

Comparative indicators of genetic variability and bark beetle infestation intensity in populations of norway spruce (*Picea abies* (L.) Karst.) in Bosnia and Herzegovina

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ABSTRACT

This study sets out comparative indicators of Norway spruce *Picea abies* (L.) Karst. populations in Bosnia and Herzegovina, based on an evaluation of the attack power of bark beetles *Ips typographus* L. and *Pityogenes chalcographus* L. and the decline of spruce within the populations sampled for genetic analysis. Twelve natural forest stands and one plantation were analyzed. The average numbers of desiccated Norway spruces per stand and isoenzyme gene markers were used for comparison purposes. The results indicate that the Norway spruce population from Mt Vlašić deviates markedly from the other sampled populations, both in genetic structure and in the recorded numbers of insects, as well as in the number of desiccated trees. These results suggest the importance of using only indigenous Norway spruce reproductive material in artificial forest regeneration to ensure its higher adaptability potential. It is also necessary to develop a dynamic management system for Norway spruce in Bosnia and Herzegovina, which will ensure the systemic stability of forest structures. Further development of the systematic monitoring system of Norway spruce bark beetles, early detection, and the introduction of effective integral protective measures are badly needed.

KEY WORDS

Norway spruce, isoenzyme, *Ips typographus*, *Pityogenes chalcographus*

INTRODUCTION

Spruce *Picea abies* (L.) Karst. is one of the most important forest tree species, both economically and ecologically, in Bosnia and Herzegovina and several central European countries. According to Fukarek (1970), its initial postglacial range in Bosnia and Herzegovina has shrunk

towards the interior from both the Adriatic Sea and the Pannonian basin. This, combined with the species' disjunct distribution, gives spruce a specific genetic structure (Ballian et al. 2007). Spruce grows in Bosnia and Herzegovina in about 586 thousand ha, or about 21% of all forests (Matić et al. 1971). These facts indicate the importance of spruce in productive forestry, just after

beech *Fagus* sp. and common fir *Pseudotsuga* sp. It is usually found in mixed beech and fir forests; pure stands are rare. These are stands of extreme habitats, such as frost-prone areas (Igman, Grmeč, Sjemeč), peatland (Bijambare) and high altitudes (Vranica) (Stefanović 1977; Stefanović et al. 1983). At high altitudes, it is often found in small groups at the upper trees, mixed with mountain pine *Pinus mugo* (Tura) (Mt. Vranica). Of particular interest is the fact that spruce has a lower capacity to regenerate naturally in mixed forests as compared with beech and common pine. As a result, more aggressive beech becomes dominant, and even common fir sometimes dominates spruce (Pintarić 2002).

Since this species provides very good quality timber with a wide range of commercial uses, it is also widely used in commercial forestry (Johann et al. 2004), including its introduction to areas to which it is not environmentally suited (Nožička 1972). Its importance may also be deduced from the fact that it constitutes about 85% of total seedling production in the Federation of BiH, as reported by Ballian (2000). It should be added that seed handling and nursery production of planting stock is much easier in the case of this species than of common pine, economically the most important species in BiH. A comparison between spruce and pine species, which are also well represented in nursery production, shows that spruce has also been used in extreme conditions. When the first state forestry offices were established in central Bosnia (Busovača) in the late 19th century, spruce plantations began to be introduced at lower altitudes, in unfavourable environmental conditions. An interesting case is that of Mt. Vlašić, where a disastrous wildfire destroyed about 18,000 ha some one hundred years ago, following which the entire area was sown with spruce (Ballian 2007). Reproductive material of unknown origin was used, as local seed production had not yet developed (Ballian et al. 2007). Plantations became increasingly common after World War II, and by 1990 there were about 125,479 ha of artificially established forests in Bosnia and Herzegovina, mainly of spruce (Mekić et al. 2002). In many European countries, unlike in Bosnia and Herzegovina, spruce is the most important species due to the policy of increasing spruce plantation over several hundred years (Klimo 2007; Schmitt and Hayder 2009).

Turbulent environmental changes that have taken place particularly in recent decades, particularly rising

temperatures and atmospheric and soil pollution, have led to a systematic decline of spruce, as reported by numerous researchers in Europe (Bergmann and Hosius 1996; Hosius and Bergmann 1993; Klimo 2007; Longauer et al. 2001; Pacalaj et al. 2002; Hlasny et al. 2010; Barka et al. 2010), including Bosnia and Herzegovina (Dautbašić 1997). This decline is most marked in secondary forests, mainly monocultures (Klimo 2007). But it is also present in natural forests, usually as a result of faulty management. The principal cause is the increasing population of bark beetles, encouraged by recent climate changes (Pernek 2000; Hlasny et al. 2010a). In Bosnia and Herzegovina, the state of spruce forests is steadily deteriorating, with steadily increasing mortality caused by bark beetles. It is caused by two major species of bark beetles: *Ips typographus* L. and *Pityogenes chalcographus* L., which have been multiplying rapidly over much of Bosnia and Herzegovina (Dautbašić and Čabaravdić 2001; Dautbašić and Treštić 2006).

The aim of the studied pointed at the demonstration that the more intensive decline of spruce population in Mt. Vlašić, compared with natural spruce populations in Bosnia and Herzegovina, is also due to its altered genetic structure, as a result of artificial establishment using seeds of inappropriate provenance. The genetic structure of Norway spruce populations was studied by means of biochemical markers, isoenzymes, while the decline of spruce was assessed on the basis of the numbers of bark beetles in pheromone traps and dead trees in the same areas, where biological material for genetic characterization was collected.

MATERIAL AND METHODS

Genetic analysis of spruce population

The genetic structures of twelve natural stands and one plantation of Norway spruce were analyzed in Bosnia and Herzegovina (tab. 1) using a standardized methodology for isoenzyme analysis.

Isoenzyme markers were used to analyze the genetic structure following Ballian et al. 2007, while the interpretation of zymograms followed the protocol provided by Konnert (2004).

The GSED statistics program (Gillet 1998) was used to compute the genetic variability parameters. The genetic distance between populations was calculated

Tab. 1. Investigated Norway spruce populations in Bosnia and Herzegovina

No.	Population	Latitude	Longitude	Altitude	No. of analyzed individuals
1	Grmeč (Bos. Petrovac)	16°37'32"	44°39'43"	1250	50
2	Preodac (Bos. Grahovo)	16°33'49"	44°09'44"	1000	50
3	Busije (Glamoč)	16°49'13"	44°02'08"	1350	50
4	Rasticevo (Kupres)	17°17'50"	44°03'52"	1300	50
5	Vlasic (Turbe) (artificial)	17°27'02"	44°19'35"	1100	50
6	Bistrica (Gornji Vakuf)	17°40'53"	43°59'22"	1650	50
7	Igman – a (frost-prone stand)	18°16'01"	43°44'48"	1200	50
8	Igman – b (normal)	18°16'19"	43°45'19"	1340	50
9	Zelegora (Kalinovik)	18°37'32"	43°39'39"	1200	50
10	Bijambare (Nišići)	18°29'39"	44°05'01"	950	50
11	Tibija (Olovo)	18°20'27"	44°20'37"	950	50
12	Romanija (Mokro)	18°39'45"	43°54'05"	1300	50
13	Han Krame (Han Pijesak)	18°53'28"	44°02'17"	1100	50
Total					650

Tab. 2. Average numbers of bark beetles trapped and number of dead trees in sampled spruce populations

No.	Population	Monitoring years	Species of bark beetle		No. of dead trees		No. of used traps
			Ips typographus	P. chalcographus	Piece	m ³	
1	Grmeč (Bos. Petrovac)	2009	293,550	nn	200	nn	24
2	Preodac (Bos. Grahovo)	2006	41,520	622,000	1,176	1,991	40
3	Busije (Glamoč)	2007–2009	322,640		269	404	73
4	Rasticevo (Kupres)	2007–2008	55,540	298,500	5	nn	23
5a	Vlasic (Skender Vakuf) (artificiala)	2006–2008	218,083	489,652	722	2,945	161
5b	Vlasic (Turbe) (artificial)	2006–2009	819,478	4,308,805	1,874	2,623	60
6	Bistrica (Gornji Vakuf)	2009	17,200	72,000	541	618	10
7	Igman – a (frost-prone stand)	2007–2009	130,881	1,332,107	372	432	105
8	Igman – b (normal stand)						
9	Zelegora (Kalinovik)	2006–2008	32,972	29,344	211	881	61
10	Bijambare (Nišići)	2005–2009	44,115	415,114	270	303	21
11	Tibija (Olovo)	2008–2009	280,147	592,932	nn	195	159
12	Romanija (Mokro)	2006–2008	63,443	98,149	nn	nn	151
13	Han Krame (Han Pijesak)	2006–2008	334,268	500,945	1,324	5,709	206

following Nei (1972), and the UPGMA method, modified for NEIGHBOR procedure in PHYLIP Version 3.5 program, was used to produce a dendrogram.

Analysis of spruce decline

The data for bark beetles were obtained by counting their numbers caught in traps during recent growing

seasons (tab. 2). We thus have data for the past five years for some populations, but for only one year in other populations. The data over longer periods reveal a dynamic insect population, the result of climatic conditions. In the absence of matching monitoring in some years, we decided to interpret mean values. In the case of Mt. Vlašić, we have data from two forestry offices belonging to different entity forestry authorities. From Mt. Igman, the data from two sampled populations, representing two different phytocenoses, are taken as one in this analysis since it was not possible to distinguish the numbers of insects and desiccated trees. Teyson and Ecotrap II traps were used to assess bark beetle population numbers in a given area. The number of trapped bark beetles was determined by the volume method, where 1 ml = 40 individuals of *I. typographus* or 400 individuals of *P. chalcographus*. The number of dead trees was arrived at on the basis of the annual stand surveys and the removal of desiccated trees from the forest for the purpose of maintaining forest health and vitality.

The samples for the genetic analysis and the numbers of dead trees and insects were collected from the same area, and represent populations in each case.

It should be noted here that the dendrograms for the insects were produced using a hierarchical model, since only mean values were available to us.

The analysis was conducted by comparing the results of the population genetic analysis with the data obtained for the numbers of bark beetles and dead trees.

RESULTS AND DISCUSSION

Without going into detail, we shall proceed immediately to a comparison of the relevant genetic parameters set out in tab. 3, and their comparison with data on the number of insects and tree decline in the populations, even though the data in tab. 2 are incomplete. The latter is due to the fact that it was very difficult to apply the same assessment methodology and principles in all studied stands. A further problem is that the number of installed traps differed from area to area, though the number of dead trees demonstrates the true state of affairs in the field. Despite certain shortcomings, this research will provide many interesting details that may play a very important part in spruce management.

Tab. 3. Indicators of genetic multiplicity, diversity and heterozygosity in 13 populations of Norway spruce in Bosnia and Herzegovina (Ballian et al. 2007)

Population	Multiplicity		Diversity	Heterozygosity	
	A/L	P(%)	n_e	$H_{st}(\%)$	$H_{te}(\%)$
Grmec	2.55	90	1.355	20.2	20.7
Preodac	2.35	80	1.330	18.4	19.2
Busije	2.30	80	1.378	21.7	21.5
Rasticevo	2.40	85	1.375	20.0	21.4
Vlasic	1.80	65	1.277	21.0	16.9
Bistrica	2.50	85	1.361	21.6	21.4
Igman – a	2.45	85	1.335	20.7	19.9
Igman - b	2.35	85	1.353	22.3	21.1
Zelegora	2.40	85	1.326	20.4	20.2
Bijambare	2.50	80	1.336	19.9	20.4
Tibija	2.40	75	1,352	19.8	19.7
Romanija	2.30	80	1.380	24.0	21.8
Han Krame	2.60	85	1.355	20.8	21.6

An overview of the situation in the field is shown by means of dendrograms. These (fig. 1, 2 and 3) reveal that population grouping differs according to genetic structure (Ballian et al. 2007) and number of individuals caught of *I. typographus* and *P. chalcographus*. It is clear, however, in all three cases, that the Vlašić population differs from every other population covered by this study. As already noted (Ballian 2007; Ballian et al. 2007), it is completely different in genetic structure and in the numbers of both insect species.

The largest average number of insects was recorded in the Vlašić population, as well as the greatest number of dead trees. Population genetic characteristics of this population also differ from indigenous populations of Norway spruce. The genetic multiplicity and diversity have very low values while observed heterozygosity is higher (tab. 3). Higher heterozygosity may be due to the mixing of the different gene pools of introduced and indigenous Norway spruce.

More than 2,500 dead trees per annum on average were recorded in the two forestry authorities that manage the Vlašić population (tab. 2). This is to be expected, given that these stands were established artificially using foreign reproductive material of unknown origin (Ballian 2007). The ravages of the 1992–1995 war have also had an effect on the state of the forests, as has the

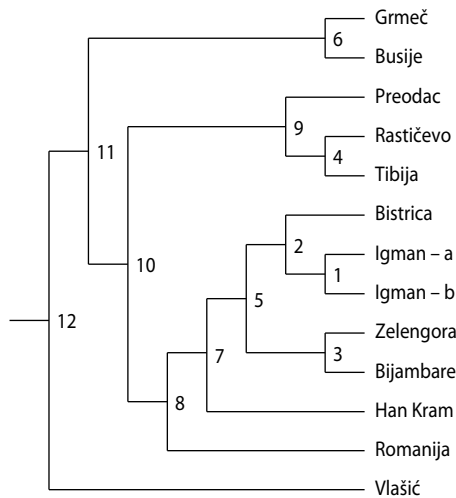


Fig. 1. Dendrogram of genetic distances Nei (1972) (Ballian i sur 2007)

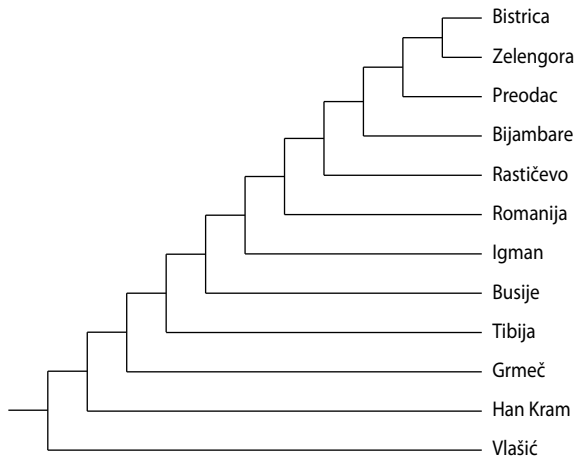


Fig. 2. Dendrogram based on number of bark beetles *Ips typographus* (total numbers caught in traps)

construction of sports facilities without planning permission. Both these factors may be regarded as the reason for the large numbers of *I. typographus* and *P. chalcographus* bark beetles, but are much more directly associated with the altered genetic structure of this population. Our analysis of the intensity of bark beetle attack in areas managed by two different forestry authorities managing the Vlašić forests reveals differences in the numbers of insects, showing that the situation in the Turbe forestry area is particularly acute. Here, a far greater number of insects were caught in fewer traps, but still more significant is the anthropogenic impact, such as the poor management system.

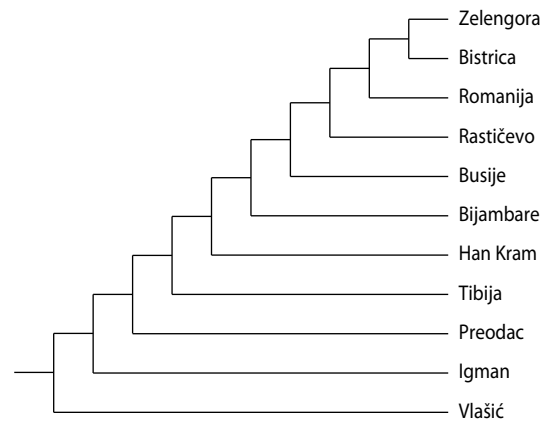


Fig. 3. Dendrogram based on number of bark beetle *Pityogenes chalcographus* (total numbers caught in traps)

By contrast, similar numbers of bark beetles were caught in the Igman population, growing in two different phytocenoses: *Piceetum illyricum montanum*, Fukarek and Stefanović 1958, emend. Horv. et al. 1974 and *Piceetum illyricum*. In this case, we are dealing with pure natural populations, so that the explanation cannot lie in the allochthonous genetic structure, as is the case with the Vlašić population. The reason should rather be sought in the severe anthropogenic impact on this population. The problem of serious spruce dieback dates back to the 1984 Winter Olympics, when the structure of the forest was disrupted by the construction of sports facilities, and many trees were damaged. This was followed by the ravages of the 1992–1995 and unplanned felling without permission. Just after the war, more than 10,000 dead trees were felled over a short period from 1996 to 1998; these trees were desiccated, but there are no data on insect action. Things improved slightly thereafter, but the site has now been laid out as a mountain resort. The population should therefore be observed with particular interest over the coming period. Reference should also be made at this point to the management system, which is probably unsuited to this type of forest on Mt. Igman.

The examples of these two populations may be complemented by another, the Preodac population in western Bosnia, where large numbers of *P. chalcographus* have also been recorded, along with a significant increase of mortality of Norway spruce. In this case, however, the number of desiccated trees (tab. 3) should be viewed with caution, since local foresters did not

distinguish between dead spruce and common fir. It is important to note that this population has a genetic structure of a very high quality, so that the basic reason for tree desiccation should be sought in the ravages of war and inappropriate management over a longer period. The extreme local habitat conditions could also be noted for this population, primarily the extensive karstification of the area, with many karst features, where spruce grows on blocks of raw limestone.

The Han Kram population is of great interest (represented by the phytocoenosis *Pino-Betuletum pubescentis* Stef. 1964, *piceetosum subass.* Beus 2007 (Mataruga et al. 2007), which also shows the high genetic values typical of eastern Bosnia, which should demonstrate its stability (tab. 3). However, large insect numbers also affect this population, with a significant number of desiccated trees as well as a large mass of felled desiccated trees. Since the site is characterized by the extreme environmental conditions of peatland combined with high altitude, one would expect insect numbers to be lower. From the mass of felled trees, which is considerable, we may conclude that the majority of desiccated trees were mature individuals already nearing the end of their lifespan, and thus less able to adapt to even the slightest changes to their environment, let alone with such major changes as the attempts to drain and convert peatland to pasture land.

Another spruce population sampled is the high altitude Bistrica population on Vranica Massif (represented by the phytocoenosis *Piceetum illyricum subalpinum* Horv. et al. 1974), where the majority of dead trees are of average thickness, with only a few very thick-trunked specimens. This population shows very high genetic parameter values, as shown in tab. 3. At the same time, the smallest number of insects were recorded in this population, along with a relatively large number of dead, thin-trunked trees. Since this is a high-altitude population, one would expect fewer insect generations (one only) per year, yet another factor favouring the stability of this population. Since the population is managed by group selection, which is the most common regeneration felling in Bosnia and Herzegovina, which is considered appropriate to spruce ecology, we shall pay specific attention to the development of this population over the coming period, and its resistance to the insect attack.

We must also refer here to a very interesting population – the Tibija population – in which a very large

number of *P. chalcographus* bark beetle has been recorded, but without any increase in the mortality of Norway spruce in this area. Since this is in a zone with very adverse climatic factors, dry and very hot winters and uneven precipitation (Stefanović et al. 1983), the fundamental reason for the stability of local spruce must lie in good management practices. It is relevant to note that forests in this area are also managed by group selection.

In the case of other sampled populations with good genetic parameters, no marked insect attack has been recorded comparable to the populations Vlačić and Igman. No major diebacks of spruce occurred there. In addition, it seems that all necessary management measures are carried out in the remaining populations – from protection to cultivation and regeneration. The Rastičevo population is the most stable, with very few desiccated trees and average attacks by both species of bark beetle.

CONCLUSION

1. Previous studies of the genetic structure of spruce stands have confirmed that the Vlačić population differs markedly from any other population, and is not indigenous. It also differs markedly from other spruce populations sampled in Bosnia and Herzegovina in the number of insects recorded and the number of trees which have died due to bark beetle attack.
2. The genetic analyses conducted in other populations have confirmed the presence of a good genetic structure in natural populations, but large numbers of insects are also present in many of these populations. In this case, the presence of insects may be associated only with severe anthropogenic impacts, including a poor spruce forest management system. In recent years the phenomenon is also the result of global warming.
3. The results obtained make it clear that only indigenous spruce material demonstrating high adaptability should be used for forest regeneration.
4. In the coming period, the system of spruce management in Bosnia and Herzegovina should be improved in order to ensure the systemic stability of spruce stands on the permanence principle.

5. To ensure the future stability of spruce stands, a standard system of permanent monitoring should be established to monitor and assess the numbers of spruce bark beetles.
6. Based on the monitoring data obtained, measures to protect spruce stands should be introduced. These should be flexible enough to ensure constant adjustment in line with the situation in the field.

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