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DESTRUCTION OF YOUNG *FRAXINUS EXCELSIOR* L. STANDS AND MINERAL NUTRITION STATUS IN LATVIA, A PILOT STUDY

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It is considered that the main reason for destruction of ash tree stands in Latvia during the last years is a pathogenic fungus *Chalara fraxinea.* The most rapid tendency to decrease is observed for young European ash stands. The aim of the study was to evaluate the content of 12 plant nutrients in soils and leaves of young *Fraxinus excelsior* stands in Latvia during 2012 and to reveal the tree nutritional problems mainly caused by pathogenic fungus *C. fraxinea*.

The investigation was done in 8 young *F. excelsior* stands located in different areas in Latvia in July 2011 and 2012. Four study sites were selected for the background level (Barkava, Rundale, Gulbene, Tervete) in *Aegopodiosa* forest type where healthy young *F. excelsior* grown under crown deck, but four study areas - in 8 to 10 years old felled areas with *F. excelsior* natural regeneration where the pathogenic fungus *C. fraxinea* development on tree branches was observed (Zalenieki, Saulkalne, Aizpurve, Fabriki). In these areas the samples for soil and leaf chemical analysis were collected both from trees with fungus development (damaged trees) and from trees without visual signs of fungus (healthy trees). The soil analyses were done using 1 M HCl extraction.

The results showed a relatively high variability in the soil chemical composition for the background level, healthy and damaged *F. excelsior.* In general, the results revealed several differences for the accumulation of nutrients in tree leaves: significant lower concentration of Ca, Mg and S in the healthy tree leaves to compare with the background level and significant lower concentrations of N, Ca, Mg and Cu in the damaged tree leaves to compare with the healthy ones, as well as a slight tendency to accumulate lower concentrations of K, S, Mn, and Zn in the damaged tree leaves.

Key words: European ash, hyphomycete, *Chalara fraxinea*, leaves, soil, nutrients, chemical analysis.

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INTRODUCTION

Currently, European or common ash (*Fraxinus excelsior* L.) is the most common (popular) broad-leaved tree species in Latvia. According to the State Forest Service data, the total area of *F. excelsior* stands was 15559.8 ha or 0.5% from the total forest area and 53.1% from the broad-leaved forest area in 2011. *F. excelsior* stand area has rapidly changed during the last 100 years. At the first half of the 20th century, *F. excelsior* area was only up to 1000 ha in Latvia (Anon 1937, Kundziņš 1937, Eihe 1940, Kronītis 1966). The area of *F. excelsior* stands started rapidly to increase due to purposed restoration and maintenance during the second half of the 20th century, the maximum reaching at the turn of the century 20 and 21: 21905 ha or 0.8% of the total forest area in 1998 (Sakss 1958).

Nowadays, there is an unambiguous tendency for the total area of *F. excelsior* stands in Latvia to decrease. During 2000-2011, the total area of *F. excelsior* stands in Latvia has decreased 1.4 times, which is 570 ha per year, on average. It is considered that the main reason for destruction of *F. excelsior* stands not only in Latvia, but throughout Europe since the turn of the century 20 and 21 is an invasive disease caused by the hyphomycete *Chalara fraxinea* T. Kowalski (Kowalski 2006, Jankovsky & Holdenrieder 2009, Rytkönen et al. 2011, Keßler et al. 2012).

F. excelsior dieback was first noticed in Poland in the 1990s (Kowalski & Holdenrieder 2009). *C. fraxinea* development damages and declines trees of all ages causing dieback of leaves, branches, shoots and twigs, facilitating circumstances for invasion of different stem and root pests and diseases (Lygis et al. 2005, Kowalski 2006, Schumacher et al. 2007, Kowalski & Holdenrieder 2008, Kenigsvalde et al. 2010).

This fungus is the most destructive to young ash plants, killing them within one growing season of symptoms becoming visible. The damage and mortality levels are much higher in afforestations, on nursery seedlings and on natural regeneration, as well as in thicket-sized and pole-sized stands (Kirisits et al. 2011). The old trees appear to be capable to endure the disease for a relatively long time, but tend to succumb eventually after several seasons of infection (Keßler et al. 2012).

In Latvia the most rapid tendency for the area to decrease is observed for young *F. excelsior* stands (younger than 40 years), where the reduction is about 63.6% during the last 11 years. Thereby there is a significant change of the age structure of *F. excelsior* stands, which could lead to decrease of grown-up stands and *F. excelsior* wood resources.

For maintenance stands infected by the pathogenic fungus and recovery of withered away *F. excelsior* stands relevant are data on mineral nutrition conditions of *F. excelsior* young stands. Sufficient supply with nutrients promotes not only productivity of tree growth, but also tree tolerance to different diseases and stress conditions. Several scientists have specially noted the importance of copper, zinc, boron, calcium etc. (Bergmann 1988, Marschner 1995). Relevant aspect for favourable tree growth condition maintenance is also soil reaction (Craul 1999, Mandre et al. 2012).

A vast range of optimal and average concentrations of nutrients in *Fraxinus* spp. leaves has been stated (Bergmann, 1988, Веретенников 2006, Hagen-Thorn et al. 2005). According to Mertens et al. (2007) there are differences in nutrient accumulation between plant species growing in the same soils, as well as differences due to regional variations, sampling time, pollution etc. Results of different soil studies are also difficult to compare due to differences in soil extraction methods. Thereby, nutrient supply for the young *F. excelsior* stands in the forest based on soil and plant chemical analysis has not been investigated sufficiently, especially in Latvia.

Therefore a pilot study has been done on nutrient content in visual healthy and infected by *C. fraxinea* young *F. excelsior* leaves and topsoil. The aim of the study was to evaluate the content of 12 plant nutrients in soils and leaves of young *F. excelsior* stands in Latvia during 2012 to reveal the tree nutritional problems probably caused by the hyphomycete *C. fraxinea*.

MATERIAL AND METHODS

Study sites

The investigation was done in 8 young *F. excelsior* stands located in different areas in Latvia in 2011 and 2012. Four study areas, each 2-3 ha, were selected in 8 to 10 years old felled areas with *F. excelsior* natural regeneration (around 2 m high) where the pathogenic fungus *C. fraxinea* development on tree branches was observed. Two felled areas are located in the southern part of Latvia or in Central Latvian Lowland Zemgale Plain – Zalenieki and Saulkalne, but two – in the north-eastern part of Latvia or in Mudava Lowland Abrene Tilted Plain near Vilaka – Aizpurve and Fabriki (Fig. 1). Besides four study sites were selected for the background level in different places in Latvia (Barkava, Rundale, Gulbene and Tervete). These sites were chosen in *Aegopodiosa* forest type where healthy young *F. excelsior* grows under crown deck. The size of each background site - 900 m^2. The soils in all the study sites are characterized as mineral soils. Coordinates of the study sites and site height above the sea level are given in Table 1.

Field work

Soil and tree leaf samples were collected in 3 study sites (Zalenieki, Saulaine, Fabriki) both from damaged and healthy *F. excelsior* in July 2011, and from all 8 study sites (including the background level) also in July 2012. Thereby the soil and leaf samples in the felled areas were collected both from trees with fungus development signs on branches (damaged trees) and from trees without visual typical symptoms of fungus (healthy trees). Each leaf sample was taken from 5-7 randomly distributed young trees at 2 m height and consisted of leaves just reaching maturity and full size. Soil samples were taken from 5 to 15 cm depth and consisted of thoroughly mixed 5-7 subsamples collected by a soil probe. In each study site 3 samples were collected separately for different status of

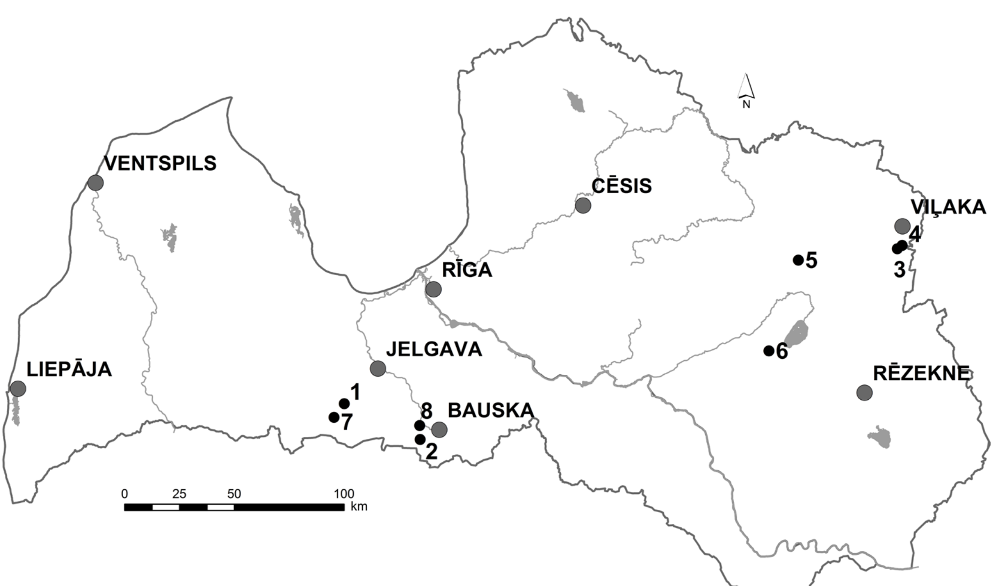

Fig. 1. The research sites of *Fraxinus excelsior* in Latvia during 2011 and 2012. Felled area: 1. Zalenieki, 2. Saulaine, 3. Aizpurve, 4. Fabriki; Forest stand (background): 5. Gulbene, 6. Barkava, 7. Tervete, 8. Rundale.

Table 1. Characteristics of the sampling sites

Location	Coordinate system				Height, m asl.
	LKS 92		Geographical coordinate system		
	X	Y	E	N	
Felled area					
Zalenieki	468330	6263090	23°29’87”	56°30’33”	26
Saulaine	502945	6246770	36°42’67”	33°55’76”	36
Aizpurve	722630	6335180	38°23’26”	33°05’44”	96
Fabriki	720460	6333640	38°21’46”	33°06’28”	98
Forest stand (background)					
Gulbene	675326	6328500	37°52’49”	33°09’33”	105
Barkava	661933	6287151	37°44’37”	33°31’58”	92
Tervete	463690	6256825	35°36’19”	33°49’27”	59
Rundale	502719	6253103	36°01’71”	33°51’30”	23

trees (healthy and damaged) and analysed in the laboratory.

Along with the soil and leaf sampling, assessment of tree vitality for 50 young *F. excelsior* in 2011 and 2012 was carried out in the studied felled areas to characterize the general physiological status of trees. This was based on the visual evaluation of crown defoliation or leaf lost in the crown with 5% precision (Anon 1994).

Laboratory analysis

The chemical analysis of soil and *F. excelsior* leaf samples were done in the Laboratory of Plant Mineral Nutrition of Institute of Biology of the University of Latvia. The soil samples were dried at room temperature and sieved <2 mm. The soil analyses were done using 1 M HCl extraction, where soil-extractant mixture was 1:5. This extraction characterizes not only the amount of element currently available for the plant uptake from the soil, but also indicates the amount of reserves of the element for the remaining vegetation season. This solution is universal and quite aggressive (Osvalde 1996). Tree leaves were quickly washed with distilled water, dried at +60 ^{o}C and ground.

The levels of Ca, Mg, Fe, Cu, Zn and Mn were determined by AAS (*Perkin Elmer AAnalyst 700*), acetylene-air flame, those of N, P, Mo, B by colorimetry, and S by turbidimetry with spectrophotometer *JENWAY 6300*, K by flame photometer *JENWAY PFPJ*, soil pH – in 1 M KCl extraction (soil-extractant micture 1:2.5), soil electrical conductivity – in distilled water extraction (soil-distilled water mixture 1:5) (Ринькис и др. 1987). Analytical replication was three times.

Statistical analysis

The statistical analysis of the research results was done using *SPSS 14.0* and *PC-ORD* software for multivariate analysis of ecological data. Standard errors (SE) were calculated in order to reflect the mean results of chemical analysis. The correlation coefficients (*Pearson*) were calculated (significant above 0.576). The Student’s t-test (*Two-Sample Assuming Equal Variances*) was used for testing the changes in the chemical element concentrations and the level of tree crown defoliation during 2011 and 2012, as well as to detect differences between the levels of chemical characteristics in the soil and leaf samples from healthy, damaged trees and the background level. Dispersion of chemical element concentrations between research sites was characterized by the variance (σ^2) parameter. To assess relationships between the chemical composition of soil, leaves, and health status

Table 2. Mean crown defoliation of young *Fraxinus excelsior* in the studied felled areas

Felled area	**Defoliation, %**		**t-test**
	2011	2012	2011-2012
Zalenieki	73.5	60.5	2.2*
Saulaine	53.2	45.3	1.3
Aizpurve	18.6	35.1	4.1*
Fabriki	24.3	31.0	1.7

* $t > t\ 0.05$

of trees in July 2012 the principal component analysis (PCA) was done using *PC-ORD Version 5* (McCune & Mefford, 1999).

RESULTS AND DISCUSSION

Young *Fraxinus excelsior* crown status in the felled areas

The results of the visual crown assessment revealed a considerable variation between the research sites (Table 2). More damaged young trees were in the felled areas in Zalenieki and Saulaine which located in the southern region of Latvia, but less damaged young *F. excelsior* were in Aizpurve and Fabriki, located in the north-eastern region of Latvia.

Differences between the levels of crown damage were also found between the felled areas in the same region in Latvia. There were significant differences for crown defoliation between Zalenieki and Saulaine in Zemgale in both of the study years (2011 $t = 2.6 > t_{0.05}$, 2012 $t = 3.4 > t_{0.05}$). Significant loss of leaves in the felled areas located in the north-eastern part of Latvia was stated only in 2012 ($t = 2.2 > t_{0.05}$). The investigations carried out in Austria also showed that damage levels vary greatly between sites (Keßler et al. 2012).

The considerable variation in defoliation intensity between research sites may be related to climatic, soil and biological factors. These factors could be, for example, stand characteristics, the amount of ash trees in the vicinity and site-dependent humidity during the infection period (Keßler et al. 2012, Kirisits et al. 2012). Observations in several countries suggest that disease intensity is lower on drier sites within the ecological amplitude of *F. excelsior* (Schumacher 2011, Keßler et al. 2012). It is known that factors like increased shading, moisture and proximity of plant tissues, limited nutrient availability particularly in dense, single-species stands may induce susceptibility in plants to fungal infections (Niemelä 1992). However the investigations carried out in other countries did not reveal clear evidence that stand density influenced the development of dieback in young, even-aged stands of *F. excelsior* (Bakys 2013). It is possible that the development of *C. fraxinea* may be less successful when subjected to competition from other fungal communities (Cleary et al. 2013).

In general, the crown condition of young *F. excelsior* has improved in the felled areas in Zemgale during 2012, revealing significant differences between the studied years for Zalenieki research site (Table 2). Whereas in the felled areas in the north-eastern part of Latvia – declined, and this tendency was significant in Aizpurve. The three year studies in Austria revealed similar results, although the disease generally increased slightly in intensity in mature ash stands, the changes from 2008 to 2010 show a wide variation and on some sites even a decrease in disease intensity was recorded (Keßler et al. 2012).

In all the studied areas healthy or trees without symptoms of fungus infection were observed. Thereby it was possible to collect leaf and soil samples for the chemical analysis both from infected and from visually healthy trees. This is consistent with the survey results in Austria where different status of trees – infected and healthy – were observed in the same research

Table 3. Mean nutrient concentrations (mg l[-1]) in soil samples using 1 M HCl extraction in Latvia for *Fraxinus excelsior* in July 2012

Tree status in study sites	Nutrient					
Macronutrients	**N**	**P**	**K**	**Ca**	**Mg**	**S**
Background, healthy * (n=12)	55.75±7.45a	101.50±17.77a	143.75±15.19a	5865.00±1032.86a	980.00±170.38a	21.25±3.71a
Healthy trees** (n=12)	66.00±3.14aa	152.00±23.13ba	201.25±23.31ba	4220.00±1072.21aa	923.75±366.57aa	24.75±0.63aa
Damaged trees** (n=12)	78.25±10.36ba	134.25±27.55aa	180.00±16.71ba	3620.00±1046.14ba	691.25±249.32aa	27.25±1.11ba
Micronutrients	**Fe**	**Mn**	**Zn**	**Cu**	**Mo**	**B**
Background, healthy (n=12)	1180.00±308.23a	208.75±142.15a	3.84±1.02a	2.58±0.28a	0.06±0.01a	0.63±0.19a
Healthy trees (n=12)	901.25±81.86aa	102.50±15.48aa	7.00±1.37ba	2.28±0.31aa	0.04±0.01aa	0.45±0.10aa
Damaged trees (n=12)	1425.00±192.43ab	90.00±25.25aa	7.25±1.09ba	2.75±0.64aa	0.04±0.01aa	0.38±0.14aa

*Barkava, Gulbene, Rundale, Tervete
** Fabriki, Vilaka, Saulaine, Zalenieki
n – number of analysed soil samples. SE – standard error. Means with the different letter for the parameter were significantly different (t-test, $p<0.05$): the first letter relates to the background level, the second – to the healthy trees.

sites (Cech 2008). It should be stressed that single genotypes of ash could be more tolerant against the infections and different stress conditions. Thereby ash will probably survive in the long term, but its population may be significantly reduced over the course of several generations (Bakys et al. 2009, Pliūra et al. 2011).

Chemical analysis

Soil

The results of soil chemical analysis are given in Tables 3 and 4. Although the results showed a relatively high variability in the soil chemical composition for the background level selected in different study sites of *Aegopodiosa* forest type, as well as for healthy and damaged *F. excelsior* in the felled areas during summer 2012, the results of P, K and Zn in the soil samples of the healthy trees in the felled areas on the average were significant higher in comparison to the selected background level.

The main differences for the soil samples of damaged trees to compare with the healthy and background level were connected with higher N, S, Fe, and lower Ca content. Consequently lower Ca resulted in significant lower level of the soil reaction for the damaged trees to compare with the background level. Notably that the found soil reaction for the damaged trees was on the lowest value appropriate for *F. excelsior*. Ash generally grows well on soils with a pH above 5-6 (Zollner & Kölling 1994), or prefers soil above pH 5.5 (Pliūra & Heuertz 2003). Probably, decreased pH level could negatively affect trees in the research sites.

F. excelsior is mesophilic and has relatively high requirements for nutrients (Diekmann 1996, Pliūra & Heuertz 2003, Kerr & Cahalan 2004).

Table 4. Soil reaction and electrical conductivity of the studied *Fraxinus excelsior* soils in Latvia in July 2012

Tree status in study sites	pH_{KCl}	EC mS cm^{-1}
Background, healthy* (n=12)	6.17±0.30a	0.88±0.13a
Healthy trees** (n=12)	5.68±0.60aa	0.88±0.21aa
Damaged trees** (n=12)	5.23±0.73ba	0.88±0.15aa

*Barkava, Gulbene, Rundale, Tervete

** Fabriki, Aizpurve, Saulaine, Zalenieki

n – number of analysed soil samples. SE – standard error. Means with the different letter for the parameter were significantly different (t-test, $p<0.05$): the first letter relates to the background level, the second – to the healthy trees.

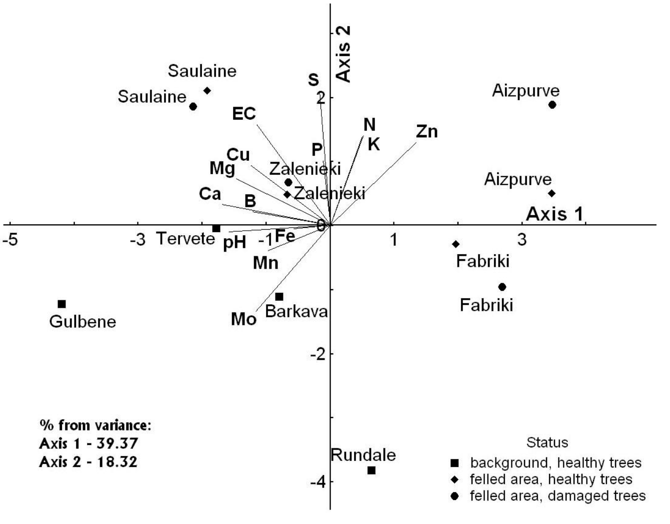

Fig. 2. Distribution of the chemical composition of *Fraxinus excelsior* soils of the studied sites withing the axes of componenet analysis for July 2012.

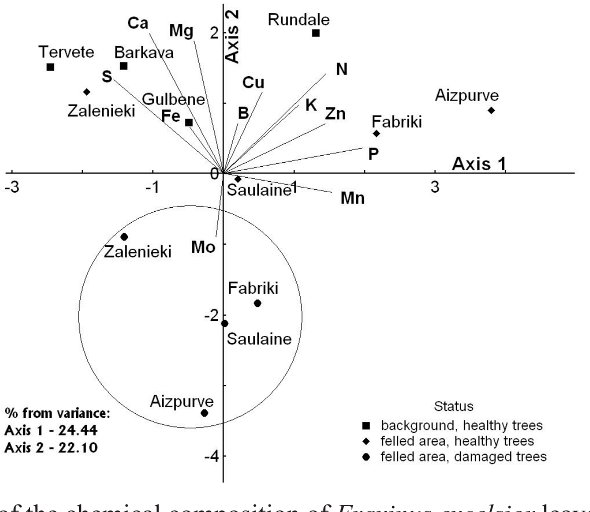

Fig. 3. Distribution of the chemical composition of *Fraxinus excelsior* leaves of the studied sites withing the axes of componenet analysis for July 2012.

Table 5. Mean nutrient concentrations (±SE) in *Fraxinus excelsior* leaf samples in Latvia in July 2012

Tree status in study sites	Nutrient					
Macronutrients (%)	N	P	K	Ca	Mg	S
Background, healthy* (n=12)	2.25±0.14a	0.17±0.01a	0.90±0.12a	1.92±0.11a	0.73±0.05a	0.31±0.07a
Healthy trees** (n=12)	2.12±0.25aa	0.19±0.02aa	0.99±0.10aa	1.59±0.21ba	0.51±0.04ba	0.23±0.06ba
Damaged trees** (n=12)	1.59±0.13bb	0.16±0.01aa	0.84±0.09aa	1.09±0.14bb	0.37±0.01bb	0.17±0.02ba
Micronutrients (mg kg^{-1})	Fe	Mn	Zn	Cu	Mo	B
Background, healthy (n=12)	186.00±86.09a	33.00±4.80a	15.30±1.41a	5.35±0.81a	0.46±0.12a	15.25±0.75a
Healthy trees (n=12)	84.00±15.38 aa	42.80±13.12aa	17.55±1.52aa	7.00±0.41aa	0.48±0.07aa	16.50±0.50aa
Damaged trees (n=12)	96.50±23.61aa	34.35±12.38aa	15.10±1.34aa	4.60±0.36ab	0.55±0.11aa	15.25±1.25aa

*Barkava, Gulbene, Rundale, Tervete
** Fabriki, Aizpurve, Saulaine, Zalenieki
n – number of analysed leaf samples. SE – standard error. Means with the different letter for the parameter were significantly different (t-test, $p<0.05$): the first letter relates to the background level, the second – to the healthy trees.

All the analysed soils could be characterized as sufficient for N, P, K, Ca, Mg, Fe, Mn, Mo, B, a slightly decreased for S, Zn, especially for the background level, as well as Cu.

An important factor affecting plant mineral nutrition is element concentration ratios, specially, Ca and Mg ratio in soil. The most optimal Ca:Mg ratio in soils using 1 M HCl extract for plant nutrition is 5-8:1 (Ринькис & Ноллендорф 1982). The average Ca:Mg ratio in the *F. excelsior* soil in Latvia (background: 6.00; healthy trees in the felled areas: 4.57; damaged trees in the felled areas: 5.23:1) was almost in this range, therefore favourable for Ca and Mg uptake from the soil. There was also a statistically significant correlation ($p<0.05$) between Ca and Mg in the soil.

In total, the PCA results revealed a relatively good structure of the individual sampling sites in the ordination space, where the first two components explained 57.69% of the total variance (Fig. 2). The research sites from the felled areas in the ordination space were mainly grouped by their geographical location showing similar chemical composition (e.g., Fabriki and Aizpurve; Zalenieki and Saulaine) despite the tree health status. The background level sites showed relatively wide dispersion in the ordination space and were located on the negative side of the Axis 2. The relatively most important factors were Ca, S and Zn.

Leaves

In general, the detected concentrations of most of the macronutrients in the background *F. excelsior* leaf samples (Table 5) corresponded well with the mean concentrations reported for *F. excelsior* leaves in different countries: N > 2.00%, P > 0.15%, Ca > 1.50%, Mg > 0.35%, S > 0.23%, but could be characterized as decreased for K (reported >1.2%) (Niinements & Kull 2003, Hagen-Thorn et al. 2004, Hofmeistar et al. 2004, Веретенников 2006). Also for micronutrients like Mn, Zn, Cu, B the stated concentrations in Latvia (Table 5) could be characterized as decreased according to studies by Hagen-Thorn et al. 2005 (Mn > 55, Zn > 19, Cu > 9, and B > 26 mg kg^{-1}). The differences in nutrient accumulation could be due to regional variations, sampling time, soil conditions, e.g. formation of hard soluble compounds in the neutral – slightly acid soil

Table 6. Mean nutrient concentrations (±SE) in *Fraxinus excelsior* leaf samples in Latvia in 2011

Tree status in study sites	**Nutrient**					
Macronutrients (%)	**N**	**P**	**K**	**Ca**	**Mg**	**S**
Healthy trees* (n=9)	1,36±0.04a	0.15±0.01a	1.09±0.13a	1.85±0.21a	0.54±0.05a	0.15±0.04a
Damaged trees* (n=9)	1.04±0.19b	0.14±0.01a	0.74±0.22b	1.40±0.14b	0.45±0.06a	0.11±0.05a
Micronutrients (mg kg^{-1})	**Fe**	**Mn**	**Zn**	**Cu**	**Mo**	**B**
Healthy trees (n=9)	116.67±7.42a	31.67±10.49a	17.47±2.34a	9.33±0.90a	0.43±0.12a	21.67±0.33a
Damaged trees (n=9)	104.67±16.34a	21.67±4.57a	13.53±2.74a	7.47±1.34a	0.43±0.02a	18.00±1.53a

* Zalenieki, Saulaine, Fabriki
n – number of analysed leaf samples. SE – standard error. Means with the different letter for the parameter were significantly different (t-test, $p<0.05$).

reaction, element antagonism, etc. Unfortunately, in the scientific literature there were no research data where content of all 12 plant nutrients in the same study and time were analysed. Therefore the comparison and interpretation of data could be complicated.

The chemical results of *F. excelsior* leaves revealed several important differences for the nutrient accumulation in healthy and damaged tree leaves. In 2012, significant lower concentrations of macronutrients like Ca, Mg and S in the healthy tree leaves if compared to the background level were stated. The results of the damaged tree leave showed significant lower concentrations of N, Ca, Mg and Cu if compared to the healthy trees in summer 2012. It was also stated a slight tendency to accumulate lower concentrations of K, S, Mn, and Zn in the damaged tree leaves to compare with the healthy trees in the felled areas in 2012. Also in 2011, similar tendencies for N, Ca, K (statistically significant) as well as Mg, S, Fe, Mn, Zn, Cu, and B were found (Table 6).

Although Fe concentration in *F. excelsior* leaf samples did not differ significantly between the studied sites, the stated concentrations for the background level could be characterized as optimal (corresponded to the upper value of optimum), while Fe level in the problem sites corresponded to the lower value of the optimum range.

All these findings indicate that *C. fraxinea* development on *F. excelsior* branches seriously disturb transport and accumulation of the most of plant nutrients. Thus reduced nutrient uptake, transport and accumulation could decrease the plant tolerance not only to *C. fraxinea* development, but also to other diseases and facilitating plant decay.

In total, the average levels of K, Zn and B in the studied tree leaves could be characterized as insufficient for normal growth of ash (less than 1.1 % K, 20 mg kg^{-1} Zn and B), as well as P (< 0.20%) in the background and damaged tree leaves, and Cu (< 5 mg kg^{-1}) – in the damaged tree leaves based on the results given by Bergmann (1988) and Hagen-Thorn et al. (2005). The reduced concentrations of Zn, B, P, and Cu could be explained by formation of hard soluble compounds in the neutral – slightly acid soil reaction and also by element antagonism. There were significant negative correlations between Ca, Mg, Cu concentration in the soil and Mn in the leaves, as well as Fe in the soil and Cu in the leaves ($p < 0.05$; $-0.60 < r < -0.77$). Plant available K in soil usually does not form insoluble compounds, therefore the decreased level of it in the leaves could be due to probably insufficient or reduced level in soil, as well as element (Mg:K) antagonism.

The decreased level of any essential nutrient could seriously disturb plant physiological processes. It is well known that B is very important in transport system development in plants, for the formation of primary plant cell walls (Matoh 1997), reproductive and height growth (Dell & Huang 1997, Möttönen et al. 2005); Zn and Cu affect the mechanisms responsible for plant tolerance and resistance to fungus, bacterial diseases; K increases osmotic pressure, water uptake from the soil, etc. (Marschner 1995). The studies have showed that the infections are suggested to primarily take place through the leaves of *F. excelsior* incited by the pathogens' ascospores (Timmermann et al. 2011). Therefore optimal supply with nutrients is very important factor in conditions of infection pressure and unfavourable environmental circumstances.

Ordinary there is no significant relationship between the nutrient content in soil and plant tissues (Roper 1992), also our research showed similar results. The only exception was Ca ($r = 0.61$). The results of *F. excelsior* leaves demonstrated a significant correlation ($p < 0.05$; $0.58 < r < 0.83$) between Ca-Mg (similar to the soil results), as well as N-P, N-Mg, K-Cu, Ca-S concentrations in the tree leaves.

In general, the PCA of the chemical composition of *F. excelsior* leaves demonstrated a relatively good structure of the research sites in the ordination space according to tree health status. The first two components explained 46.54% of the total variance. The leaf samples of the background level and healthy trees from the felled areas were clearly located on the positive side of the Axis 2, but the damaged trees - on the opposite side. The most important factors were concentrations of macronutrients as Ca, P, Mg, N, and S, which also revealed significant differences in the different status of trees.

CONCLUSIONS

The first research results revealed a relatively high variability in the soil chemical composition for the background level, healthy and damaged *F. excelsior*. In general, the results revealed several differences for the accumulation of nutrients in tree leaves: significant lower concentration of Ca, Mg and S in the healthy tree leaves if compared to the background level and significant lower concentrations of N, Ca, Mg and Cu in the damaged tree leaves if compared to the healthy ones, as well as a slight tendency to accumulate lower concentrations of K, S, Mn, and Zn in the damaged tree leaves. In total, the average levels of K, Zn and B in the tree leaves from the all studied sites could be characterized as insufficient for *F. excelsior*, P – decreased in the background and damaged tree leaves, as well as Cu – in the damaged tree leaves.

Although the results of the pilot study revealed several problems of mineral nutrition of *F. excelsior*, further research is necessary to test more precisely the tendencies in nutrient accumulation in both infected and healthy stands to work out measures to improve the tree nutrition and vitality, especially under pressure of *C. fraxinea* infection.

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