotypes BA3, BA9, HO2, HO8, HO15, and HO3, the second containing the rest of the material except

**TABLE 4.** Frequencies of the private alleles detected at the corresponding locus per sub-group

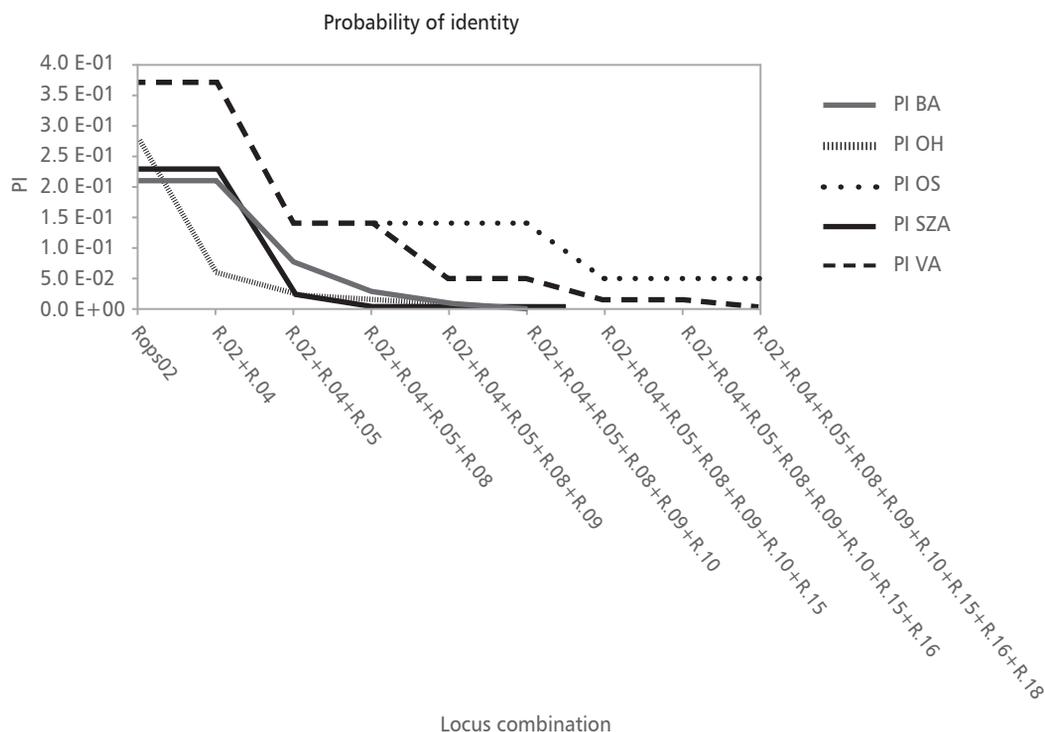
Sub-group	Locus	Allele	Frequency
BA	Rops02	136	0.500
BA	Rops05	150	0.500
BA	Rops 16	222	0.500
OH	Rops02	122	0.250
OH	Rops04	127	0.500
OH	Rops05	144	0.750
OH	Rops09	118	0.250
OH	Rops15	125	0.500
OH	Rops 16	220	0.250
OS	Rops02	128	0.500
OS	Rops05	166	0.500
OS	Rops15	233	0.500
OS	Rops 16	234	1.000
SZA	Rops02	140	0.500
SZA	Rops05	135	0.167
SZA	Rops08	209	0.333
SZA	Rops08	217	0.333
SZA	Rops09	86	0.167
SZA	Rops09	95	0.667
SZA	Rops09	116	0.167
SZA	Rops10	195	0.333
SZA	Rops15	149	0.167
SZA	Rops 16	216	0.500
SZA	Rops18	227	0.333
VA	Rops09	148	0.500
VA	Rops15	178	0.033
VA	Rops18	159	0.500

OS14, OS2 and OS3. The absence of genetic distance within the genotypes HO15 and HO3, most of the plants from VA excluding VA5, SZA17 and SZA8, and OS14, OS2 and OS3, may indicate their clonal origin. The genetic distance detected between the genotypes in BA3 and BA9 (Dist=1.80), within the two plants HO2 and HO8 (Dist=0.90), and among this sub-

group with the sub-cluster HO15-HO3 (9.01), may demonstrate that the plants have different origins despite their supposed clonal origins. The same situation was observed for the plant VA5 (genetic distance from the rest of VA genotypes of 0.90). An erroneous attribution could be the reason of the separation of SZA16 from the other SZA plants.

**TABLE 5.** Frequency based test of assignment [28] to assign the genotypes to the five candidate or to other sub-groups following the "leave one out" procedure. (A) Assignment values with the log-likelihoods converted to positive. The lowest value indicates the most likely group (gray boxes). (B) Summary of the number of samples assigned to "self-candidate" or different sub-group.

Sample	Sub-group	A						B			
		BA	OH	OS	SZA	VA	Assigned sub-group	Sub-group	Self sub-group	Other sub-group	
BA3	BA	4.408	12.941	28.194	24.153	23.097	1	BA	2		
BA9	BA	5.505	11.941	24.194	25.676	24.495	1	BA	OH	4	
HO15	OH	21.028	7.060	24.495	25.977	24.495	2	OH	OS	3	
HO2	OH	12.232	8.583	24.194	22.454	26.495	2	OH	SZA	2	1
HO3	OH	21.028	7.060	24.495	25.977	24.495	2	OH	VA	15	
HO8	OH	12.533	7.310	22.796	22.756	26.796	2	OH	Total	26	1
OS14	OS	22.408	19.959	0.903	21.659	22.000	3	OS	Percent	96%	4%
OS2	OS	22.408	19.959	0.903	21.659	22.000	3	OS			
OS3	OS	22.408	19.959	0.903	21.659	22.000	3	OS			
SZA16	SZA	22.250	19.755	20.796	20.301	18.030	5	VA			
SZA17	SZA	28.620	23.505	26.194	5.367	23.097	4	SZA			
SZA8	SZA	28.620	23.505	26.194	5.367	23.097	4	SZA			
VA1	VA	22.426	19.260	22.796	17.391	1.537	5	VA			
VA10	VA	22.426	19.260	22.796	17.391	1.537	5	VA			
VA11	VA	22.426	19.260	22.796	17.391	1.537	5	VA			
VA12	VA	22.426	19.260	22.796	17.391	1.537	5	VA			
VA14	VA	22.426	19.260	22.796	17.391	1.537	5	VA			
VA15	VA	22.426	19.260	22.796	17.391	1.537	5	VA			
VA16	VA	22.426	19.260	22.796	17.391	1.537	5	VA			
VA2	VA	22.426	19.260	22.796	17.391	1.537	5	VA			
VA3	VA	22.426	19.260	22.796	17.391	1.537	5	VA			
VA4	VA	22.426	19.260	22.796	17.391	1.537	5	VA			
VA5	VA	22.426	19.260	22.796	18.613	3.204	5	VA			
VA6	VA	22.426	19.260	22.796	17.391	1.537	5	VA			
VA7	VA	22.426	19.260	22.796	17.391	1.537	5	VA			
VA8	VA	22.426	19.260	22.796	17.391	1.537	5	VA			
VA9	VA	22.426	19.260	22.796	17.391	1.537	5	VA			



Probability of identity for increasing locus combinations

Sub-group	N	R.02	R.02+ R.04	R.02+ R.04+R.05	R.02+R.04 +R.05 + R.08	R.02+R.04 +R.05+R.08 + R.09+ R.10	R.02+ R.04+ R.05+ R.08+ R.09+ R.10	R.02+R.04+ R.05+R.08+ R.09+R.10+ R.15	R.02+R.04+ R.05+R.08+ R.09+R.10+ R.15+R.16	R.02+R.04+ R.05+R.08+ R.09+R.10+ R.15+R.16+ R.18
BA	2	2.1E-01	2.1E-01	7.9E-02	3.0E-02	1.1E-02	4.2E-03	1.6E-03	5.9E-04	2.7E-04
OH	4	2.8E-01	6.0E-02	2.7E-02	1.3E-02	2.7E-03	5.6E-04	1.2E-04	1.3E-05	4.9E-06
OS	3	3.8E-01	3.8E-01	1.4E-01	1.4E-01	1.4E-01	1.4E-01	5.3E-02	5.3E-02	5.3E-02
SZA	3	2.3E-01	2.3E-01	2.9E-02	5.4E-03	1.6E-03	6.6E-04	8.4E-05	1.9E-05	7.8E-06
VA	15	3.8E-01	3.8E-01	1.4E-01	1.4E-01	5.3E-02	5.3E-02	1.7E-02	1.7E-02	6.5E-03

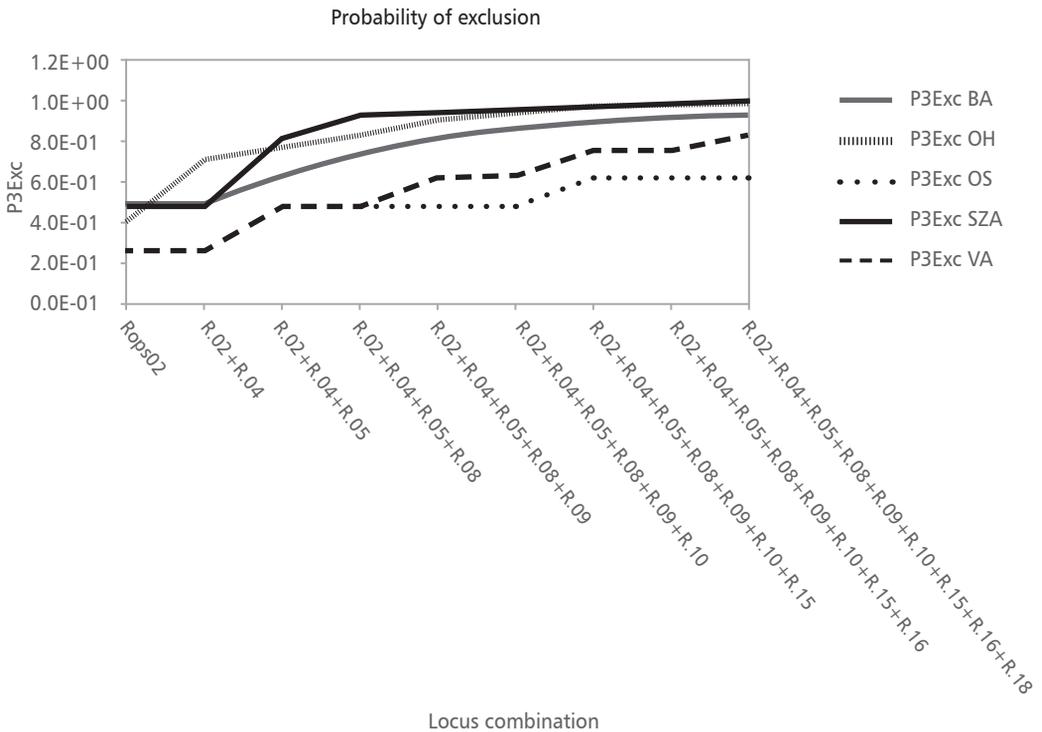
E-01 =  $\times 10^{-1}$ ; E-02 =  $\times 10^{-2}$ ; E-03 =  $\times 10^{-3}$ ; E-04 =  $\times 10^{-4}$ ; E-05 =  $\times 10^{-5}$ ; E-06 =  $\times 10^{-6}$ 

Probability of identity by locus

Sub-group	N	Rops02	Rops04	Rops05	Rops08	Rops09	Rops10	Rops15	Rops16	Rops18
BA	2	2.1E-01	1.0E+00	3.8E-01	3.8E-01	3.8E-01	3.8E-01	3.8E-01	3.8E-01	4.6E-01
OH	4	2.8E-01	2.1E-01	4.6E-01	4.6E-01	2.1E-01	2.1E-01	2.1E-01	1.1E-01	3.8E-01
OS	3	3.8E-01	1.0E+00	3.8E-01	1.0E+00	1.0E+00	1.0E+00	3.8E-01	1.0E+00	1.0E+00
SZA	3	2.3E-01	1.0E+00	1.3E-01	1.9E-01	3.0E-01	4.1E-01	1.3E-01	2.3E-01	4.1E-01
VA	15	3.8E-01	1.0E+00	3.8E-01	1.0E+00	3.8E-01	1.0E+00	3.3E-01	1.0E+00	3.8E-01

E-01 =  $\times 10^{-1}$ ; E+00 = 0

FIGURE 3. Probability of identity (PI) - for each locus and for increasing combinations of the 9 Loci (R. = Rops)



Probability of exclusion for increasing locus combinations

Sub-group	N	R.02	R.02+ R.04	R.02+ R.04+R.05	R.02+ R.04+ R.05+ R.08	R.02+ R.04+ R.05+ R.08+ R.09+ R.0.10	R.02+ R.04+ R.05+ R.08+ R.09+ R.10	R.02+R.04+ R.05+R.08+ R.09+R.10+ R.15	R.02+R.04+ R.05+R.08+ R.09+R.10+ R.15+R.16	R.02+R.04+ R.05+R.08+ R.09+R.10+ R.15+ R.16+ R.18
BA	2	4.90E-01	4.90E-01	6.40E-01	7.40E-01	8.10E-01	8.60E-01	9.00E-01	9.30E-01	9.50E-01
OH	4	4.20E-01	7.10E-01	7.80E-01	8.30E-01	9.10E-01	9.60E-01	9.80E-01	9.90E-01	9.90E-01
OS	3	2.80E-01	2.80E-01	4.80E-01	4.80E-01	4.80E-01	4.80E-01	6.30E-01	6.30E-01	6.30E-01
SZA	3	4.70E-01	4.70E-01	8.10E-01	9.10E-01	9.50E-01	9.60E-01	9.90E-01	9.90E-01	9.90E-01
VA	15	2.80E-01	2.80E-01	4.80E-01	4.80E-01	6.30E-01	6.30E-01	7.60E-01	7.60E-01	8.20E-01

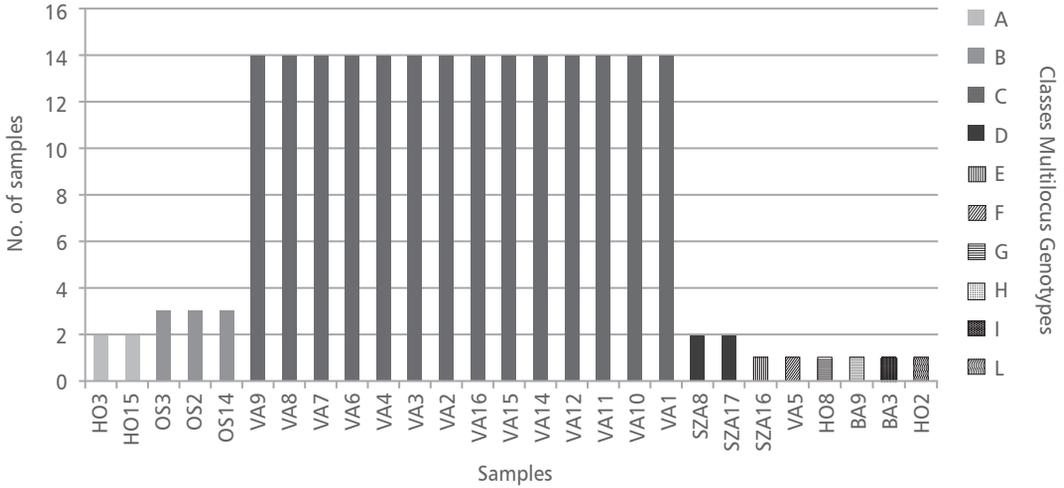
E-01=  $\times 10^{-1}$

Probability of exclusion by locus

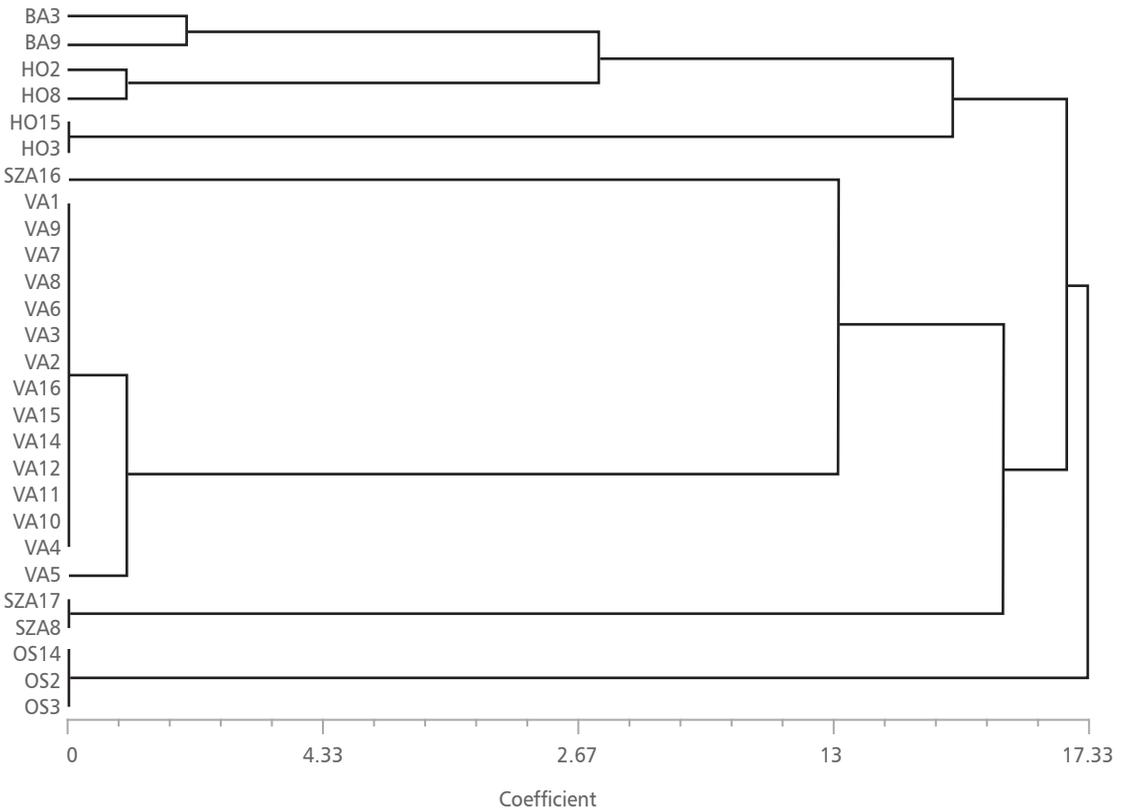
Sub-group	N	Rops02	Rops04	Rops05	Rops08	Rops09	Rops10	Rops15	Rops 16	Rops18
BA	2	4.90E-01	0.00E+00	2.80E-01	2.80E-01	2.80E-01	2.80E-01	2.80E-01	2.80E-01	2.40E-01
OH	4	4.20E-01	4.90E-01	2.40E-01	2.40E-01	4.90E-01	4.90E-01	4.90E-01	6.80E-01	2.80E-01
OS	3	2.80E-01	0.00E+00	2.80E-01	0.00E+00	0.00E+00	0.00E+00	2.80E-01	0.00E+00	0.00E+00
SZA	3	4.70E-01	0.00E+00	6.40E-01	5.20E-01	4.10E-01	2.60E-01	6.40E-01	4.70E-01	2.60E-01
VA	15	2.80E-01	0.00E+00	2.80E-01	0.00E+00	2.80E-01	0.00E+00	3.40E-01	0.00E+00	2.80E-01

E-01=  $\times 10^{-1}$ ; E+00= 0

**FIGURE 4.** Probability of exclusion (P3) - excluding a putative parent pair for each locus and for increasing combinations of the 9 loci (R.=Rops)



**FIGURE 5.** The representation of the repeated matching Multilocus Genotypes (MLG) that may represent ramets of the same clone/genet. The 27 analyzed plantlets are clustered in a total of 4 classes. In addition, 6 private multilocus genotypes have been identified.



**FIGURE 6.** UPGMA cluster based on Genetic Distance coefficient [32] calculated for 27 *Robinia pseudoacacia* multilocus genotypes obtained with 9 SSR loci.

**TABLE 6.** Putative clones based on repeated multilocus genotypes (MLG) detected for the 27 samples, arbitrary clustered in a unique group, using 9 SSR loci. The matching MLG, listed first, are labeled with letters. The number of samples showing the same MLG has been indicated as well as the MLG frequency. Single MLG, listed at the end of the table, are labeled with numbers.

Sample	Sample	Population	Multilocus Genotype (MLG)	No. of samples	Group label	Frequency
5	HO3	OH	122134127127138144221221106118197199125125220226229237	2	A	0.074
3	HO15	OH	122134127127138144221221106118197199125125220226229237			
9	OS3	OS	128134123123146166221221100100199199146233234234237237	3	B	0.111
8	OS2	OS	128134123123146166221221100100199199146233234234237237			
7	OS14	OS	128134123123146166221221100100199199146233234234237237			
27	VA9	VA	132156123123133146221221100148197197142180226226159229	14	C	0.519
26	VA8	VA	132156123123133146221221100148197197142180226226159229			
25	VA7	VA	132156123123133146221221100148197197142180226226159229			
24	VA6	VA	132156123123133146221221100148197197142180226226159229			
22	VA4	VA	132156123123133146221221100148197197142180226226159229			
21	VA3	VA	132156123123133146221221100148197197142180226226159229			
20	VA2	VA	132156123123133146221221100148197197142180226226159229			
19	VA16	VA	132156123123133146221221100148197197142180226226159229			
18	VA15	VA	132156123123133146221221100148197197142180226226159229			
17	VA14	VA	132156123123133146221221100148197197142180226226159229			
16	VA12	VA	132156123123133146221221100148197197142180226226159229			
15	VA11	VA	132156123123133146221221100148197197142180226226159229			
14	VA10	VA	132156123123133146221221100148197197142180226226159229			
13	VA1	VA	132156123123133146221221100148197197142180226226159229			
12	SZA8	SZA	1401561231231331382092179595195199142146216218227229	2	D	0.074
11	SZA17	SZA	1401561231231331382092179595195199142146216218227229			
10	SZA16	SZA	13214012312313514622122186116199199149180216224229229	1	1	0.037
23	VA5	VA	132156123123133146221221100148197197142178226226159229	1	2	0.037
6	HO8	OH	134134123125144144219221120120199200142191218224229237	1	3	0.037
2	BA9	BA	136134125125146150219221106120199200142191222226229237	1	4	0.037
1	BA3	BA	136334125125146150219221106120199200142191222226229229	1	5	0.037
4	HO2	OH	334134123125144144219221120120199200142191218224229237	1	6	0.037
Total				27	10	1.000

## DISCUSSION

Our study has been carried out for the genotyping of plantlets of *Robinia* raised from root cuttings of five selected mother plants located in a damaged experimental trial. The bad condition of the trial and the loss of the original

field layout caused unsure information about the relationship of the sampled roots to a peculiar mother clone. In addition, under controlled conditions, a low percentage of sprouting by the root cuttings was obtained, and the number of the analyzed plantlets was limited. Nevertheless, our study provided useful results.

First, the low percentage of root cuttings germination verifies that without a previous appropriate treatment not all roots of black locust can generate plantlets [21, 34]. Barret *et al.* [6] reported that black locust can be vegetatively propagated from shoots and root cuttings, but success rates vary among clones; our data confirmed this knowledge and highlighted the importance of the quality of the materials. This evidence should be considered for practical purposes in nursery industry and in the trade of clones. The second important aspect is the problematic harvest of the root cuttings. We have analyzed plantlets raised from roots supposedly belonging to five clones (five selected mothers), and we expected five clusters, each one showing homogeneous multilocus genotypes. However, we observed genetic variability within the assumed clones. We detected four main groups instead of five and six plants with unique independent genotypes. These results may be due to different reasons. Black locust is a plastic species with a large environmental adaptability [12]; it is subject to frequent mutations and has high somatic instability [19]. This characteristic, observed in other species as well, has been found mainly for vegetative material propagated *in vitro* [35, 36]. However this explanation seems not appropriate for our samples since they were not obtained from *in vitro* cultures or somatic embryogenesis but from root cuttings collected directly in field. Thus, in normal conditions, they should have genotypes identical to the respective mother tree. The second possible explanation for the observed variability within plantlets could be an involuntary error during the sampling step. It is well known that black locust has an extensive, fibrous root system. It is quite weak, fine and shallow, but the roots extend radially from 1 to 1.5 times tree height [37]. Accidental sampling of roots from an adjacent tree, with roots that overlap the desired clone, is an obvious possible source of error in propagation. Considering the difficult condition of the plantation where our samples have been collected, the displacement of undesired genotypes, due to the sprouting of neighboring cultivars, can be supposed. We

should also consider that the high out-crossing rate and the limited seed dispersal [38] could generate roots from progenies of neighbor clones or wild genotypes. Obviously, the probability of error during the collection of roots cuttings will increase in a damaged plantation, where it is difficult to distinguish mother trees from suckers and seed progenies. The sampling of related plants could explain why several common alleles are found in more than 50% of the groups and why the Paetkau's assignment test (Figure 2) clustered three OS samples and the plant SZA16 with the sub-group VA. However, without a larger sample it is hard to know if the presence of alleles at high frequency represents a high level of relatedness or simply a random assortment. We can presume that the original population does not have a large number of founders, so some alleles could be commonly based on genetic drift. In Hungary, black locust has a long tradition of cultivation in short rotation plantations established using seedlings. Rédei [39] reports that seeds are harvested by sifting about 10 cm of soil under selected clones that are themselves derived from previous seedling progeny trials. This method, useful and fast, allows the collection of large quantities of (mostly) half-sib seeds. Although the resulting progenies may be similar morphologically, they are likely to be genetically diverse. We could assume that the root cuttings collected and analyzed in this study are from plants obtained by this method. The above procedure could also explain the result of the cluster UPGMA that showed two different and genetically distant groups and variability within the sub-clusters (Figure 6).

## CONCLUSION

Our study points to the need for genetic confirmation of the identity of clones, selected by breeders and nursery enterprises, not only before the sale to farmers, but also after plantation. Indeed, certified clones are also important in mosaic clonal plantations designed to avoid failures due to the narrowing of the

genetic base (monoclonal culture) [40]. Ritchie [41] reports that the identification of unique or off-type individuals within a mixed plantation is very difficult unless each tree is labeled. It means that it is difficult to identify a maladapted clonal genotype when it is scattered throughout the plantation, and removing it from the production base could be difficult. Similarly, it can be difficult to identify a certain clone with special performance.

Special care should be applied to the collection of black locust root cuttings because of the overlapping of the root system from different plants. This is particularly important when the material is devoted to the agricultural market for farmers. However, in spite the attention, collection errors could happen especially in damaged field trials, and the identity of clones could be unsure. Molecular tests may support traditional breeding to resolve questions about the identity of ramets for trade

avoiding regrettable mistakes, and for farmers to obtain sustainable wood plantations using certified clones. Our study shows that molecular markers can be successfully employed for the early and post selection of clonal material.

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# Survey Research in the Forest Science Journals - Insights from Journal Editors

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## Abstract

**Background and Purpose:** Survey research is one of the most commonly applied approaches in the social sciences. In the forest research it has been used for more than five decades. In spite of that or the fact that the amount of survey-based articles in the forest science journals has increased during the last decade, their share in all articles published in 20 forest science journals (9,372 articles, 2005-2014) is quite modest (3.2%). In our paper we look at the opinions and attitudes of forest science journal editors towards survey research, as their perspective might enlarge our understanding of the use of this approach in the field of forestry.

**Materials and Methods:** We selected 20 forest science journals - 15 from the SCI list and five non-SCI journals and contacted editors of these journals with the self-administered e-mail questionnaire. Data were collected in October 2014 and analyzed by descriptive statistics. The overall response rate was 75%. The assumptions for the study were based on the evidence addressing opinions and attitudes of journal editors from other research fields (finance) since no similar study was found in the field of forestry.

**Results:** The majority of editors reported the same review process for survey-based articles as for all others. In two journals, articles with the survey-based content are screened more rigorously and in two journals their publishing is generally discouraged. 40% of journal editors hold the view that no difference should be made between survey research and other types of original research, and another 40% think that survey research should in the first place play a complementary role. As the main strength of survey research editors see the possibility to obtain data unavailable from other sources. They perceive adverse selection and the difficulty to generalize results as the main weaknesses.

**Conclusions:** Editors of forest science journals have similar opinion on survey research as those from the field of finance. In both fields, survey-based articles typically undergo the same review process

as all other original research articles. Journal editors were evenly split in their views if survey research should be considered equal or complementary to other types of original research. The two most commonly identified strengths and weaknesses differed just by the order.

**Keywords:** survey research, journal editors, forest science, forest policy

## INTRODUCTION

From all approaches the survey research is probably one of the most common ones that the social science employs in order to empirically disclose characteristics and interrelations of psychological and sociological variables [1]. It is used to document existing conditions, the characteristics of a population, opinions or attitudes in a way that the data is collected from a sample of elements by the use of a questionnaire [2-4]. The sample is typically drawn from a well defined study population and it is large enough to allow extensive statistical analyses, on the base of which generalizable statements are drawn about the object of the study [4-6].

Generalizability has become the most advantageous characteristic of the survey research [3, 5] together with efficiency [4, 7] and the ability of providing data inaccessible by other methods [7-9]. On the other hand, surveys are one of the approaches that are used for revealing people's opinions and attitudes, the main criticism of which refers to the respondents: they can do very different things than what they say they do – and even if they do what they say, their real reasons for doing things can be different from the ones they cite [9]. Besides, once the survey work is underway, there is little one can do upon realizing that some crucial item was omitted from the questionnaire, or upon discovering that a question is ambiguous or is being misunderstood by respondents [5, 10]. Finally, evidence shows that survey research calls for better implementation [10-12] and a more adequate interpretation of results [10-13].

In the forest research surveys have been used for more than fifty years [14]. During the last decade 9,372 articles were published in 20 forest science journals, out of which

304 were survey-based, what makes the share of 3.2% [15]. Compared to the articles using other methods inherent to life sciences, the modest contribution of survey-based articles may perhaps be explained by a still traditional research interest in the field of forestry. This means that many research topics and questions often do not take into account the human dimension, i.e. people's attitudes, opinions and values. Furthermore, in the forest research social science methods are mainly used in the forest policy and forest governance, which are rather young disciplines and as such they are not frequently in the scope of traditional forestry journals. When looking at other research fields that also adopted survey from the social sciences and have been using it for many decades, then the field of management accounting counts for 30% [12] and management information systems for 17% [11] of survey-based articles respectively. Although this evidence dates back to 1993 and 2005, it shows the sparse contribution of survey-based articles in the forest science journals [15] and indicates that survey research does not yet belong to the mainstream approaches in the forest research, although it has already been used for five decades [15]. On the other hand, the number of survey-based articles published in forest science journals has been increasing over the last decade, which leads to the expectation that this trend will also continue also in the future [15].

In our paper we focus on the survey research from the viewpoint of science journal editors. Editors' potential ability to provide insightful commentary about particular research and its role makes them very valuable respondents [16]. Editors act as "channel members (and, some would argue, gatekeepers) who assemble product assortments (academic papers) from various producers (researchers) and deliver

them to appraisers (reviewers) who deem them acceptable or unacceptable for public consumption (publication)" [16], and in that way they are obviously exposed to more papers in their respective disciplines than most other scholars [16]. Besides, editors are assumed to be the leaders in their respective areas of research and their viewpoints are assumed to reflect a mainstream disciplinary bias in these areas [16].

The views of science journal editors have already been examined in several disciplines. For example, applied linguistics looked on how editors view the issue of nonnative speakers publishing in their journals [17], criminology and criminal justice examined editors' opinion with respect to the use of qualitative and quantitative methods in published research [18] and the medicine revealed editors' views on the overall medical research publication [19] or policies, practices and attitudes of medical journal editors [20]. There are also discipline-independent studies including all journals of the one particular publisher, as that of the Wiley-Blackwell, looking for the editors' views on publication ethics [21]. With respect to the survey research it is the field of finance where editor views were examined [9, 22]. However, no studies were undertaken within the forestry or natural resources research.

By assuming that in the process of publishing research results the science journal editors play a crucial role [9, 16-18, 21, 22] we looked into what attitudes occur in the "population" of forest science journal editors with respect to survey research. While our analysis aimed to ascertain facts, not to test the theory, we used plain assumptions based on the already existing research results.

Our findings aim to broaden the understanding of survey research and its use in the field of forestry, complementing that way the already existing evidence on the amount, development, quality and maturity of survey research [15]. All these different aspects together have the potential to provide critical insights into the nature, structure, and behavior of a research field in question [21], which is that of forest research in this particular case.

## MATERIALS AND METHODS

For our analysis we first selected forest science journals and then approached their respective editors. By the journal selection we used the SCImago Journal & Country Rank (SJR), as this has already been a proven approach [15]: the SJR web portal offers a list of peer-reviewed journals ranked according to the SJR-indicator<sup>1</sup> from the category Q1 (best ranked) to the Q4. From the list of 150 journals (Subject area "All", subject category "Forestry", year 2013) we excluded: journals having too broad (e.g. Applied Geography) or too narrow focus (e.g. Forest pathology); journals dominated by natural-sciences (e.g. Trees – Structure and Function) or bio-technical journals (e.g. Tree Genetics and Genomes; Wood Science and Technology); book-type journals; journals with no current editor or defunct journals; and journals ceasing publication. From the remaining 58 journals we took a sample of five journals per quality groups Q1 to Q3, by trying to: maintain a sort of a geographical distribution (if that is possible in today's international publishing); include both journals with male and female editors; have a full perspective on forests (e.g. journals addressing urban forests); forest journals specialized in publishing research based on the social-science approaches (e.g. forest policy) as well as those of a wider forestry scope. We focused on the categories Q1 to Q3 while assuming that apart from the SCI-journals (listed in Web of Science® Citation Index Expanded™, issue august 2014) the study would benefit from the inclusion of non-SCI journals as well (instead of Q4), i. e. "traditional" and open access journals outside the SCI-list [23]. Those journals are peer-reviewed, national or international, they accept papers from all forestry disciplines (including social-science research), publish more than one issue per year (in one of the languages authors are fluent in), and have accessible online database.

Based on criteria mentioned above we took a sample of 20 journals, 15 from the SCI-list (categories Q1, Q2 and Q3) and the

five “non-SCI” journals (Table 1). We analyzed all survey-based papers published in these journals, which we address elsewhere [15], and approached journal editors for finding out their opinion on survey research. The editors of the selected journals were identified by reviewing the journals’ website. The e-mails were sent to them in October 2014 containing a short explanation and the questionnaire. The self-administered questionnaire consisted of six closed-ended questions (see results section). In the case of two questions there was a possibility of multiple answers, and in case when none of the answers applied, the editors had a possibility to add their views and comments for each.

Since there is no previous similar research related to forestry, and since there is some in other disciplines, we applied the “earlier method to a different group of people” in order to learn whether in that case the conclusions derived from the earlier study apply equally well to our conclusions [24]. By knowing that replication is more common in natural than in social sciences [16] our analysis aimed to ascertain facts, not to test the theory, and so we used plain assumptions based on the already existing research results [22]. These assumptions are as following:

- (i) review process of survey-based articles is the same as for all other articles,
- (ii) editors have divided opinions on the role survey research should play in comparison to other types of original research,
- (iii) according to journal editors, there is no one major strength or weakness of survey research that obviously prevails over others.

Data were collected in October 2014 and inserted into the MS Excel database. We proceeded with the analysis with the help of descriptive statistics. Due to the small sample size, the tests of statistical significance, determining whether genuine differences exist between the responses of editors of different journal categories, were not possible.

## RESULTS

We asked editors of 20 forest science journals (11 male and 3 female editors) that are geographically distributed as shown in Table 1, to answer 6 questions from the questionnaire we sent them via e-mail. We got 15 answers, which makes an overall response rate of 75%, whereas response rates among different journal categories varied (Table 1). Most editors replied within the first three days (15 journals), one questionnaire was returned after one week and one after two weeks. Five editors did not respond, even though they were sent the reminder twice.

By answering the first question, if their particular journals have an established policy involving the publication of survey research, all journal editors selected “No”. It means that in their respective journals survey-based manuscripts go through the same procedure as the rest of the submitted articles.

While reporting that their journals do not have an established policy involving the publication of survey research, journal editors were asked to indicate the path that is followed when survey-based manuscripts are considered for publication. Table 2 shows that majority of editors (74%) follow the same review process for the survey-based articles as for all other articles, whereas in two journals, the publishing of manuscripts with the survey content is generally discouraged. One of these journals is from the Q2 category and one from the non-SCI category.

On the question about the role survey research should play in the forestry literature 40% of editors hold the opinion of equal position, which means that no difference should be made between the survey research and other types of original research (Table 3). The same amount of them (40%) think that this role should be a complementary one, meaning that survey research should in the first place complement information and knowledge created through other types of original research. Although the small sample size does not allow generalization of results,

**TABLE 1.** Forest science journals included in the study, journal categories, responses from journal editors and respective response rates

Nr.	Forest science journals	Journal category*	Responses from journal editors
1	Canadian Journal of Forest Research	Q1 - SCI	Yes
2	Silva Fennica	Q1 - SCI	No
3	International Forestry Review	Q1 - SCI	Yes
4	Forest Policy and Economics	Q1 - SCI	Yes
5	Urban Forestry and Urban Greening	Q1 - SCI	Yes
6	Small Scale Forestry	Q2 - SCI	No
7	German Journal of Forest Research ("AFJZ")	Q2 - SCI	Yes
8	Revista arvore (Brazil)	Q2 - SCI	Yes
9	New Zealand Journal of Forestry Science	Q2 - SCI	Yes
10	iForest (Italy)	Q2 - SCI	No
11	Croatian Journal of Forestry ("Šumarski list")	Q3 - SCI	Yes
12	Baltic forestry	Q3 - SCI	Yes
13	Journal of forestry research (China)	Q3 - SCI	No
14	Reports of forestry research ("ZLV") – Czech Republic	Q3 - SCI	Yes
15	Southern Forests: a journal of forest science (S. Africa)	Q3 - SCI	Yes
16	South-East European Forestry (SEEFOR)	Non-SCI	Yes
17	Open journal of forestry	Non-SCI	No
18	Poplar ("Topola") – Serbia	Non-SCI	Yes
19	Works ("Radovi") – Croatia	Non-SCI	Yes
20	Forestry Journal ("Lesnícky časopis") - Slovak Republic	Non-SCI	Yes

\* Q1, Q2 and Q3 categories from the SCImago Journal & Country Rank (see the text for explanation); SCI - appearing in the Web of Science® Citation Index Expanded™, issue august 2014 [23]; Non-SCI - not appearing in the mentioned list [23].

**TABLE 2.** The review process of survey-based papers (n=15, one answer possible)

Question:	Editor's responses Number (%)
<i>Although my journal does not have an established policy, it has followed the following path when considering survey-based manuscripts for publication:</i>	
A. Survey-based manuscripts go through <u>the same</u> review process as other manuscripts.	11 (74)
B. Survey-based manuscripts are screened <u>more rigorously</u> than other manuscripts before they go through the review process.	2 (13)
C. Survey-based manuscripts are generally <u>discouraged</u> and only those with the greatest potential for making a contribution to the finance literature go through the review process.	2 (13)
D. My journal uses the <u>following</u> review process for survey-based manuscripts ( <i>please describe:</i> )	0 (0)
TOTAL:	15 (100)

foremost when different journal categories are concerned, it is worth to mention that the half of the editors from SCI-journals (Q1-Q3) think that survey research should play a complementary role, whereas the majority of editors from non-SCI journals hold the view of equal role of all manuscripts (Table 3). From two editors who hold the view that the role of survey research is limited when compared to other types of original research, one is from a SCI and other from a non-SCI journal. The editor who did not select any of offered answers wrote: "All research is considered on its individual merits. It is the value of the research rather than the method which is the key issue".

The last two questions were about strengths and weaknesses of survey research. Answers were multiple so that their number does not match to the number of editors, which is 14 in this case. From Table 4 it is obvious that all journal editors consider survey research as having particular strengths. Their views about these strengths are rather dispersed, although one third of the editors think that survey results are valuable for suggesting "new avenues for future research" (Table 4) and another quarter of them thinks that surveys are valuable for gathering the "data unavailable from other sources" (Table 4). "Survey research can, at times, be the most appropriate approach to

**TABLE 3.** The role that survey research should play in the forestry literature (n=15; one answer possible)

Question: <i>Which of the following statements best describes your view on the role that survey research should play in the forest literature.</i>	SCI-list* (Q1-Q3)	Non-SCI*	TOTAL
	Number (%)	Number (%)	Number (%)
A. Survey research should be considered <u>equal</u> to other types of original research.	3 (27)	3 (75)	6 (40)
B. Survey research should play a <u>complementary</u> role to other types of original research.	6 (55)	0 (0)	6 (40)
C. There is a <u>limited</u> (or no) role for survey research relative to other types of original research.	1 (9)	1 (25)	2 (13)
D. The role of survey research should be <u>as follows</u> : ...	1 (9)	0 (0)	1 (7)
<b>TOTAL</b>	<b>11 (100)</b>	<b>4 (100)</b>	<b>15 (100)</b>

\*SCI - appearing in the Web of Science® Citation Index Expanded™, issue august 2014 [23]; Non-SCI - not appearing in the mentioned list [23].

**TABLE 4.** Perceived strengths of a survey research (n=14, multiple answers possible)

Question: <i>The strengths of survey research are as follows:</i>	Editor's responses Number (%)
A. None, because survey research does not add value	0 (0)
B. Surveys produce data unavailable from other sources	8 (25)
C. Survey responses can suggest new avenues for future research	10 (30)
D. Direct responses from decision makers add value	5 (15)
E. Sometimes there is no other way to answer a research question	7 (21)
F. Other:	3 (9)
<b>TOTAL</b>	<b>33 (100)</b>

addressing a research issue", was noted by one editor. Another editor added that survey data can, depending on the question, provide information on perceptions and preferences quite efficiently.

The results summarized in the Table 5 transmit the message that editors do not see one major weakness of survey research as prevailing over others. By more than 20% of answers survey research is viewed as suffering from adverse selection problems and from the difficulty to get research results generalized in a required manner. Other potential weaknesses were more or less evenly distributed (Table 5). Editors also provided additional views (Table 5). One editor sees the way of interpreting the data as problematic, together with the poor description of a method: "All too often we find people interpret responses to questions as a measured fact and not as a perception. It is seldom found that perceptions as established through questionnaires are verified by some measurement and too often we find that the survey results are used to establish some quantitative result which is misleading and incorrect. (...) The other common problem is that the methods used are generally poorly described and thus not repeatable or testable. Generally, authors don't even supply a list of the questions asked in the survey". Indeed, another editor observed that survey methodology is

often quite weakly implemented, making this approach "rather superficial" and unable to grasp the very essence of a problem.

## DISCUSSION

When our results are compared to that of finance [22], then one can recognize great similarities. Neither surveyed journal editors from finance (all 25 of them) nor from forest science report any difference when survey-based articles are submitted for publishing. In most cases, which means 81% in finance [22] and 74% in forest science journals, the review process for survey-based articles follows the same procedure as all other articles. It is two editors from finance [22], the same as in forest journals (see above), that indicated a more rigorous screening of survey-based articles. In finance however none of the editors indicated that survey-based articles are discouraged of publishing.

The attitudes of surveyed finance and forest journal editors, regarding the role survey research should play in the literature, are also similar. The editors' opinions are in the true sense evenly split when it comes to whether survey research should be considered equal or complementary to other types of research (Table 3 and Table 6 in appendix). The remaining results

**TABLE 5.** Perceived weaknesses of a survey research (n=14, multiple answers possible)

Question: <i>The weaknesses of survey research are as follows:</i>	Editor's responses Number (%)
A. Generalizing results from survey research is often difficult.	8 (23)
B. Survey research has major adverse selection problems because those who take the time to respond may not be the best respondents.	8 (23)
C. Survey research often suffers from non-response bias.	5 (15)
D. Noise reduces the statistical power of results.	4 (12)
E. A respondent may not have the full knowledge of how to respond to a question.	5 (15)
F. Other:	4 (12)
<b>TOTAL</b>	<b>34 (100)</b>

related to this question show that perception differs with respect to the journal category. Almost 70% of the "core" finance journal editors stated that survey research should play a complementary role (Table 6, appendix), whereas in the forest science 75% of non-SCI journal editors indicated that survey research should be considered equal to other types of original research (Table 3). It, however, needs to be underlined that the sample size is rather small and as such limits the generalization of results. Also, the small sample size has to be kept in mind when comparing the results among two research fields.

All responding journal editors agree that survey research has its strengths as well as weaknesses. According to finance journal editors the two highly ranked strengths are the production of data unavailable from other sources and the opinion that survey responses can suggest new avenues for future research (Table 7, appendix). Editors of forest science journals have very similar views that differ only by the ranking order (Table 4). The same applies to editors' views toward the potential survey weaknesses as well. As seen by finance journal editors [22], those are the difficulty of generalizing the results and a non-response bias (Table 7, appendix), whereas by forest journal editors it is vice versa (Table 5).

The editors' scepticism about the generalization of results might be related to the fact that survey typically asks for a collection of data from a fraction of the study population [1, 5] so that conclusions can be "transferred" back to a target population only if sample respondents truly reflect this population [1, 10, 22]. This should therefore clearly be demonstrated in the paper [1, 25]. Also, probability sampling should be adequately accomplished or an otherwise selected method should be justified and reported in detail [4, 7, 26].

In the survey research, a non-response bias can occur if some respondents included in the sample do not respond so that the error is caused by the absence of respondents rather than by the collection of erroneous data [3,

6, 7, 26]. This concern can among others be eliminated by looking for the ways of reducing existing non-response bias or by having that bias discussed and analysed [7]. This is especially relevant in the forest research where not enough attention has been paid to it [15].

Essentially, actual strengths and weaknesses of any selected approach will depend upon research questions in place, the specifics of the research design and the nature of the knowledge being sought [5]. That is because every approach has its advantages and disadvantages [3] and selection depends on the set of cumulative factors [6]. Another aspect is whether survey research is adequately implemented and whether the conclusions are properly drawn. The responsibility in this case lies with the researcher, using proper sampling methods and testing a non-response bias among others [22]. Later in the review process the evaluators are those assessing the methodological rigor and the quality of a paper based on survey results.

## CONCLUSIONS

In our paper we were interested into attitudes and opinions of forest science journal editors as related to survey research. We approached editors of 20 forest science journals with the self-administered e-mail questionnaire (75% response rate) and analyzed the obtained data with the help of descriptive statistics. While our analysis aimed to ascertain facts, not to test the theory, we used plain assumptions based on the already existing research:

- The review process of survey-based articles is the same as for all other articles: indeed, according to editors' responses, survey-based articles in the forest science journals typically undergo the same review process as all other original research articles. Two from 15 editors stated that this kind of articles are generally discouraged in their journals.
- Journal editors have divided their opinions

on the role survey research should play in comparison to other types of original research: indeed, opinions of responding editors were evenly split between equal (40%) and complementary role (40%).

- According to journal editors, there is no one major strength or weakness of survey-based research that obviously prevails over others: indeed, editors' views were rather dispersed. As the most commonly identified strength was the ability of survey research to suggest avenues for future research. Main weaknesses were seen in adverse selection problems and the difficulty to generalize results.

By comparing our results to those obtained

from finance journal editors we could conclude that in both cases attitudes and opinions about survey research were very similar. We should however point out that our results are only indicative due to a small sample.

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## APPENDIX

**TABLE 6.** Views of finance journal editors on the role survey research should play in the finance literature [22]

Views of finance journal editors on the role survey research should play in the finance literature	Core*	Non-core*	TOTAL
	Number (%)	Number (%)	Number (%)
A. Survey research should be considered <u>equal</u> to other types of original research.	0 (0)	10 (58.8)	10 (43.5)
B. Survey research should play a <u>complementary</u> role to other types of original research.	4 (66.7)	6 (35.3)	10 (43.5)
C. There is a <u>limited</u> (or no) role for survey research relative to other types of original research.	2 (33.3)	1 (5.9)	3 (13)
D. The role of survey research should be <u>as follows</u> : ...	0 (0)	0 (0)	0 (0)
<b>TOTAL</b>	<b>6 (100)</b>	<b>17 (100)</b>	<b>23 (100)</b>

\* One distinguishing characteristic of a "core" journal is its perceived quality. Another is that "core" journals have been publishing longer, on average, than have the "non-core" journals [22].

**TABLE 7.** Perceived strengths and weaknesses of a survey research as seen by finance journal editors [22]

Strengths	Number (%)
A. None, because survey research does not add value	0 (0)
B. Surveys produce data unavailable from other sources	17 (30.4)
C. Survey responses can suggest new avenues for future research	15 (26.8)
D. Direct responses from decision makers add value	10 (17.9)
E. Sometimes there is no other way to answer a research question	13 (23.2)
F. Other:	1 (1.8)
Weaknesses	Number (%)
A. Generalizing results from survey research is often difficult.	16 (27.6)
B. Survey research has major adverse selection problems because those who take the time to respond may not be the best respondents.	13 (22.4)
C. Survey research often suffers from non-response bias.	15 (25.9)
D. Noise reduces the statistical power of results.	4 (8.3)
E. A respondent may not have the full knowledge of how to respond to a question.	10 (17.2)
F. Other:	0 (0)

# Social and Policy Aspects of Climate Change Adaptation in Urban Forests of Belgrade

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## Abstract

**Background and Purpose:** Climate change has an impact on economic and natural systems as well as human health. These impacts are particularly visible in urbanised areas. Urban forests, which are one of the main natural features of the cities, are threatened by climate change. Generally, the role of forests in combating climate change is widely recognised and its significance is recognised also in urban areas. However, appropriate responses to climate change are usually lacking in their management. Climate change adaptation in relation to urban forests has been studied less often in comparison to climate change mitigation. Adaptive capacity of forests to climate change consists of adaptive capacity of forests as an ecological system and adaptive capacity of related socio-economic factors. The latter determines the capacity of a system and its actors to implement planned actions. This paper studies social and policy aspects of adaptation processes in urban forests of Belgrade.

**Materials and Methods:** For the purpose of this study content analysis of urban forest policy and management documents was applied. Furthermore, in-depth interviews with urban forest managers and Q-methodology surveys with urban forestry stakeholders were conducted. Triangulation of these data is used to assure validity of results.

**Results:** The results show weak integration of climate change issues in urban forest policy and management documents, as well as weak responses by managers. A comprehensive and systematic approach to this challenge does not exist. Three perspectives towards climate change are distinguished: (I) 'sceptics' - do not perceive climate change as a challenge, (II) 'general-awareness perspective' - aware of climate change issues but without concrete concerns toward urban forests, (III) 'management-oriented perspective' - highlights specific challenges related to urban forest management. Awareness of urban forest managers and stakeholders towards climate change adaptation is characterized by assumptions and uncertainties, which are the result of poor knowledge, lack of data of local impacts and weak communication.

**Conclusions:** The results indicate the need for building urban forestry institutional and human capacities for creating effective climate change adaptation responses, which will lead to better understanding of challenges posed by climate change and ability to make the trade-offs between possible decisions.

**Keywords:** awareness, urban forest management, climate communication, adaptation measures, institutional and human capacity, Serbia

## INTRODUCTION

In the last century the global urban population has increased rapidly from 746 million in 1950 to over 3.9 billion in 2014 [1]. In Europe, 73% of the population lives in urban areas [1]. Population growth, together with technological development and increased consumption levels, has increased the pressure on urban centres, its natural resources and ecological systems [2]. Climate change is a major challenge today's society needs to cope with, especially as it has been proven that human activities have been the dominant cause of it [3]. The Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) has confirmed the important role of cities in the development and delivery of climate change responses, as cities are in many ways affected by climate change and are a focal point of vulnerability, as their functioning relies on complex infrastructures [3, 4]. Urban forests are part of a green infrastructure which is one element that can contribute to climate change adaptation in cities [5]. However, the role of cities [3] and its green infrastructure is still marginally studied in relation to variety of issues related to climate change. So far mitigation actions have been supported by a wide range of policies in various sectors [6], while adaptation has only become prominent lately.

'Urban forests' in this paper implies all forests and other tree-based green areas (e.g. parks, tree alleys) that are situated within the administrative border of a city [7, 8]. Urban forests contribute to mitigating climate change in many ways: by controlling greenhouse gases (GHGs) emissions, the shading effect

on buildings (reduces energy use and carbon emissions), by regulating the urban microclimate (reducing albedo, providing shade and cover) and the hydrological regime of cities [9-11]. At the same time, urban forests are becoming highly threatened by climate change [4]. Assessing the vulnerability of urban forestry systems is essential for adaptation processes and their long-term sustainable development [5]. Adaptation mainly is a matter of local importance [12, 13] and promotes the implementation of measures which are useful in the present, and at the same time reduce the risk of unacceptable losses in the future [13, 14]. FAO [15] sees adaptive forest management as an essential for addressing arising challenges and reducing forest vulnerability. In adaptive management various measures can be included: selection of drought-tolerant or pest-resistant species, use of stock from a range of provenances, assisting natural regeneration of functional species, and measures targeted to individual requirements of single species [15-17]. All of these measures need to be adapted to site specific forest conditions [15].

Adaptive capacity of urban forests comprises adaptive capacity of forests as ecological systems and adaptive capacity of socio-economic factors of urban forestry. Adaptive capacity of socio-economic factors determines the capability of systems and its actors to implement planned actions. "Adaptive organizations that incorporate organizational learning enhance social capital through internal and external linkages, partnerships, and networks, and make room for innovation and multi-directional information flow" are needed nowadays [7, p6]. Effective urban forest management with

regard to climate change must be responsive to a wide variety of economic, social, political and environmental circumstances [13]. Thus, effective communication on climate change is very important [18]. Developing a dialogue within and out of the urban forest management community is essential, and will increase the range of possible actions [13]. It is recognised that climate change ultimately requires a national response, and that much more attention must be given to how decisions are made [19] and how decision-makers value expected risks and benefits [20]. Planning for climate adaptation requires comparison of decision options, and these should be based on relevant scientific results which are effectively communicated and perceived [20]. Perception is recognised as an active process of understanding, through which people construct their own version of reality [19] and therefore influences decisions.

Belgrade is the capital of Serbia and has faced enlargement and an intensive urbanisation process in the last decades, mainly at the expense of green areas [21]. In the last 50 years, an increase in mean annual temperature has been observed in all parts of Belgrade (up to 0.04 °C/yr), as well as for precipitation (up to 1.7 mm/yr) [22], which demonstrate a demand for climate change adaptation strategies in all sectors (including urban forestry) in Belgrade [23]. The protection of existing and the planning of new urban forests, as well as creation of responses to climate change, is identified as need by city administration [24].

This paper focuses primarily on social and policy aspects of adaptation processes in urban forestry in Belgrade. By applying mixed methods research, it aims to understand: (i) current climate change adaptation practices in urban forest management of Belgrade, and (ii) perceptions of various urban forestry stakeholders toward the issue. Following research questions are addressed: 1) to what degree do urban forest management plans integrate climate change adaptation measures?; 2) how do urban forest related

policy documents take climate change in consideration?; 3) and what are the perceptions of urban forestry stakeholders regarding climate change adaptation in urban forestry of Belgrade?

## METHODS

In this research following methods were applied: content analysis of relevant policy and management documents, in-depth interviews and Q-methodology surveys. Triangulation of the data is used to assure the validity of results, and to control possible weaknesses and biases. For the purpose of this research a case study approach has been implemented [25], with urban forests of the city of Belgrade as a selected case.

### Analysis of Documents

Urban forest related policy and management documents (Table 1) are analysed by content analysis [26]. Searched aspects were: (i) the contribution of urban forests to mitigating/adapting to climate change; (ii) the vulnerability of urban forests to climate change; (iii) climate change impacts on urban forests and (iv) climate change adaptation measures considered as important for urban forests management. It is analysed whether these aspects appear directly or indirectly in the documents and how detailed they are presented.

### In-Depth Interviews

In-depth interviews were conducted with six urban forestry managers of two main management bodies in Belgrade. Two interviews were conducted with the heads of the management units and other four interviewees were chosen with the snowball technique. The selection criterion was that all were in charge of developing management plans for forests in Belgrade. The interviews were conducted in May 2012, with an average length of 45 minutes. All interviews started with the question whether the managers were

TABLE 1. Aspects related to climate change from analysed urban forest management and policy documents

Name of document	Year of passing	Level	Mitigation aspects	Adaptation aspects	Type of urban forest mentioned	Quotes from documents in relation to mitigation or adaptation to climate change
Law on Forests of the Republic of Serbia	2010	national	+	+/-	+/- (forest in general)	"...potential of the forests must be enhanced for mitigation of climate change..." [39]
Forest Development Strategy of the Republic of Serbia	2006	national	+	+/-	+/- (forest in general)	"Forests have an irreplaceable role in the climate change mitigation ... and, in this sense, carbon sequestration. Efforts should be made to increase permanently the forest capacity" [40] "The Government will (...) support the research and analysis of the potential scope and methods of carbon sinks in forests, promote the efficient generation and consumption of bio-energy, pursuant to the UNFCCC and Kyoto Protocol, and thus create the conditions for application for the international funds" [40]
Afforestation Strategy of Belgrade	2011	local	+	+/-	+	Climate change, as one of the main environmental challenges, is described in detail in section 7 of the Strategy [24]
Urban forest management plans (management districts: Kosmaj, Kosutnjak, Bežanijska Kosa, Milicevo brdo)	2006-2009	local	+/-	-	-	Climate change is not directly mentioned in any of the four UFMPS. One of the general aims of forest management is: "forests have important role in improving climatic conditions"
Law on Spatial Planning of the Republic of Serbia	2010	national	+	+	+	Aim is: "to include climate change as a factor for sustainable development and the environment into sectoral strategies, and to develop sustainable system of risk management for climate change in Serbia" [36] Identified operational aims for adaptation to climate change: - "adoption of sectoral plans/programs regarding climate change adaptation measures, by harmonization of sectoral strategies with the EU strategies, White Paper on adapting to climate change and relevant EU directives" [36] - "reduce the risk of climate change by strengthening capacity to adapt in the most vulnerable social groups and sectors of the economy" [36]
Regional Spatial Plan for the Administrative Territory of the City of Belgrade	2004	local	+/-	+/-	+/- (all types of green areas in cities)	Aim is: "to protect nature and landscape in order to affect the protection and improvement of the environment, including microclimate. It is highly important to: preserve natural ecosystems, especially forest in the urban environment (e.g. Topčider, 'Košutnjak', 'Avala') and forests along river banks and on islands (e.g. 'The Great War Island', 'Ada Ciganlija', 'Ada Hujaj'), establish new forest areas and green areas in the city" [37]

TABLE 1. Aspects related to climate change from analysed urban forest management and policy documents - countinuation

Name of document	Year of passing	Level	Mitigation aspects	Adaptation aspects	Type of urban forest mentioned	Quotes from documents in relation to mitigation or adaptation to climate change
Master Plan of Belgrade 2021	2003	local	+/-	+/-	+/- (green infrastructure)	"Unlike many other urban sectors, which are regulated by various financial and construction measures, a system of public greenery, although it represents the climatic infrastructure, is not supported by any financial or legal mechanisms. In this regard, Belgrade will have to make a series of regulations to ensure the improvement of this system" [38]
National Sustainable Development Strategy of the Republic of Serbia	2008	national	+	+	+/-	The main problems that are identified are: "absence of a national inventory of GHGs emissions; the lack of strategic documents related to climate change; inconsistent legislation, relating to emissions, with the regulations of EU". Therefore, the main aims are: <ul style="list-style-type: none"> <li>- harmonization of national regulations in the field of climate change and ozone depletion with EU regulations;</li> <li>- adaptation of existing institutions in relation to the needs for active implementation of climate protection policy and fulfilment of obligations under international agreements;</li> <li>- adaptation of business entities in the energy, industry, transport, agriculture and forestry, utility and housing business policy of climate protection and compliance with international agreements;</li> <li>- developing an action plan for adaptation to the climate change of all economic sectors;</li> <li>- the design, development and implementation of an adequate health response system on the effects of global climate change [41]</li> </ul> Climate change have been indirectly mentioned throughout the document [42]
Development Strategy of the city of Belgrade	2011	local	+/-	+/-	-	
Tourism Development Strategy of Belgrade	2008	local	+	+/-	+/- (green infrastructure)	One of the key challenges for sustainable tourism, are: "reducing the impact of tourist transport on climate change and pollution" [43] and "...protection and valorisation of natural and cultural heritage...", where "...impacts of climate change on natural heritage, and the lack of adequate resources (human and financial) for the protection and preservation of heritage" are identified [43] Quote related to climate change and green areas: "Climate change imposes an obligation to improve the microclimate conditions, by conservation of the existing and establishment of new green infrastructure (alleys and green areas) along all pedestrian paths and cycling routes, wherever possible in the existing urban setting. This problem is almost completely neglected in the past two decades of development" [43]

+ directly addressed; +/- indirectly addressed; - missing

confronted with climate change in their work, and how important this issue was to them. The next question raised issues of communication and policy-making and led into specific details of climate change adaptation of urban forests. In the end, main challenges for adaptation processes in urban forests were stressed.

## Q-Methodology

The aim of the Q-methodology is to analyse subjectivity in a statistically interpretable form [27]. In this research, the Q-methodology surveys were used to extract stakeholders' individual perceptions [28] of climate change adaptation in urban forests of Belgrade, and to differentiate which aspects of adaptation processes are seen as the most important.

They were addressed to a variety of urban forestry stakeholders (urban forest managers, employees in ministries, research organisations, NGOs, etc.), including those targeted in the in-depth interviews. In total 23 respondents from 14 organizations were interviewed (five at local, eight at national and two at regional level). 20 of the Q-surveys were conducted face to face in June 2012, with an average length of 50 minutes. Three additional Q-surveys were completed through an on-line application of the Q-methodology by using Q-Assessor.

The application of the Q-methodology implied formulating statements about climate change adaptation in urban forests based on the in-depth interviews and literature review. After the test phase, a concourse of 48 statements was created. The Q-surveys consisted of respondents sorting 48 statements, based on their subjective point of view, along a scale of +4 (strongly agree) to -4 (strongly disagree) using provided score sheets. The results of these 23 Q-sorts were then analysed using PQMethod 2.33 factor analysis software (available at: <http://schmolck.userweb.mwn.de/qmethod/>). As a result shared perspectives are identified and described. Each Q-survey was complemented by a brief follow-up interview, revealing why respondents have agreed/disagreed the most with certain statements [29].

## Case Study Description

### *Research Area: Urban Forests of the City of Belgrade*

Belgrade is the biggest city in the Republic of Serbia by area (3,222 km<sup>2</sup>) [30] and population (1.66 million) [31]. In the period from 1948–2002 the total population of Belgrade has increased by 2.5 times [30, 32], which was followed by significant enlargement of the city [18]. Urbanization has had a major impact on the green areas, many forests had to be cut-down and very little has been done to prevent this situation [33, 34].

Belgrade has in total 35,980.00 ha of forests in the administrative area. The Public Enterprise (PE) 'Serbia Forests' manages 32,322.70 ha of forests, while the Public Utility Company (PUC) 'Greenery Belgrade' manages 610.75 ha of forests and 2,900 ha of other green areas. These two management organizations are the most important at the city level. Other forests are managed by other organizations (water management companies, military, agricultural organizations, churches) according to 10-year management plan approved by the Ministry [33]. Urban forests of Belgrade are mostly small in size, fragmented and scattered [24]. Deciduous tree species prevail (96.2%) [33]. General assessment of the forests in Belgrade shows unfavorable conditions of forests, and the main management goals identified are the conversion of coppice forests into high forests, timely and adequate maintenance of artificially established stands, increasing the share of autochthonous species, and responding to upcoming challenges (e.g. climate change) [24].

### *Background Information on Climate Change Policy in Serbia*

The assessment of climate change for Serbia by a regional climate model shows that annual temperature is expected to rise from 0.8–1.1°C (according to A1B scenario) to 3.4–3.8°C (A2 scenario) per decade [23]. Precipitation is projected to decrease by 1% each decade, which will be followed by a decrease in the number of days with snow cover [35].

In 2001 Serbia became Party to the United Nations Framework Convention on Climate Change (UNFCCC), and in 2008 it ratified the Kyoto Protocol [23], thus focusing mainly on mitigation activities. So far, no climate change adaptation strategy has been developed at any level.

The Ministry of Agriculture and Environmental Protection is a national coordination body for the realisation of the UNFCCC convention. In collaboration with other ministries and governmental bodies (e.g. Republic Hydro-meteorological Service, EU Integration Office), Serbia formed a working group for fulfilling obligations ratified by the UNFCCC. The Initial National Communication (INC) to UNFCCC represents one output of this working group and is the first state-of-the-art report in the field of climate change at national level [23].

The development of the INC indicated several obstacles for the effective identification and implementation of climate change adaptation measures. The main problems identified were: (i) a lack of systematic data collection and databases, (ii) a deficient structure of the sector and (iii) a lack of financial and technological capacities. The main goal of the state therefore is to build new and strengthen existing capacities of experts who are involved in (sectoral) policy-making in relation to climate change and the development of the National Action Plan for Adaptation [23].

## RESULTS

### Climate Change Adaptation Aspects in Urban Forest Policy Documents

Urban forest management in Belgrade is influenced by various national and local policy documents. Content analysis of these policy documents demonstrated weak integration of climate change aspects. Climate change mitigation aspects are more prominent compared to adaptation (Table 1).

Of all analysed documents, the Spatial Plan of the Republic of Serbia (2010) is the most

advanced in terms of the integration of climate change issues. A specific chapter focuses on climate change effects in various sectors (e.g. forestry, nature protection) and identifies main problems:

- "Climate data and information used in the planning are developed with application of standard methods and guidelines based on stationarity of climate;
- Low awareness of the need to include climate change as a factor of sustainable development into sectoral strategies, particularly sectors vulnerable to climate change (agriculture, water management, forestry, energy, tourism, health, construction, transportation);
- Lack of adequate support for the implementation of multidisciplinary research programs on climate change, vulnerability and adaptation options;
- Lack of a special state program for solving problems of climate change;
- Limited funds for capacity building (institutional and individual), education, training" [36].

According to the latest Spatial Plan (2010), existing lower level urban planning documents (e.g. Regional Spatial Plan for the Administrative Territory of the City of Belgrade [37] and Master Plan of Belgrade [38]) still require adjustments related to climate change.

The analysed forestry-related policy documents (e.g. Law on Forests [39], Forest Development Strategy [40] and Afforestation Strategy of Belgrade [24]) have generally been harmonised with various international regulations, including climate change regulations. However, content analysis revealed that climate change issues are weakly integrated and mainly appear as general and indirect statements throughout the documents. The Afforestation Strategy of Belgrade (2011) has been the most advanced, primarily by integrating climate change mitigation aspects [24], while the Forest Development Strategy (2006) only briefly introduces these aspects.

Other documents (National Sustainable Development Strategy [41], Development

Strategy of Belgrade [42] and Tourism Development Strategy of Belgrade [43]) recognise climate change as a future challenge and call for development of thoughtful approaches. The National Sustainable Development Strategy identifies the main problems in this regard (Table 1).

### Climate Change Adaptation Aspects in Urban Forest Management Plans

Four analysed urban forest management plans (UFMPs) were developed for different forest areas (municipalities) and urban forest types (urban and peri-urban forests), managed by PE "Serbia Forests" or PUC "Greenery Belgrade". In all four UFMPs climate change mitigation and adaptation aspects were not directly covered and related terms were not used<sup>1</sup>. Implications could only be found in the description of general aims of forest management, such as "forests have an important role in improving climatic conditions" or "forests have positive impacts on the environment". Parts of the UFMPs describing climate conditions in Belgrade are abundant with information of all climate parameters (e.g. annual average air temperature, minimal/maximal annual temperature/precipitation), but future impacts of climate change are not mentioned (Table 1).

### Urban Forest Stakeholders' Perception towards Climate Change Adaptation

The results obtained by the in-depth interviews and Q-methodology offer insights into the current state of urban forest management and policy regarding climate change. We therefore interlink the findings from both sources of information, as they complement and explain each other (detailed findings from each method are presented in Table 2 and 3).

The application of the Q-methodology in this study revealed three shared perspectives

regarding climate change adaptation in urban forests, which are named: 'sceptics', 'management-oriented perspective' and 'general-awareness perspective' [29].

'Sceptics' do not perceive climate change as a challenge. They hold the opinion that climate variations are normal and that there is a lack of data and evidence on existing change at the local level. This perspective reveals a very low level of awareness and communication regarding climate change, both inside and between various urban forestry organisations. Moreover, sceptics are of an opinion that urban forests will naturally adjust to future climate variability. They perceive other problems as more important (e.g. economic crises, governance issues, lack of information and technical assistance). However, it can be said that this perception is not so rigid, as more scientific evidence regarding climate change impact and information would be needed for this group to change opinion (follow-up interviews).

The two other perspectives are aware of challenges posed by climate change, and both selected statements regarding importance of education, public awareness, individual and collective actions in tackling climate change as important. However, they are also revealing different standpoints.

The 'management-oriented perspective' is aware of concrete needs related to improvement of urban forest management in the light of climate change (e.g. introduction of monitoring and modeling tools, obtaining more funds for research, improving legislation). The in-depth interviews revealed that urban forests vulnerability to climate change was noticed in the last ten years in practice (Table 3), but was not addressed in management plans. Vulnerabilities are seen through: (i) lower physiological state of trees due to frequent droughts/water stress, (ii) more frequent weather accidents, (iii) changes

<sup>1</sup> we use following terms in Serbian: *klimatske promene* (climate change), *ublažavanje klimatskih promena* (climate change mitigation), *prilagodavanje klimatskim promenama* (climate change adaptation), *osetljivost/ranjivost na klimatske promene* (vulnerability to climate change), *uticaj klimatskih promena* (impact of climate change)

**TABLE 2.** List of statements used in Q-methodology with normalized factor scores for each statement and perspective [29]

No.	Statement	Sceptics	Management-oriented perspective	General-awareness perspective
		Normalized factor scores		
1	Introducing monitoring system and modelling tools for forest management will be of great importance for adaptation to climate change.	1	4	2
2	The establishment and development of a dialogue about climate change adaptation among various actors is highly important for management of urban forests.	-2	3	0
3	Scientific knowledge about adaptation of urban forests to climate change would help in the adaptation process.	4	1	4
4	Public institutional money is needed to deal with the adaptation of urban forests to climate change.	0	2	1
5	The protection of biodiversity and forest habitats will depend on how well we adapt forests to climate change.	-1	1	1
6	Adaptation of urban forests to climate change is important for preserving forests for future generations.	2	3	2
7	Management of urban forests should adapt to more frequent natural disasters that are consequences of climate change.	-4	-1	1
8	Public awareness about climate change is already high; there is no need for more educational programs and trainings.	-2	-4	-3
9	Climate change adaptation actions in urban forests are costly; it does not make sense to invest in them.	3	-4	-4
10	Selection of climate-resilient species in urban forests management is needed for urban forests adaptation to climate change.	-2	0	2
11	Popularization of climate change topic can be done through greater involvement of experts.	2	1	1
12	Taking into account adaptation to climate change in urban planning will help the process of adaptation.	-1	3	3
13	Lack of interest of urban forestry actors toward climate change will not influence adaptation process.	0	-2	-2
14	We should aim at planting as many species as possible in order to make forests resilient to climate change.	-3	-1	0
15	Climate change adaptation actions in urban forests are not needed because forests will naturally adjust to future climate variability.	4	-2	-3
16	Education of employees in urban forestry in climate change is needed for adaptation process.	-2	1	2
17	Employees in forestry are informed about climate change only through informal sources (e.g. internet, newspapers).	-1	-2	-1
18	Actions aimed at reduction of impacts of climate change are expensive for companies and enterprises.	1	-2	-2
19	Forest management enterprises and agencies should make main decisions in adaptation of urban forests to climate change.	1	0	-4
20	Stronger political leadership would be of great importance in initiating climate change adaptation actions.	-4	2	2
21	Local authorities should play a crucial role in developing climate change adaptation strategies in various sectors.	-3	0	-1

**TABLE 2.** List of statements used in Q-methodology with normalized factor scores for each statement and perspective [29] - continuation

No.	Statement	Sceptics	Management-oriented perspective	General-awareness perspective
		Normalized factor scores		
22	More funds from national funds should be secured for doing research on climate change adaptation.	1	4	4
23	Companies and enterprises need to consider climate change in all their activities (corporate responsibility).	2	2	4
24	We need legislation that addresses climate change adaptation in urban areas.	-1	4	0
25	There are many other problems in the city than climate change adaptation which should be solved.	3	-1	1
26	Natural forests are best suited to adaption for climate change.	2	0	-2
27	It is already too late to do anything, as any action to adapt urban forests to climate change will take a long time to produce an effect.	0	-3	-4
28	Everybody has to contribute to tackling climate change through individual actions.	2	1	2
29	Water supply will be endangered if nothing is done about adaptation of urban forests to climate change.	-2	0	0
30	Failure to address climate change is the fault of political leaders.	-2	0	0
31	As effect of climate change we have more trees that are drying now in urban forests.	-1	1	1
32	Climate variation is normal, so we cannot say that there is global climate change.	4	-1	-3
33	In future invasive species will become a big problem due to climate change.	-3	3	-1
34	Adapting forests to climate change should be done because of sustainable development of the city.	0	-2	-3
35	There is not enough information to say definitely that climate change exists.	2	-2	-2
36	Climate change adaptation in urban forests will not help in regulation of city microclimate.	-1	-3	-2
37	Urban scale and local adaptations are not important part of national and international policy agenda of climate change.	0	-3	-2
38	Only when negative effects of climate change become evident, it will be acted in finding a resolution.	0	0	0
39	Non-native (alochtone) species in urban forests are not negatively influenced as a consequence of climate change.	-1	-1	-1
40	Media is insufficiently covering climate change.	0	1	1
41	Urban forest management has other priorities then climate change adaptation.	3	-1	-1
42	When schools/universities include climate change in their curriculum, young generations will know what to do in the end.	1	2	3
43	Climate change adaptation in urban forests is an urgent issue that requires an immediate change of forest management.	-4	0	0
44	Climate change adaptation policy for urban forests should be top-down mandated.	1	2	3

**TABLE 2.** List of statements used in Q-methodology with normalized factor scores for each statement and perspective [29] - continuation

No.	Statement	Sceptics	Management-oriented perspective	General-awareness perspective
			Normalized factor scores	
45	Adaptation to climate change in urban forest will not contribute to minimizing climate change effects if other sectors in the city (e.g. transport, energetic) are not adapted as well.	2	-1	3
46	The cost and effects of climate change adaptation in urban forests need to be calculated before actions are taken.	1	2	-1
47	The organizations responsible for climate change and other enterprises involved in urban forestry have good communication about climate change.	0	-4	-1
48	Enforcement and implementation of international agreements on climate change are more important at the global level rather than separately at the national or local level.	-3	-3	0

in forest structure, (iv) changes in forest increment. In social terms, vulnerability is seen through higher use and changed demands toward forests, while in economic terms vulnerability is expected by increased costs of maintenance and introduction of measures related to climate change. The concept of green infrastructure (e.g. forests, parks, green corridors) is identified as important regarding the climate change by some managers, who are trying to introduce this concept into city planning and thus secure higher visibility and importance of urban forests from other sectors (in-depth interview).

The 'general-awareness perspective' values statements which highlight general challenges to climate change as the most important, such as the need for more scientific evidence, better education, more funds for conducting research and improved cross-sectoral cooperation.

One of the main weaknesses, which was stressed in both in-depth and Q-surveys, is the low level of communication and coordination between urban forestry actors. National level organizations responsible for climate change issues and agencies involved in urban forest management (mainly at local level) do not cooperate. Managers stressed that climate change was not set as an important issue at

the management level, that possible existing data and findings are not shared and used in management and that communication around the issue is a matter of individual interest (Table 3).

According to 'general-awareness perspective', climate change adaptation policy for urban forests should be top-down mandated by leading national bodies. However, 'Management-oriented perspective' perceives that management bodies, due to their practical knowledge and experience, should be involved in this process as well.

## DISCUSSION

Climate change is a serious challenge for future urban forest management in Belgrade. For the last 50 years climatic changes have already been recognised [22]. Forest resources in Belgrade are facing similar problems as other forest resources in the temperate continental zone due to the climate change [17]. The notion of local risks and negative influences of climate change have been recognized in the everyday practice of forest managers, but are not analysed and tackled in future management plans. This means that many of

**TABLE 3.** Summary of main aspects revealed through in-depth interviews

Climate change adaptation aspects (related to questions of in-depth interview)	Main aspects identified related to urban forests in Belgrade
Climate change issue in the work of forest managers	<ul style="list-style-type: none"> <li>– become a topic of discussion in the last few years</li> <li>– not perceived as a strategic aim of urban forest management</li> <li>– managers are concerned with challenges caused by climate change in everyday practices</li> <li>– some managers were involved in the development of national planning and climate change documents by giving expert opinion in regards to urban forests</li> </ul>
Importance of climate change in urban forest management	<ul style="list-style-type: none"> <li>– varies among managers</li> <li>– uncertainty exists due to the lack the data on the local impact</li> <li>– seen as less important challenge than other challenges (e.g. land use conflicts, governance issues, lack of information and technical assistance)</li> <li>– becomes more important because of perceived changes in forest resources, changes in maintenance operation, or in management practices</li> <li>– mitigation measures (e.g. afforestation) have been better understood</li> </ul>
Policy and legislation regarding climate change issues	<ul style="list-style-type: none"> <li>– climate change aspects mainly indirectly covered</li> <li>– managers’ knowledge about existing national climate change regulatory framework is based only on individual interest</li> <li>– the lack of legislation is one of the biggest constraints for the integration of climate change adaptation measures in management</li> </ul>
Communication about climate change	<ul style="list-style-type: none"> <li>– the level of communication is very low (inside and between various organizations) is happening on individual level</li> <li>– need for information on financial sources and other opportunities that exist at the national level for tackling climate change</li> <li>– internet and grey literature are mostly used as the source of information</li> </ul>
Urban forest contribution to climate change in FMPs	<ul style="list-style-type: none"> <li>– not specifically described in UFMPs (it is seen as one of the main forest functions)</li> <li>– terminology related to climate change is hardly used</li> <li>– the long-term monitoring data regarding the contribution of urban forest to climate change are missing</li> </ul>
Vulnerability of urban forests to climate change	<ul style="list-style-type: none"> <li>– noticed in the last ten years in Belgrade, but not analysed adequately</li> <li>– database of resulting changes does not exist</li> <li>– in ecological terms the vulnerability of forests is seen through negative effects of drought periods on various tree species, water stress, worse physiological state of trees and more frequent natural disasters</li> <li>– management and maintenance operations have been changed (more frequent irrigation and mowing are needed, planting is done in autumn)</li> <li>– the structure of forests has been changed (coniferous species have been replaced with deciduous; <i>Fagus sylvatica</i> L. heavily impacted, invasive tree species have become more frequent in urban areas)</li> <li>– change in forest increment due to long dry periods is noticed</li> <li>– all the changes in management and maintenance operations have been made as a consequence of already experienced negative influences (reactive adaptation measures)</li> </ul>
Monitoring of climate change impact on urban forests	<ul style="list-style-type: none"> <li>– monitoring of climate change impact on urban forests has not been done</li> <li>– the necessity for comprehensive monitoring practices was expressed by all managers</li> </ul>

**TABLE 3.** Summary of main aspects revealed through in-depth interviews - continuation

Climate change adaptation aspects (related to questions of in-depth interview)	Main aspects identified related to urban forests in Belgrade
Climate change adaptation measures in urban forests	<ul style="list-style-type: none"> <li>– have not been applied as such in urban forest management plans</li> <li>– most urgent and valuable adaptation measures are selection of climate-resilient species, optimizing species mixture, creation of ecological corridors and enhanced forest protection measures</li> <li>– in social and economic terms many actions are identified: adjustment of institutions, inclusion and empowerment of various actors, capacity building of employees in forestry, provision of finances</li> </ul>
Constraints to climate change adaptation in urban forests	<ul style="list-style-type: none"> <li>– lack of political will and legislation, financial resources, knowledge and skills about climate change</li> <li>– inadequate coordination and communication between urban forestry actors</li> <li>– passiveness of employees</li> <li>– absence of foresight planning in urban forest management</li> <li>– management practices applied currently are very old, and new methods and approaches are needed</li> </ul>

the currently applied forestry measures are mostly characterized by reactive adaptations [44]. Introducing new measures and tools, as well integrating different tools (species suitability maps, decision support systems) in management, and making them available to a larger community of forest practitioners [45], could improve current urban forestry practice in Belgrade.

Even though, the forest sector in Serbia has been harmonized with international climate change regulations during the last decades, integration of climate change aspects in forest policy and management is still weak. Main goal of the Forest Development Strategy (2006) and the Law on Forests (2010) is securing sustainable forest management [39, 40], which provide basis for further improvements and modifications towards the integration of climate change adaptation aspects in urban forest management. The Afforestation Strategy of Belgrade is one example where climate change becomes prominent [24]. Also the significance of urban planning documents for urban forest management has been emphasised. However, frequent changes of the government and legislation in Serbia prevent the adequate implementation of existing

ordinances into urban forest management. Such an instable system of passing legislation is directly connected to limited reactions of lower level governments and management. Harmonization of legally binding planning and management documents is necessary for an appropriate planning of activities.

Current urban forest policy and management in Belgrade is traditionally top-down, dominated by decisions made by the national body and characterized by low levels of communication among actors. Hence, there is need for better communication between actors, as well organization of training sessions and outreach activities for forest professionals [45, 46]. Furthermore, the coordination of activities and interaction of various stakeholders at different levels and from different sectors is needed [7, 47]. Stakeholder's awareness of potential risks needs to be raised to set up conditions for well-informed and timely actions. [45]. Bottom-up initiatives by local actors (e.g. managers) addressing specific local risks to climate change could be valuable. Moreover, interactive discussions on measures [46] and the involvement of various experts (e.g. climate experts, decision scientists, social and communications specialists) are important

as this might lead to better communication and agreement on selected measures and evaluation of trade-offs [20].

Even though many urban forestry stakeholders recognize the importance of climate change, their actual response can be characterized as low and passive. This indeed represents one of the major challenges for climate change adaptation. The presence of sceptics among employees in forestry regarding this issue proves that climate change awareness is still not as high as needed. Hence there is an urgent need in Serbia to raise awareness among experts and improve capacities that are needed for adequate responses, as suggested in other European studies [45, 46]. Empowering decision-makers and citizens is an important step, and can be done through formal education programs but also public service announcements [18].

## CONCLUSION

This study gives broad overview of current situation related to climate change adaptation in urban forest management and policy, thus it represents the first analysis on this topic in Serbia. It can serve as a basis for more detailed quantitative and qualitative analysis of specific urban forests and problems imposed by climate change, in both ecological and socio-economic terms, as a result of which more practice-oriented recommendation could be drafted.

At the moment, the integration of climate change adaptation measures in urban forest management in Belgrade is a big challenge dependent on decisions of distinct actors who hold different perceptions. These distinctions of opinions indicate existence of complex urban forestry system, where various needs should be harmonized in order to overcome

existing and forthcoming challenges. Due to this complexity, adaptive forest management is seen as an adequate approach for urban forest management under climate change. Traditional urban forest management with a narrow sector-specific focus, dominated by decisions of few actors, cannot meet the increasing challenges that urban forests face nowadays. In practical terms, adapting urban forests to climate change should aim at reducing their vulnerability to undesirable effects while preserving a full range of ecosystem services. This mainly involves the reduction of urban forest exposure to risk and increased urban forest resilience to disturbances. Adapting socio-economic aspects of urban forestry system are thus necessary, which assume involving various stakeholders and establishing coordination and interaction at all levels, as well as developing necessary policy and management plans and programmes. Furthermore, urban forestry stakeholder's awareness and knowledge of risks imposed by climate change are necessary prerequisite in order to implement adaptation measures. Strengthening research, communication and fostering discussion around climate change, as well as building a stronger network of urban forestry actors, both at the local and national level, are therefore urgently required.

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# Prospect of *Milicia excelsa* (Welw.) C. Berg for Multi-Tree Species Agroforestry

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## Abstract

**Background and Purpose:** The population of most of our economically indigenous tree species in Nigeria is declining. Human activities and agricultural practices have been the ultimate contributors to this decrease. In order to ameliorate the conflict between agriculture and forestry, agroforestry was introduced. However, most of the practiced agroforestry is based on single tree species. Agroforestry practiced using single tree species have been reported to be ecologically staggered and therefore it is pertinent that phytosociology of trees with agroforestry potential is studied in order to improve the sustainability of human livelihood.

**Materials and Methods:** This study was carried out in the University of Ibadan's campus forest. The data were collected on *Milicia excelsa* (Welw.) C. Berg by enumerating the tree species and also by identifying and enumerating the tree species associated with the subject tree (*Milicia excelsa*). Statistical analysis was done using percentages, Chi-square and charts.

**Results:** A total of 49 individual *Milicia excelsa* were encountered in the study area. The results show 31 woody tree species associated with *Milicia excelsa*. Of all the associates *Azadirachta indica* A.Juss. happened to be the best one, having an average distance of 5.4 m to the subject tree. The sex ratio of *Milicia excelsa* was discovered to be approximately 1:1.

**Conclusions:** Based on the obtained results of this research it may be concluded that *Milicia excelsa* has the prospect of being used in agroforestry in multi-tree species systems.

**Keywords:** phytosociology, sex ratio, biodiversity, conservation, *Milicia excelsa*

## INTRODUCTION

Agroforestry practices have considerable potential in solving some of Africa's main land-use problems [1-2] through provision of a wide range of tree products for domestic use or sale [3]. Agroforestry plays a significant role in increasing agricultural productivity by nutrient cycling, reducing soil erosion, improving soil fertility and enhancing farm income compared to conventional crop production [4-6]. Agroforestry can also potentially reduce deforestation while increasing food, fodder and fuelwood production [5, 6]. The benefits of agroforestry practice include food and nutrition security, increased income and assets, improved land management [7]; it also creates environmental and management synergies [8].

Traditional agroforestry has been practiced for millennia by agrarian-based societies throughout the world [7]. The World Bank estimated that 1.2 billion people practice some form of agroforestry on their farms and in their communities [9]. Although agroforestry has been practiced by these farming communities for a long time, there is inadequate study about the ecological compatibility of the tree components.

The sustainability of any agroforestry practices depends largely not only on the compatibility of the agricultural crops with forest trees, but also on the effectiveness of the tree components vis-à-vis contribution to food crops' yield and soil management [10].

Over the years, many agroforestry practices embarked upon by rural farmers and agroforestry experts have been dominated by mono-specific tree components. Using one tree species in tandem with the agricultural crops has been previously preached against. However, this is based on the reason that single tree species have not been able to meet the need of people. This is also coupled with the fact that population of those who depend on agroforestry output for livelihood is geometrically increasing. This has not only led to the depletion and disappearance of many valuable trees through deforestation but has

also aggravated food insecurity, especially in the rural areas. However, multi-tree-species agroforestry has been suggested [11-13] as a means of creating a more environmentally friendly system compared to the mono-specific tree pattern. Practicing multi-tree-species agroforestry can also improve the sustainability of biodiversity, the production of good quality timber and non timber species, the control of pest and diseases and climate change amelioration. In order to identify the tree species compatible for a multi-tree-species agroforestry, the phytosociology of such trees needs to be studied. Due to lack of pre-assessment of the phytosociology of some so-called agroforestry trees, lots of agroforestry practices have been jeopardized. This is because some trees happened to inhibit the growth of other trees and agricultural crops growing around them [14].

Apart from the phytosociology, the reproductive ability of the tree species is also very important in determining the sustainability of the agroforestry. In many instances, the reproductive ability of any dioecious tree species is determined by sex ratio, which also in the long run affects its agroforestry potential. This study therefore examined the agroforestry potential of *Milicia excelsa* (Welw.) C.C. Berg in the University of Ibadan. According to Borokini *et al.* [15], the University of Ibadan houses the bulk of the *M. excelsa* in Ibadan because of more considerable level of protection from the tree felling on the campus. *Milicia excelsa* is commonly known as Iroko in Nigeria. Iroko as a member of the family Meliaceae is a wind-pollinated dioecious tree species, threatened by overuse and areal fragmentation with typically low density. Iroko is a large deciduous tree that reaches up to 50 m height and 4 m diameter at breast height (dbh), with high umbrella-like crown growing from a few thick branches [15]. It is native to tropical Africa, mostly West and East Africa. Currently, this species is categorized as one of the endangered valuable timber species under the International Union for Conservation of Nature (IUCN Red Data List). Some of the threats to this species include

heavy exploitation, iroko gall (*Phytolyma fusca* Alibert, 1947) which attacks especially at an early growing stage, and an easy loss of seeds' viability. A number of countries have formulated policies toward its protection. For instance, it is protected by law in Ghana, Ivory Coast and Mozambique, while in Kenya, a Presidential ban on logging of indigenous timber was implemented in 1986.

Apart from the fruit production, male *M. excelsa* is different physiognomically from the female counterpart in terms of the crown position (Figure 1). On male trees, branches are more or less arranged in an orthotropic pattern, with an angle from lateral branches to the main stem below 90°, while on female trees branches are arranged in a plagiotropic position. In addition, female iroko produces

lengthier and knot-free merchantable bole and this has undoubtedly made it more suitable for timber production than male. Presently, there is a lack of information on the phytosociology of indigenous trees with agroforestry potential. According to Zubair *et al.* [16], *Milicia excelsa* is one of the pioneer species mostly preferred on farmlands for agroforestry purposes. This is due to its positive effect on soil fertility, soil-nutrient cycling and exhibition of favourable interactions with crops [17]. Populations of *Milicia excelsa* in the natural forests have been in decline over the years, resulting in the fact that only areas which still have a considerable stands of *Milicia excelsa* are Universities' Forests, Strict nature reserves and cemeteries [15]. This therefore makes the present study important.



FIGURE 1. Female (left) and male (right) *M. excelsa* in the University of Ibadan

## MATERIALS AND METHODS

This study was carried out in the University of Ibadan's forests. These forests are located around the school's administrative and residential area. The University of Ibadan's campus is located north of Ibadan along Oyo road (latitude 7°28'N, longitude 30°52'N, altitude 277m a.s.l.). The climate is the West Africa monsoon with dry and wet seasons. The dry season lasts usually from November through March and is characterized by dry cold wind of harmattan. The wet season usually lasts from April to October with occasional strong winds and thunderstorms. The annual rainfall in the area is 1258 mm - 1437 mm with mean daily temperature ranging from 22°C - 31°C. Soil type is ferric luvisols.

The collection of data was accomplished through identification, enumeration and measurement of distances between the subject tree (*M. excelsa*) and its associates' woody tree species. The closer a woody tree species is to *M. excelsa*, the more associated is the species with the subject tree. To identify the tree species associated with the subject tree, a search radius method according to Sabiiti and Cobbina [18] was used. Crown diameter of the subject tree was measured using 50m tape for the estimation of the search radius. An associate woody tree species is a single or multi-stemmed individual with a dbh  $\geq$  10cm located within the search radius of the subject tree [18]. Search radius (SR) is the distance from the subject tree within which all other trees are considered associate trees. It can be calculated as:  $SR = 7/4 \times CD$ , where CD is a crown diameter of the subject tree.

Data were analyzed using percentages, graphs and Chi-square test. Microsoft excel 2003 and IBM SPSS Statistics 20 are the statistical software used for the data analysis.

## RESULTS

### Phytosociology of *M. excelsa*

A total of 31 associated woody tree species with *M. excelsa*, belonging to 14 families (Table 1) were encountered in the study area. Of the

entire tree species associated with *M. excelsa*, *Azadirachta indica* A.Juss happened to be the first associate, having an average distance of 5.4 m to the subject tree (*M. excelsa*). Other tree species also closely associated with *M. excelsa* are *Entandrophragma cylindricum* (Sprague) Sprague, *Trichilia heudelotii* Planch. Ex Oliv., *Elaeis guineensis* Jacq., *Leucaena leucocephala* (Lamk.) De Wit., *Antiaris africana* Engl., *Anogeissus leiocarpus* (DC.) Guill.&Perr., *Spondias mombin* L., *Pycnanthus angolensis* (Welw.) Warb., *Lecanodiscus cupanioides* Ex Benth., and *Ficus exasperata* Vahl, having association distances of 6.3 m, 6.5 m, 6.9 m, 7.1 m, 7.2 m, 7.5 m, 8.3 m, 8.5 m, 9.4 m and 10.5 m respectively. On the other hand, tree species that have the weakest association with *M. excelsa* are *Enterolobium cyclocarpum* (Jacq.) Griseb (29.8 m), *Petrophorum pterocarpum* (DC.) K. Heyne (29.3 m), *Bosqueia angolensis* Ficalho (28.4 m), *Albizia zygia* (DC.) J. F. Macbr (26.6 m), *Cascabela thevetia* (L.) Lippold (26.4 m) and *Lannea welwitschii* (Hiern) Engl. (25.3 m). Based on the frequency and percentage of occurrence of the species, *N. laevis* has the highest population of 12 individual trees, representing 14.5% of the total species population. This is followed by *A. africana* and *B. sapida*, having a population of 7 individual trees (8.4%) each. Tree species such as *Azalia bella* Harms. Caes., *Anogeissus leiocarpus* (DC.) Guill.&Perr., *Ceiba petandra* L., *Entandrophragma cylindricum*, *Enterolobium cyclocarpum*, *Ficus mucuso* Welw. ex Ficalho., *Holarrhena floribunda* (G. Don) Durand & Schinz., *Lannea welwitschii* (Hiern) Engl., *Leucaena leucocephala*, *Petrophorum pterocarpum*, *Plumera rubra* L., *Pycnanthus angolensis*, *Rauvolfia vomitoria* Afzel. Ex Spreng., *Thevetia nerrifolia* Juss. Ex Steud. And *Trichilia heudelotii* Planch. exOliv. are represented with a single tree each accounted for just 1.2% of the total population.

The percentage distribution of trees into families (Figure 2) shows that Leguminosae (16.1%) is the highest represented family. This is closely followed by Apocynaceae (12.9%). Next to Apocynaceae are Fabaceae, Meliaceae and Moraceae having the same representation

**TABLE 1.** Tree species associated with *M. excelsa*

Tree species	Common/Local Name	Family	Average distance (m)	Frequency (n)	Proportion (%)
<i>Azelia bella</i> Harms. Caes.	Apa	Leguminosae	22.3	1	1.2
<i>Albizia lebeck</i> (L.) Benth.	Igbagbo	Leguminosae	14.3	2	2.4
<i>Albizia zygia</i> (DC.) J. F. Macbr	Banabana	Leguminosae	26.6	2	2.4
<i>Anogeissus leiocarpus</i> (DC.) Guill. & Perr.	Axlewood	Loganiaceae	7.5	1	1.2
<i>Antiaris africana</i> Engl.	False iroko	Polygonaceae	7.2	7	8.4
<i>Azadirachta indica</i> A.Juss.	Neem tree	Meliaceae	5.4	6	7.2
<i>Blighia sapida</i> K. Konig	Akeeapple	Sapindaceae	11.7	7	8.4
<i>Bombax buonopozense</i> P. Beauv.	Red cotton tree	Bombacaceae	15.1	2	2.4
<i>Bosqueia angolensis</i> Ficalho	Saworo	Moraceae	28.4	4	4.8
<i>Ceiba petandra</i> L.	Kapok	Bombacaceae	15.4	1	1.2
<i>Daniella ogea</i> (Harms) Rolfa ex Holland.	Balsam tree	Leguminosae	17.6	3	3.6
<i>Elaeis guineensis</i> Jacq.	Oil Palm	Palmae	6.9	6	7.2
<i>Entandrophragma cylindricum</i> (Sprague) Sprague	Cedar mahogany	Meliaceae	6.3	1	1.2
<i>Enterolobium cyclocarpum</i> (Jacq.) Griseb	Ear pod	Leguminosae	29.8	1	1.2
<i>Ficus exasperata</i> Vahl	Sand paper tree	Moraceae	10.5	2	2.4
<i>Ficus mucoso</i> Welw. ex Ficalho.	Fig	Moraceae	11.0	1	1.2
<i>Gliricidia sepium</i> (Jacq.) Kunth	Gliricidia	Fabaceae	17.9	5	6.0
<i>Holarrhena floribunda</i> (G. Don) Durand & Schinz.	False rubber tree	Apocynaceae	16.2	1	1.2
<i>Lannea welwitschii</i> (Hiern) Engl.	Muumbu	Anacardiaceae	25.3	1	1.2
<i>Lecanodiscus cupanioides</i> Ex. Benth.	Akika	Sapindaceae	9.4	2	2.4
<i>Leucaena leucocephala</i> (Lamk.) De Wit.	Jumpy bean	Fabaceae	7.1	1	1.2
<i>Morinda lucida</i> Benth	Oruwo	Rubiaceae	19.7	2	2.4
<i>Newbouldia laevis</i> (P. Beauv.) Seem. ex Bureau	Tree of life	Bignoniaceae	20.1	12	14.5
<i>Petrophorum pterocarpum</i> (DC.) K. Heyne	Rain tree	Fabaceae	29.3	1	1.2
<i>Plumera rubra</i> L.	Frangipani	Apocynaceae	25.8	1	1.2
<i>Pycnanthus angolensis</i> (Welw.) Warb.	African nutmeg	Myristicaceae	8.5	1	1.2
<i>Rauvolfia vomitoria</i> Afzel. ex Spreng.	Swizzler stick	Apocynaceae	18.5	1	1.2
<i>Spondias mombin</i> L.	Hog plum	Anacardiaceae	8.3	4	4.8
<i>Sterculia tragacantha</i> Lindl.	Star chesnut	Sterculiaceae	13.4	2	2.4
<i>Thevetia nerrifolia</i> Juss. ex Steud.	Bush milk	Apocynaceae	26.4	1	1.2
<i>Trichilia heudelotii</i> . Ex Oliv.	Rere	Meliaceae	6.5	1	1.2

of 9.7%. The families with the lowest representation of 3.2% include Bignoniaceae, Loganiaceae, Myristicaceae, Palmae, Polygonaceae, Rubiaceae and Sterculiaceae.

### The Distribution of *M. excelsa* and Sex Ratio

The results show that a larger population (32 stands), which accounted for 65.3% of *M. excelsa* is located in the forest in the residential area of the study location, while 17 stands (34.7%) are distributed within the forest around the school area. Chi-square result (Table 2) shows that the sex distribution of the species does not significantly depend on the location. The relative number of males (11) in the school area is lower than that of the relative number of males in the residential area (15). Similarly, the relative number of females in the school area (6) is lower compared to the number of females in residential area (17). The overall number of males (26) is slightly larger than females (23), resulting with approximate sex ratio of 1:1.

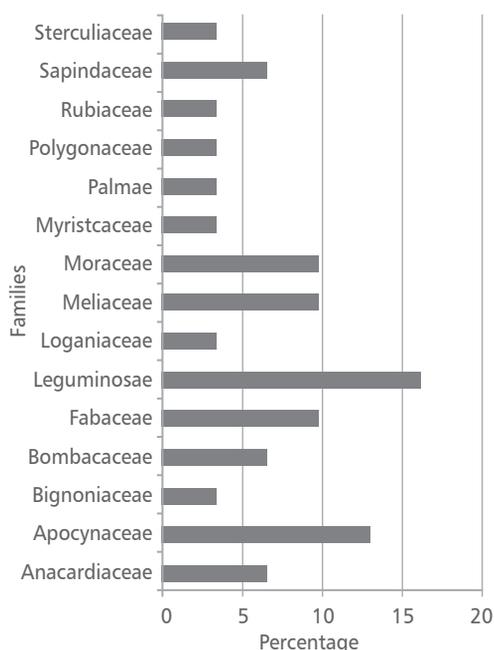


FIGURE 2. The distribution of the *M. excelsa*'s associates by family

TABLE 2. The effect of location on sex distribution of *M. excelsa*

Sex	Location		Chi-square value	df	p-value
	School	Resident			
Male	11	15	1.417	1	0.234ns
Female	6	17			
Total	17	32			

### DISCUSSION

This study's findings support assertion by Garrity [7] claiming that a woody tree species is justified to be used for multiple tree agroforestry if it supports the existence of other woody species around its niche. Hence, the observed associates of *M. excelsa* in this study indicate its potential for agroforestry uses. In addition, the diversity of the *M. excelsa* associates encountered in this study appeared to be in agreement with White [18], who found that tree of similar evolutionary history tend to be adapted to a peculiar location and niche. For instance, the best three associates (i.e. *A. indica*, *E. cylindricum* and *T. heudelotii*) discovered in this study are in the same family of Meliaceae with *M. excelsa*. This is in agreement with the work of Oni and Hall [19] on *Parkia biglobosa*. These authors discovered that similar tree species share similar edaphic and other growth requirements.

Having tree species like *E. guineensis*, *E. cylindricum*, *L. leucocephala* and *P. angolensis* as associates of *M. excelsa* additionally confirms its potential for agroforestry purpose. This is based on the fact that the named species are economically important tree species used on many occasions to support human livelihoods, especially in rural areas. For example, all parts of *E. guineensis* are used for various purposes [17]. According to Neupane and Thapa [5], *L. leucocephala* is one the most important tropical tree species and has been variously employed to improve soil fertility in the tropics through alley agroforestry practice.

The differences in the population of the associated trees in this study may be attributed to the aftermath effect of human impacts on the environment and the identified economic importance of the species. For instance, *N. laevis*, *A. africana* and *Blighia sapida*, which had the highest population among the associates, have been previously identified as less known species [19] and therefore, less prone to exploitation by humans. Tree species such as *P. rubra*, *R. vomitoria* and *T. nerrifolia*, which are also less known species [19] are also less represented, which may be due to the fact that their regeneration ability is low. The family distribution of the tree associates of *M. excelsa* recorded in this study supports the previous study [20] claiming that Leguminosae is among the most numerous tree species families.

The least disturbed stands had a greater proportion of female trees. This is probably because female iroko tree (Figure 1, left) have better quality trunks in terms of physiognomy and length than the male counterpart (Figure 1, right), which can be easily converted to sawn timber for utilization purposes.

The unbiased ratio of the male and female population of *M. excelsa* recorded in this study agrees with Ndakidemi and Ndakidemi [21]. They reported that balancing the number of male and female in a dioecious plant is very important for the rapid regeneration in agroforestry system. This fact coupled with others such as the presence of matured mother trees, the reduced logging of the subject tree and the absence

of significant ecological threat may have been the reasons why the population of *M. excelsa*'s sex is unbiased in the University of Ibadan compared to the biased population in Ibadan metropolis as reported by a previous study [15].

## CONCLUSION

This study showed that *M. excelsa* has the potential of being used for multi-species agroforestry projects. This conclusion is based on the association of iroko with a large number of tree species. The associated tree species also happened to be economic trees upon which the users can depend for livelihood and sustenance. The results of this study revealed that *Azadirachta indica*, *E. cylindricum*, *T. heudelotii* and *E. guineensis* can be combined with iroko in multi-tree species agroforestry. This is based on the fact that these tree species have been found to grow naturally where iroko survived and are also in the same family with iroko. Hence, their edaphic requirements are similar to that of iroko, which will favour the growth of the adjoining agricultural crops as well as the entire agroforestry system. The findings revealed that evolutionary relationship has a key role to play in determining the phytosociology of a tree species population.

The population size of iroko is under strong influence of human activities. The number of female trees is under larger pressure due to better quality timber.

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- Early View papers (published online, waiting to be published in print version):
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*c) Book:*

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*d) Chapter in a book:*

9. CURTIS PS 2008 Estimating Aboveground Carbon in Live and Standing Dead Trees. *In: Hoover CM (ed) Field Measurements for Forest Carbon Monitoring. Springer, New York, NY, USA, pp 39-44*

e) *Thesis:*

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f) *Non-scientific literature:*

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