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Dying of ash (Fraxinus excelsior L.) in the 'Jesionowe Góry' Nature Reserve

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Abstract. The aim of this study was to analyse changes in the health status of ash stands belonging to the age classes VI and VIII growing in optimal site conditions and to compare the ash dieback with other age classes in the Jesionowe Góry Nature Reserve. Our research was conducted during the growing season in 2006 and 2007 on 22 permanent and 54 temporary sample plots. The health condition of the stands and the structure of natural regeneration were determined with a one-year interval. The amount of damage was defined using leaf loss, pest infestation and shoot dieback. Based on our estimation of natural regeneration, the capability for stand evolution in the future was determined.

Keywords: ash stands, 'Jesionowe Góry' Nature Reserve, dying of ashes

1. Introduction and aim of work

The symptoms of trees' and forest stands of European ash (*Fraxinus excelsior* L.) dieback are observed throughout Poland (literature). The present size of damage leads, in extreme cases, to cease in the production of planting material and elimination of this species in forest inspectorate's aims of silviculture.

Forest stands' durability in reserves, in which ash is an important green woodogenic species, is also problematic.

The dieback processes occur usually after appearance of unfavourable environmental factors. It is caused by the fact that ash is a species sensitive to any changes in humidity and temperature. The lowering of ground water level, weather anomalies, extreme temperatures, and also change in acidity of substrate weaken the forest stands and cause further development of symptoms of the disease. Dieback is particularly intensive during drought. According to Jaworski (2011), the only exception are the ashes growing on soils rich in calcium carbonate. Ashes should demonstrate more resistance in such habitat conditions. Reserve 'Jesionowe Góry' is an example of such environment. It is located on early post-glacial hills rich in calcium carbonate (Czerwiński 1981). Ash forest stands' dieback has been observed in Poland since 1992 (Sierota et al. 1993; Grzywacz 1995; Stocki 2001; Kowalski 2006; Przybył 2002). The disease processes of ashes have intensified in years 1995-1996 in all age classes (Stoicki 2006). Presently, the majority of acreage of natural European ash presence in Europe is threatened (Pautasso et al. 2013). A signalled slow decrease in the area of ash forest stands with symptoms of dieback (Forest Research Institute (FRI) 2014) does not necessarily mean better a health condition of this species. It can be a result of change in the species composition of forest stands in which ash that died back has lost the function of dominant species. Ash forest stands constitute almost half of the area of all dying back deciduous forests (FRI 2014). Initially, due to high sensitivity of ash to abiotic factors (Jaworski 2011), ash dieback was linked with unfavourable influence of inanimate factors (Bakys et al. 2013). However, constantly expanding acreage and intensification of disease process started to indicate on infectious character of this phenomenon. Kowalski's (2007) research in this range, has led to ascertainment that necrosis of ash tissues is strongly connected with the activity of fungi Chalara fraxinea T. Kowalski. A significant reduction in the increment of trees' thickness was noted, 3-5 years before the occurrence of dieback symptoms

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(Cieśla, Gil 2008). Kowalski and Czekaj (2010) noticed that the intensification in disease symptoms is changeable and depends on the age and method of regeneration. Young forest stands are prone to the disease.

Oszako (2006) distinguishes typical disease symptoms connected with the dieback process: leaves atrophy, shoot tip dieback, disruption in fructification, necrotic stains on the bark, cracks in the bark, and root dieback. The observed symptoms of ash dieback show features of multi-factor disease like in case of oaks, birch, poplars, and other endangered species.

The aim of this thesis was the characteristics of health condition of tress on permanent sample plots established in 2006 and 2007. This characteristics were supplemented with the analysis of dieback process progress on a system of randomly chosen sample plots established in the remaining forest stands of examined reserve.

2. Object of research

Reserve 'Jesionowe Góry' (53°20'47"N, 23°17'28"E) of area 376,66 ha was established in 1987 (Ordinance 1987).

Presently, it is located in the area managed by Czarna Bialostocka forest inspectorate. The aim of protection was to preserve a fragment of Knyszyn forest with diversified series of communities on swamp and mixed sites of high level of naturalness and with mature, multi-species old forest. Ash forest stands of older age classes originate probably from natural regeneration, younger, however, originate from artificial regeneration. All ash forest stands grow in optimal environment conditions (Czerwiński 1981). The durability of the old forest was threatened, however, by the process of dieback of main green woodogenic species that are reaching largest sizes among all trees of the reserve. The area of reserve is rolling, covered by oak-hornbeam forest (*Aceri-Tilietum* Faber 1936 = *Tilio-Carpinetum* Tracz 1962) (Reserve Protection Plan 2004).

3. Methodology

In forest stands with significant share of ashes of VI and VIII age class growing on early post-glacial hills, the evaluation of health condition was made twice. For the first time, the research

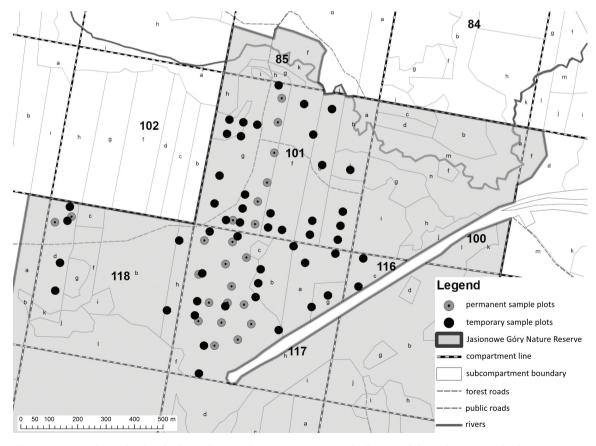


Figure 1. Map of sample plots in the Jesionowe Góry Nature Reserve in Czarna Białostocka Forest District

was conducted in the vegetative season in 2006 (in half of August) and then repeated in the same period in 2007. The spatial scope of the research was limited to trees on 22 circular sample plots (each area of five acres), randomly located in the reserve's ash forest stands of area 20.41 ha (Fig. 1). This part of the sample plots was located on the hills. The joint area of the research amounted 1.1 ha what constituted 5.4% of the examined area. Measured were: 226 water elms, 156 European hornbeams, 79 European ashes (therein 8 dead trees), 21 Norway spruces, 12 Norway maples, 8 small-leaved limes, 5 common oaks, 5 European aspens, and 4 common birches.

The second stage of health condition characteristics was the comparison of ash forest stands growing also on the area of the reserve, but not on the hills. In order to do so, established were 54 circular sample plots on junctions of criss-cross of squares located on area of 56.32 ha of ash forest stands in age classes IIa, IIb, IIIa, VI, and VIII. The acreage of sample plots was adjusted to the age of forest stands (Forest Management Instruction 2003). The total research area amounted 1,275 ha what constituted 2.26% of the examined area. Measured were: 283 water elms, 152 European hornbeams, 133 European ashes, 89 Norway spruces, 62 common birches, 41 Norway maples, 31 common oaks, 28 small-leaved limes, 14 black alders, 11 common hazels, 4 European aspen, and 1 European larch.

All ashes on the sample plots were measured in detail towards the evaluation of: the degree of assimilation apparatus damage, size, discolouration and damage of leaf blade, the degree of shoot dieback, the degree of colonisation by insect secondary invaders, and the visible signs of pathogenic fungi presence.

The defoliation degree was noted with accuracy to 5%. Helpful turned out be tables of oak and beech defoliation degree, developed by Borecki and Keczyński (1992), due to the fact that ash was not included in atlases evaluating the loss in assimilation apparatus. Additionally, photographs of tree crowns with different degrees of defoliation were taken. Then, the photographs were compared with the ones in atlas. This comparison has increased the accuracy of determining the loss in assimilation apparatus in the field.

For evaluation of the degree of damage accepted was European classification: International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forest – ICP (Borecki et al. 1995). The size of leaf blades was estimated on different heights of crown of each ash with the use of binoculars.

Comparative photographs from Hartmann elaboration (1992) were used for determining the discolourations and damage in leaf blades and for determining the degree of shoot dieback.

The intensity of the shoot dieback was expressed in 4-degree scale (Dmyterko 2006). The evaluation of the degree of insect colonisation intensity involved counting holes (feeding ground of ash bark beetle or elm bark beetle) or clusters of sawdust on the bark (feeding grounds of elm bark beetle) from root swelling to the height of around 2m on the whole circumference of the trunk. The evaluation of pathogenic fungi presence was limited to the evaluation of fructifications.

Additionally, also an analysis of the number of natural seeding of all species was made. In 2006, natural seeding was measured on the whole circular plot. In the following year, natural seeding was measured on 2m wide zone, on the north radius of the sample plot (Fig. 2). The evaluation of breeding usefulness of young generation was made on the base of methodology from principles of silviculture (2003).

The complex evaluation of ash damage used three characteristics: the degree of assimilation apparatus reduction, the degree of shoot dieback, and the degree of colonisation by insect secondary invaders. To each of listed aforementioned characteristics assigned were four degrees of changes intensification: 0, 1, 2, and 3 (FRI 1994). Calculated was the average indicator of trees' damage as an average from all trees. The average indicator of damage (*W*) was a base for establishing the degree of damage, which is as follows (FRI 1994): 0 – no damage ($W \le 0.50$), I – weak damage ($0.50 < W \le 1.50$), II – average damage ($1.50 < W \le 2.50$), III – strong damage ($W \ge 2.50$).

In order to determine the significance in difference between two measuring dates used was the t-student's test.

4. Results

4.1. Research on permanent sample plots

In terms of quality evaluation, examined were only living trees. Two samples in 2006 and 2007 included were 71 ashes in each year. The study of assimilation apparatus loss showed that in 2006, the most of the trees had an average degree of crown damage (defoliation 26–60%), while after 1 year, the most trees had a strong degree of damage (defoliation over 60%). The percentage of tress defined as tress with no damage to the assimilation apparatus, has decreased by half in 2007 (Fig. 3). Only one ash, from those examined in

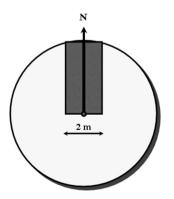
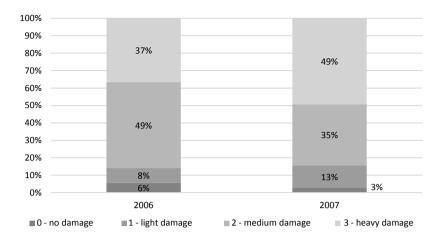
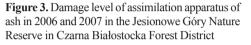


Figure 2. Area for seedling assessment on sample plot





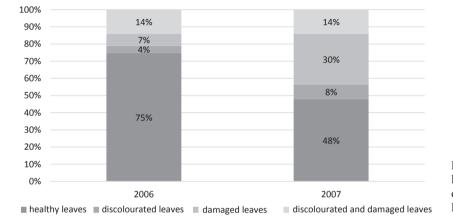


Figure 4. Discolouration and damage level of leaf blades of ash in 2006 and 2007 in the Jesionowe Góry Nature Reserve in Czarna Białostocka Forest District

2006, has improved its crown condition and was transferred to the class of weak damage in 2007.

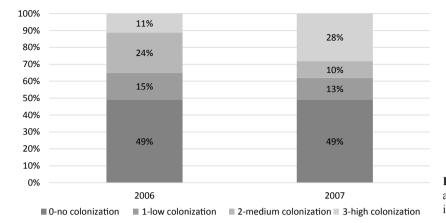
The most common defect of leaves in 2006 was the joint occurrence of discolouration and damages, while in 2007 dominated leaf damage, although the examination was made in the same phenological period. Observed was the decrease in the number of trees with healthy leaves, from 75% in 2006 to 48% in the following year. Trees with healthy leaves were transferred in majority to the group of trees with discoloured leaves, what increased the number of trees in this group from 7% to 30%. The group of trees with damaged leaves has doubled its number and in 2007, it amounted 8%. The number of trees with both discoloured and damaged leaves, remained unchanged and amounted 14% (Fig. 4).

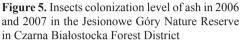
The observations conducted in 2006 indicated that 66% of the ashes did not show reduced leaf blades. The remaining part of ashes had leaves reduced in different degrees. After 1 year, no significant changes were noted. This characteristic can indicate from individual features of a given specimen and is not a good indicator of ashes dieback.

Majority of ashes (99%) showed symptoms of shoot dieback, of which strong dieback was stated in over 60% of the examined ashes in both measuring dates (62% in 2006 and 65% in 2007), average dieback in around 30% (respectively 31% and 28%), and weak dieback in 6% in both measuring dates. No significant statistical changes were stated.

Around half of the examined in both dates, ashes did not show any signs of colonisation by insect secondary invaders (Fig. 5). The share of trees of weak colonisation has decreased by 2%. Ashes colonised in an average degree has decreased its share by 14%. The highest share had 3rd degree – large colonisation. It increased its share by 17%. To this group, transferred were the trees included in 2006 to trees of average colonisation. Only one ash from group of trees of weak colonisation was transferred to the group of large colonisation. In the remaining cases, the transfer to different degrees of colonisation consisted in moving one class up. Observed was the deteriorating state of trees already colonised by insect secondary invaders. The changes were statistically significant.

During the research, observed was only one specimen of macroscopic pathogenic fungi – sulphur polypore (*Laetipo*-





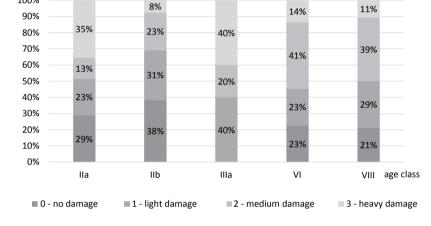


Figure 6. Damage level of assimilation apparatus by age classes of ash in the Jesionowe Góry Nature Reserve in Czarna Białostocka Forest District

rus sulphureus). The specimen was located in oblong crack of the trunk on a height of around 4 m.

4.2. Research on the remaining sample plots

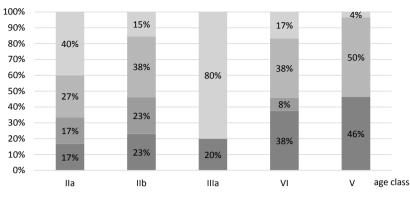
For comparison of dieback intensity in age classes (on temporary plots), examined were 101 living ashes. The biggest share of trees with no damage to the assimilation apparatus and with an average damage showed younger age classes. However, there were some exceptions (Fig. 6). Ashes with the highest degree of assimilation apparatus had the largest share in IIa and IIIa age classes (35% and 40%), whereas the smallest in IIb class – 8%, VI – 14%, and VIII – 11%.

Percentage analysis of share of discolourations and damage to leaf blades in the division to age classes show that the largest share of trees with healthy leaves had older age classes, VI – 38% and VIII – 46%. The share of healthy leaves in the remaining age classes was on similar level (20%). Discoloured leaves occurred in the IIa class with share 17%, IIb class – 23%, and VI age class with 8% share. The most damaged leaves were noted in the VIII class – the half of all the trees in this age, 38% - in VI and IIb classes. The youngest examined forest stands had leaves damaged in 27%. The most tress with discoloured and damaged leaves were observed in the IIIa age class – 80%, in IIa class – 40%, while in IIb class, only 15%. The oldest age classes in this group of tress had smaller share VI – 17%, and VIII only 4% (Fig. 7).

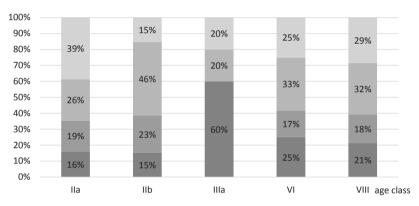
The atrophy of leaf blades was present in over 30% of trees in the IIa, IIb, and VIII age classes, while in the IIa and VI age class, only in around 20%. The reduction of leaf blades in around 30% of the population may indicate from the characteristic of this population of ashes and may not be a symptom of dieback.

An analysis of the degree of shoot dieback showed no symptoms in 60% of tress from the IIIa age class, around 15% in the IIa and IIb age classes. In older age classes, over 20% of trees did not show any shoot dieback. Dieback in small degree, both in younger and older age classes amounted around 20%. The share of trees with the highest degree of shoot dieback increased with age. The only exception was the youngest age class in which, stated was the highest share of trees belonging to this group -39% (Fig. 8).

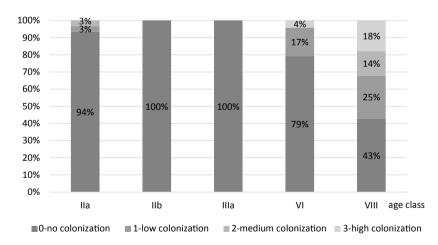
100%



■ healthy leaves ■ discolourated leaves ■ damaged leaves ■ discolourated and damaged leaves



■ 0-no deterioration ■ 1-low deterioration ■ 2-medium deterioration ■ 3-high deterioration



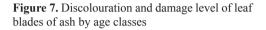


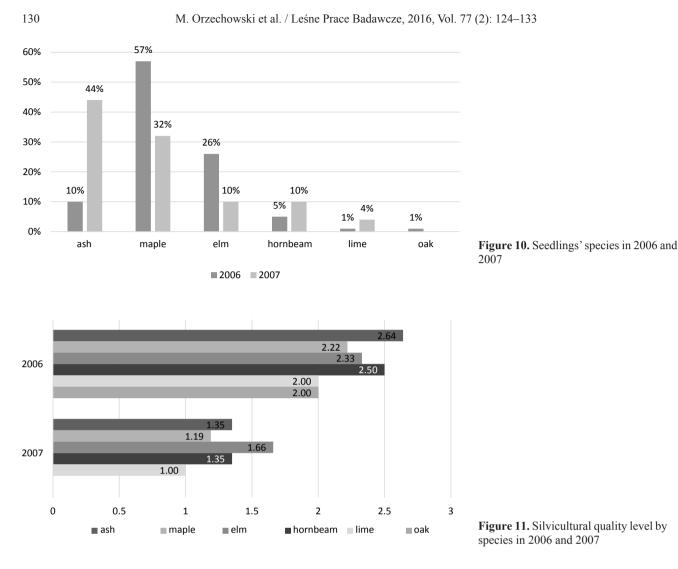
Figure 8. Shoots deterioration level of ash by age calsses

Figure 9. Insects colonization level of ash by age classes

The trees in the youngest age class did not show any symptoms of colonisation by insect secondary invaders. The exception were the trees in the IIa age class, in which stated were trees colonised in weak and average degree. In the older age classes, the share of uncolonised trees has decreased. The oldest forest stands (VIII class) in more than half were colonised in different degrees (Fig. 9).

4.3. Natural seeding

In 2006, during the first measurements, natural seedings covered on average 24% of the sample plots. In the following year, the surface covered with natural seeding has increased to 34%. The highest increase in share was noted in case of natural seeding of ash. It increased from 10% to 44%, horn-



beam – by 5%, and lime – by 4%. The decrease in the number was noted for maple (by 25%) and elm (by 16%). In 2007, no significant share of oak was noted what confirms the difficulties in regeneration of this species on fertile habitats (Fig. 10).

The evaluation of breeding usefulness showed improvement, especially in case of ash (average evaluation from 2.64 decreased to 1.35, what means visibly better quality of natural seeding), maple (from 2.22 to 1.19), elm (from 2.33 to 1.66) and hornbeam (from 2.50 to 1.35). Ash and maple showed the best characteristics when taking into account the percentage share of species and the degree of breeding usefulness. Ash has significantly improved its share and its breeding usefulness has improved. Elm, despite the decrease in its share, has constituted a significant element of natural seeding. Hornbeam and elm were a background for dominating in natural seeding ash and maple (Fig. 11).

On temporary sample plots (established in 2007), the largest cover of natural seeding was noted in the IIb class - 40%. In the IIIa class and in the oldest classes, it amounted

30%. In younger forest stands, ash has regenerated definitely more intensive than other species. Ash has definitely dominated in the IIa, IIb, and IIIa age classes. In the older age classes, predominated different species such as maples, elm, and hornbeam, but still, even here, ash was a species often occurring in natural seeding (Fig. 12).

The worst quality in the IIa class had elm and hornbeam, in the IIb class – maple, elm, and hornbeam. In the IIIa class, occurred only ash of a very good quality and elm. In the older age classes, the natural seeding was characterised with a similar quality condition (Fig. 13).

4.4. Results of complex evaluation

In 2006, the average indicator of damage amounted 1.89 point, whereas in 2007, already 2.01. Both indicators qualify forest stands as damaged on average.

While examining the ash trees from the temporary sample plots, determined was the degree of ash damage as a com-

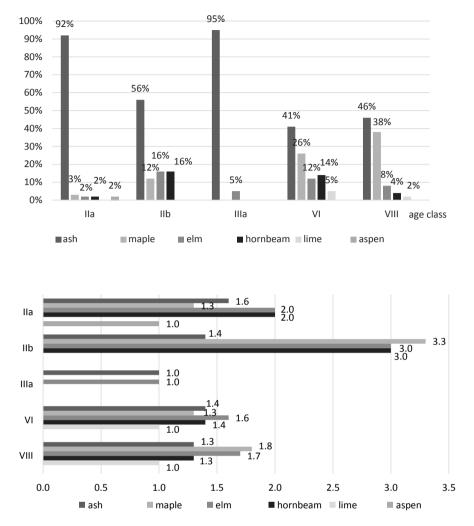


Figure 12. Seedlings' species by age classes of stand

Figure 11. Silvicultural quality level by species and age classes

plex evaluation of the damage degree for particular age classes. The damage indicator for the IIa class amounted 1.17, for the IIb class -0.87, and for the IIIa class -1. Older classes have respectively in the VI class 1.08, and in the VIII class -1.38. All indicators qualify forest stands as damaged in small degrees.

5. Discussion

On the base of the research, it can be stated that the ashes in the reserve show bad health condition and their state keeps getting worse. The trees have shown visible symptoms of dieback.

Cieśla and Gil's (2008) research allow to think that trees with symptoms of dieback do not show any chances for recovery. A confirmation of this hypothesis could have had been a control of increment to thickness (test of so-called increment death) with the use of increment borer, but due to its invasiveness, this method was not used in this research.

The degree of intensification of particular symptoms of ash forest stands' dieback is different for each tree. Resistance to dieback is probably shaped by genotype (Stener 2012).

The low level of natural seeding cover can be an effect of complex structure (two-storied stand) and strong closure what influences on shading of the forest floor, although the health condition of the young generation indicates a possibility of ash regeneration in this position.

Oszako (2006), on the base of oak forest stands, stated that the most important characteristics, showing the pace of dieback are: indicator of trees colonisation by insect secondary invaders, defoliation indicator, and discolouration of leaf blades. Those characteristics are also mentioned in the ash forest stands that undergo the phenomenon of dieback. The research of dieback pace was based only on 1-year period in which the changes in occurrence of feeding traces of insect pests could have been dependent on a number of factors only indirectly connected with ashes' health condition. The influence of the factors controlling the number of examined species cannot be eliminated: intra-population processes or external influence (Oszako 2006). It cannot be ruled out that the evaluation of leaf blades' discolouration in this research was burdened with an error, indicating from the date of examination (August), when phenologically natural discolouration occur.

The evaluation of occurrence of macroscopic fungi fructifications did not allow to observe the intensification in the presence of those organisms. Expectation on fructifications can be very misleading, because majority of the discovered recently fungi suspected of causing dieback of ashes, does not belong to macrofungi (Kowalski 2007). However, in order to examine the level of colonisation by fungi, necessary is laboratory analysis of wood samples. The examination of the inside of ashes' trunk would be very helpful in the evaluation of trees' condition. Useful in this case may be non-destructive methods (in order not to cause any damage in the reserve), such as, examination of the inside of the trunk on the base of sound propagation. Data from such measurements can show fuller image of trees' health condition, show the quality of the inside of the trunk (type of rot and its size). Another method, from the non-invasive ones, that can be applicable at examining ash dieback at young age, is the method using minirhizotron (Sas-Paszt et al. 2007). By using this method, examined can be the processes occurring in roots in natural site of the examined plant. An examination of course of root dieback in case of young ash trees can allow to fully understand the process of dieback.

The evaluation of forest stands of different age classes was aimed for capturing the difference in the degree of dieback of forest stands belonging to different age classes. It can be noticed that the age of forest stand is significant when it comes to the intensity of occurrence of factors proving ashes dieback. The youngest forest stands, as a result of accumulated stress factors and additionally under pressure of species competition, show dieback to a large extent. Forest litter is also treated as a reservoir of infectious material, which increases the risk of young generation's dieback. It should be noticed, however, that in Bakys' et al. (2013) research, no relation between the trees density (higher in the younger forest stands) and development of ash forest stands' disease was stated. The oldest forest stands also dieback to a large extent. These forest stands are forest stands of advanced age and this also can be a cause of their general weakness.

6. Summary and conclusions

Ash forest stands growing in optimal habitat conditions on the area of the reserve showed strong symptoms of this species dieback. Dieback had an intensifying character, and health condition of those forest stands kept getting worse during research period.

Such diagnostic characteristics were subjected to the changes as: increase in defoliation, occurrence of insect secondary invaders, and change in discolouration and damage of leaf blades.

A characteristic feature of this population of ashes is the occurrence in one-third of the specimens, leaf blades with atrophy symptoms. It needs explanation, whether those symptoms have any connection with disease process. Observed was also the differentiation in the intensity of dieback in age subclasses. In the older age classes, noted was a higher intensity in occurrence of features proving the ash dieback process. A large share of trees dying back in the youngest subclass can be caused by strong inter-species or inter-specimen competition.

In the process of ash dieback in all age classes on the area of the reserve, no significant presence of visible symptoms of pathogenic fungi was stated – macrofungi and no cancer occurrence was stated.

It is advisable to conduct the research in order to identify fungi colonising leaves, shoots, and trees' shafts.

The primary cause of ashes' shoots dieback is probably general weakness of the trees' condition as a result of influence of unfavourable abiotic factors. For further weakening of ashes, health condition contribute phyllophagous pests and secondary invaders, such as ash bark beetle and elm bark beetle. One should conduct monitoring of occurrence, examination of the dynamics of the population, and the possibility of reduction in the number of those bark beetles.

Conflict of interest

The authors declare lack of potential conflicts.

Gratitude and sources of funding

The research were conducted within authors' own researches.

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Author's contribution

M.O. – The concept of work, literature review, preparation and revision of the manuscript; W.K. – Review of the literature, the preparation of the manuscript; J.K. – Fieldwork, data analysis and preparation of the manuscript.

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