PROMOCIJAS DARBS
Zinātniskā doktora grāda
zinātnes doktors (Ph.D.) Lauksaimniecības un
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PARASTĀ OŠA *FRAXINUS EXCELSIOR* L. DESTRUKCIJA LATVIJĀ: AUDŽU SUKCESIJA UN OŠA ATJAUNOŠANĀS

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DIEBACK OF COMMON ASH *FRAXINUS EXCELSIOR* L. IN LATVIA: SPECIES SUCCESSION AND ASH REGENERATION

DOCTORAL THESIS
for the doctoral degree
Doctor of Science (Ph.D.)
in Agriculture, Forestry and Fisheries

LATVIJAS VALSTS MEŽZINĀTNES INSTITŪTS “SILAVA”
LATVIAN STATE FOREST RESEARCH INSTITUTE “SILAVA”

LATVIJAS LAUKSAIMNIECĪBAS UNIVERSITĀTE
LATVIA UNIVERSITY OF LIFE SCIENCES AND TECHNOLOGIES

Mg. geogr. **ILZE MATISONE**

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Promocijas darba zinātniskie vadītāji / *Supervisors*:

1. PhD Natālija BURŅEVIČA
2. Dr.biol. Roberts MATISONS
3. Dr.silv. Tālis GAITNIEKS

Promocijas darbs izstrādāts Latvijas Valsts mežzinātnes institūtā “Silava”, doktorantūras studiju ietvaros Latvijas Lauksaimniecības universitātes Meža fakultātē laika periodā no 2014. līdz 2020. gadam. Pētījums izstrādāts AS “Latvijas valsts meži” pētījuma “Ošu mežu destrukcija un atjaunošanās Latvijā”, Meža nozares kompetences centra projekta “Metodes un tehnoloģijas meža kapitāla vērtības palielināšanai” (ERAF, L-KC-11-0004), kā arī ERA-NET SUMFOREST projekta REFORM “Mistrotu mežu apsaimniekošana: risku mazināšana un stabilitātes paaugstināšana” ietvaros.

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1. Dr.silv. Olga MIEZĪTE, Latvijas Lauksaimniecības universitāte / *Latvia University of Life Sciences and Technologies*;
2. Dr.silv. Dagnija LAZDIŅA, Latvijas Valsts mežzinātnes institūts “Silava” / *Latvian State Forest Research Institute “Silava”;*
3. PhD Vaidotas LYGIS, Dabas izpētes centrs, Lietuva / *Nature Research Centre, Lithuania.*

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ANOTĀCIJA

Neskatoties uz to, ka parastais osis aizņem tikai 1–2% no kopējās meža platības Eiropā un 0.3% Latvijā, tā ir ekoloģiski nozīmīga koku suga. Kopš deviņdesmito gadu sākuma Austrumeiropā un desmit gadus vēlāk arī Centrāleiropā un Rietumeiropā novērota strauja oša audžu kalšana. Oša kalšanas cēlonis – invazīvā patogēnā sēne *Hymenoscyphus fraxineus* (vēlāk precizēts zinātniskais nosaukums) – noteikts 2006. gadā. Latvijā intensīva oša audžu kalšana novērota sākot ar 2000. gadu. Darba mērķis bija noskaidrot parastā oša *Fraxinus excelsior* L. audžu kalšanas dinamiku un faktorus, kas ietekmē koku uzņēmību pret patogēnu; novērtēt slimības skarto audžu sukcesiju un atjaunošanās potenciālu.

Oša jaunaudžu platība laikā no 2000. līdz 2015. gadam Latvijā samazinājās 4.4 reizes. Ilglaicīgās inventarizācijas dati liecina, ka šajā laika posmā kopējā oša mirstība bijusi 6.9%, un oša audžu krāja samazinājusies par 171 $m^3 ha^{-1}$. Veikto pētījumu rezultātā secināts, ka oša dabiskā atjaunošanās jaunaudzēs, kā arī pieaugušās audzēs, salīdzinot ar laiku pirms oša kalšanas, ir krasi pasliktinājusies. Vitālās paaugas īpatsvars ir apmēram ⅓, un, kokiem augot, tas turpina samazināties. Notiek oša audžu pārkrūmošanās un oša pakāpeniska nomaiņa ar citām platlapju sugām. Pētījumā noskaidrots, ka uzņēmību pret patogēnu ietekmē ošu un oša audžu morfometriskie parametri, meža tips un meteoroloģiskie faktori.

Lai gan Latvijā oša kalšana novērota visu vecumu un dažādu sastāvu audzēm, noteiktas individuālas un reģionālas atšķirības. Augstāka vitalitāte konstatēta vecākiem/lielākiem kokiem; jauktās audzēs; labi drenētās un sausās minerālaugsnēs; vairākkārt koptās audzēs; Latvijas austrumu daļā.

Darba rezultāti ir izmantojami oša audžu apsaimniekošanas plānošanā, esošo oša ģenētisko resursu mežaudžu novērtēšanā un jaunu mežaudžu izveidošanā, kā arī pamatinformācijai turpmākajiem pētījumiem.

Šis promocijas darbs sastāv no tematiski vienotām deviņām zinātniskām publikācijām.

ABSTRACT

The area of common ash forest is only 1–2% of the total forest area in Europe and 0.3% in Latvia. Prior to dieback, ash was an economically and still is ecologically highly important tree species. In Eastern Europe since the beginning of the 1990s, and a decade later throughout Central and Western Europe, rapid dieback of ash stands caused by an unknown reason was observed. In 2006 the causal agent of ash dieback – an anamorphic fungus *Hymenoscyphus fraxineus* (species name later refined) was determined. In Latvia, intensive dieback of ash stands has been observed since 2000. The aim of the thesis was to assess the dieback dynamics of common ash *Fraxinus excelsior* L. stands and determine the main factors affecting susceptibility of the trees, as well as future succession and potential regeneration of the affected common ash stands in Latvia.

In Latvia, the area of young ash stands decreased by 4.4 times during 2000–2015. The ten-year-inventory (2005–2015) revealed that the overall mortality of ash was 6.9% and standing volume of ash stands decreased by 171 m^3 ha^{-1}. The obtained results indicated that regeneration of ash saplings in ash young stands as well as in mature stands was lower than in in the pre-dieback times, but regeneration was still successful. Furthermore, survival and health condition of ash saplings was relatively poor, as only ca. ⅓ of individuals were healthy, and the health condition decreased with aging. Overgrowth of ash stands with shrub species has begun, suggesting formation of broadleaved stands with a small admixture of ash in the future. We found that, susceptibility to ash dieback is significantly affected by morphometric parameters of ash trees and stands, site conditions and meteorological factors.

In Latvia, ash dieback was present in ash stands of all ages and composition, yet some individual and regional differences were observed. Lower susceptibility to ash dieback was observed for older/larger dimensions trees, mixed stands on well-drained and dry mineral soils, stands thinned more intensively, and in the eastern part of Latvia.

The findings of the thesis are applicable for the planning of ash stand management, assessment of the existing situation, for the designation of additional genetic resource forests, as well as provide basic information for future studies.

SATURS / *CONTENTS*

PUBLIKĀCIJU SARAKSTS / *LIST OF PUBLICATIONS*

Promocijas darba rezultāti ir apkopoti deviņās publikācijās, uz kurām atsauces tekstā veidotas, izmantojot romiešu ciparus:

The results of thesis are compiled in nine publications, referred to by Roman numerals in the text:

I Laiviņš M., Priede A., **Pušpure*I.**[1](2016) Spread of *Hymenoscyphus fraxineus* in Latvia: analysis based on dynamics of young ash stands. Proceedings of the Latvian Academy of Science. Section B Natural Exact and Applied Sciences, 70(3), 124–130. 10.1515/prolas-2016-0020

II **Matisone I.**, Matisons R., Laiviņš M., Gaitnieks T. (2018) Statistics of ash dieback in Latvia. Silva Fennica, 52(1), article id 9901. 10.14214/sf.9901

III **Pušpure*I.**, Matisons R., Kenigsvalde K., Gaitnieks T., Burņeviča N. (2018) Seasonal development of lesions caused by *Hymenoscyphus fraxineus* on young *Fraxinus excelsior* trees in Latvia. IForest, 11(1), 17–23. 10.3832/ifor2283-010

IV **Pušpure*I.**, Gerra-Inohosa L., Matisons R., Laiviņš M. (2017) Tree-ring width of European ash differing by crown condition and its relationship with climatic factors in Latvia. Baltic Forestry, 23(1), 244–252.

V Rungis D., Korica A., Gailite A., **Puspure*I.**, Veinberga I. (2016) Analysis of the Genetic Diversity and Population Structure of Latvian Ash (*Fraxinus excelsior* L.) Stands using Nuclear and Chloroplast SSR Markers. Proceedings of the Latvian Academy of Sciences, Section B: Natural, Exact, and Applied Sciences, 70(3), 101–108. 10.1515/prolas-2016-0017

VI **Pušpure*I.**, Laiviņš M., Matisons R., Gaitnieks T. (2016) Understory changes in *Fraxinus excelsior* stands in response to dieback in Latvia. Proceedings of the Latvian Academy of Science. Section B Natural Exact and Applied Sciences, 70(3), 131–137. 10.1515/prolas-2016-0021

VII **Pušpure*I.**, Gerra-Inohosa L., Arhipova N. (2015) Quality assessment of European ash *Fraxinus excelsior* L. genetic resource forests in Latvia. Research for Rural Development, 2, 37–43.

VIII **Pušpure*I.**, Matisons R., Laiviņš M., Gaitnieks T., Jansons J. (2017) Natural regeneration of common ash in young stands in Latvia. Baltic Forestry, 23(1), 209–217.

IX **Matisone I.**, Matisons R., Jansons A. (2019) Health condition of European ash in young stands of diverse composition. Baltic Forestry, 25(1), 59–62.

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Autoru ieguldījums publikācijās / *The contribution of the authors*

	Ideja / *Original idea*	Pētījuma plāns / *Study design*	Datu ievākšana / *Data collection*	Datu analīze / *Data analysis*	Manuskripta sagatavošana / *Manuscript preparation*
I	ML	ML	ML	ML, AP	ML, AP, **IM***
II	IM, TG	IM, RM	IM, ML	IM, RM	IM, RM
III	**IM***, TG	**IM***, RM	**IM***	**IM***, KK, RM	**IM***, RM, NB
IV	RM, ML	RM	**IM***, RM, LG-I	**IM***,RM	**IM***,RM
V	DR	DR	**IM***, AK, AG, IV	AK, AG, IV	DR, AK, AG, IV, **IM***
VI	ML, TG	**IM***	**IM***, ML	**IM***, RM	**IM***,RM
VII	**IM***	**IM***	**IM***, LG-I	**IM***	**IM***, NB**
VIII	**IM***, ML, JJ	**IM***, TG	**IM***	**IM***, RM	**IM***, RM
IX	RM, ĀJ	IM, RM	IM	IM, RM	IM, RM

AG – Agnese Gailīte, ĀJ – Āris Jansons, AK – Anna Korica, AP – Agnese Priede, DR – Dainis Ruņģis, **IM – Ilze Matisone**, IV – Ilze Veinberga, JJ – Jurģis Jansons, KK – Kristīne Kenigsvalde, LG-I – Linda Gerra-Inohosa, ML – Māris Laiviņš, NB – Natālija Burņeviča, RM – Roberts Matisons, TG – Tālis Gaitnieks.

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SAĪSINĀJUMI / *ABBREVIATIONS*

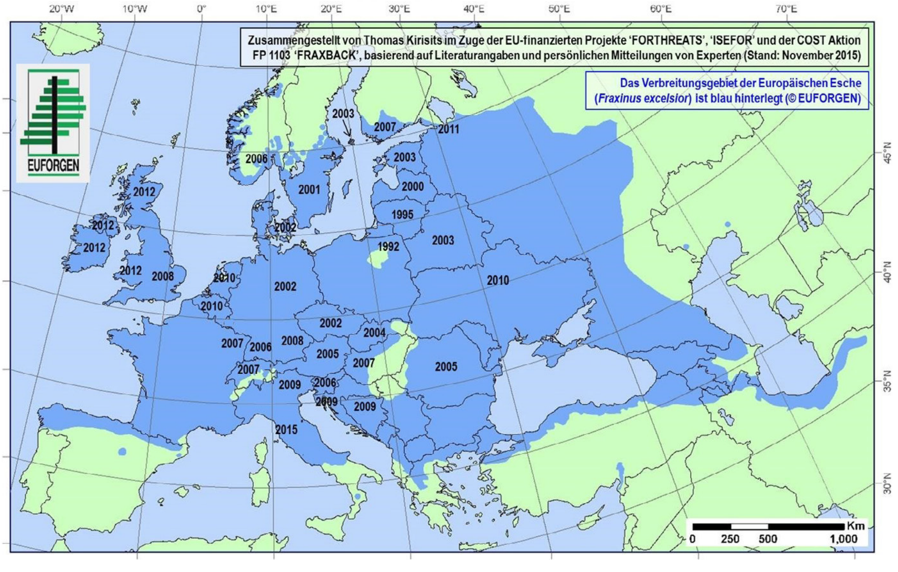
ADB	– oša audžu kalšana / *ash dieback*
PSP	– ilglaicīgs parauglaukums / *permanent sampling plot*
DBH	– stumbra diametrs krūšu augstumā / *stem diameter at breast height*
H	– koka augstums / *tree height*
UG	– *undergrowth*
AG	– *advance growth*
GRF	– ģenētisko resursu mežs / *genetic resource forest*
TRW	– koka gadskārtu platums / *tree-ring with*
FI	– meža inventarizācija / *forest inventory*
EL	– eksistējošs bojājums / *existing lesion*
NL	– jauns bojājums / *new lesion*

1. DARBA VISPĀRĪGS RAKSTUROJUMS

1.1. Parastā oša vispārīgs raksturojums

Parastais osis *Fraxinus excelsior* L. (Oleaceae) ir viena no četrām vietējām ošu sugām Eiropā (Thomas, 2016), kas sastopams 64% Eiropas teritorijas (Grime et al., 2007). Oša dabiskais izplatības areāls plešas no Fenoskandijas dienvidu daļas līdz Grieķijai un no Spānijas ziemeļu daļas līdz Volgas upei Krievijas rietumos (Thomas, 2016; 1.1. att.). Tas lielā mērā sakrīt ar parastā ozola *Quercus robur* L. izplatības areālu, kas ir raksturīga suga Eiropas nemorālo mežu zonai (Dobrowolska et al., 2011). Oši kalnu rajonos (Pireneji, Alpi) sasniedz 2200 m v.j.l. augstuma robežu (Beck et al., 2016). Eiropā parastais osis ir sastopams plašās teritorijās dažādu vecumgrupu primārajos un sekundārajos mežos. Tipiskas augtenes ir platlapju meži, ieleju meži, upju krasti un pļavas. Prastais osis Eiropas valstīs aizņem 1–2% no kopējās mežaudžu platības vai koksnes krājas (Fischer & Lorenz, 2011; Rozsypálek et al., 2017). Oša mežaudžu platība tā ziemeļu izplatības areāla tuvumā ir daudz mazāka nekā Centrāleiropā un Rietumeiropā (Cleary et al., 2017; Drenkhan et al., 2017; Heinze et al., 2017).

Pirms oša kalšanas Zviedrijā (Dobrowolska et al., 2011) un Apvienotajā Karalistē (Wardle, 1961), osis tika pieskaitīts pioniersugām, bet Dānijā uzskatīts kā starpposms starp mežaudzes pionierstadiju un klimaksa stadiju (Ahlberg, 2014).



1.1. att. **Parastā oša *Fraxinus excelsior* dabiskās izplatības areāls (koši zils)**
Gadi norāda patogēna *Hymenoscyphus fraxineus* izplatību Eiropā (BFW, 2020).

Centrāleiropā un Ziemeļeiropā osi pielīdzināja invazīvajām sugām (Wagner, 1990), un tika lietots termins “*fraxinisation*” (Fraxigen, 2005).

Parastais osis ir ekoloģiski plastiska suga, kas aug dažādās augsnēs (izņemot skābas) un dažādos augšanas apstākļos (Wardle, 1961). Dabiskā izplatības areāla austrumu daļā oši dominē palieņu mežos un mitrās zemienēs māla-smilšmāla augsnēs (Dufour & Piegay, 2008) vai gravu mežos un samērā sausās vietās akmeņainās kalnu nogāzēs (Weber-Blaschke et al., 2008). Tādēļ vēsturiski izdalīti divi oša ekotipi: tā sauktais “ūdens osis”, kas piemērojies mitriem augšanas apstākļiem, un “kaļķa osis” kas pielāgojies sausām, kaļķainām vietām (Münch & Dietrich, 1925). Eiropā parastais osis lielākoties dominē vietās, kas ir mazāk piemērotas dižskābarža *Fagus* spp., ozola *Quercus* spp. un zināmā mērā arī alkšņu *Alnus* spp. augšanai (Jahn, 1991; Ellenberg, 1996). Lielākoties osis ir sastopams mežaudžu piemistrojumā (kopā ar dažādām platlapu sugām); tīraudzes veido reti (Dobrowolska et al., 2011; Pautasso et al., 2013).

Parastā oša populācijai Eiropā raksturīgs zems polimorfisma līmenis: kopumā no 201 oša populācijas izdalīti 12 hloroplastu haplotipi, bet, analizējot 62 populācijas, konstatēti divi kodola haplotipi (Heuertz et al., 2004a). Salīdzinājumam – Eiropas platlapju koku sugu izpētē 22 populācijās atklāti 15 haplotipi, ieskaitot septiņus haplotipus no 24 oša populācijām (Petit et al., 2003). Pēcleduslaikmeta veģetācijas rekolonizācijas rezultātā ir radušās ģenētiski atšķirīgas parastā oša līnijas, uz ko norāda mātes pārmantotie hloroplastu haplotipi, kuru izplatība ir heterogēna (Heuertz et al., 2004a). Augstāka haplotipu daudzveidība konstatēta Centrāleiropā un Dienvideiropā, turpretī Ziemeļeiropā un Austrumeiropā dominē viens haplotips (H01) (Tollefsrud et al., 2016), norādot uz ģenētiskās daudzveidības ģeogrāfiskām atšķirībām. Rietumeiropā un Centrāleiropā starp populācijām novērota samērā augsta homogenitāte, alēļu bagātība un ģenētiskā daudzveidība (Heuertz et al., 2004b), turpretī oša ziemeļu izplatības areāla tuvumā novērota populāciju fragmentācija un zema alēļu daudzveidība (Heuertz et al., 2004a; Tollefsrud et al., 2016; Børja et al., 2017; Cleary et al., 2017). Osi apputeksnē vējš, tādēļ populāciju fragmentācijas samazināšanu ziemeļu izplatības areāla tuvumā varētu veicināt ziedputekšņu plūsma starp populācijām (Bacles et al., 2005).

Osis pirms kalšanas bija ekonomiski nozīmīga koku suga (Dobrowolska et al., 2011; Pratt, 2017; Enderle et al., 2019 un atsauces tajā). Osis ir ātraudzīgs, ar izturīgu un elastīgu koksni, kas piemērota augstas kvalitātes kokmateriālu ražošanai, piemēram, mēbelēm, finierim, grīdas segumam, sporta aprīkojumam un citiem mērķiem (Fraxigen, 2005; Enderle et al., 2017; Rozsypálek et al., 2017). Vairākās Eiropas valstīs osim ir liela ainaviska nozīme, tas ir galvenā sastāvdaļa dārzu, parku un dzīvžogu veidošanā (Pautasso et al., 2013; McCracken et al., 2017). Tāpat osis ir ekoloģiski nozīmīga suga, kas nodrošina augstu bioloģisko daudzveidību, īpaši ar to saistītām sugām (Lõhmus & Runnel, 2014; Mitchell et al., 2014). Piemēram, Lielbritānijā 953 sugas ir cieši saistītas ar parasto osi, bet 44 no tām ir pilnībā atkarīgas no oša (Mitchell et al., 2014).

Latvijā osis atrodas tuvu tā ziemeļu izplatības areāla robežai, kas nosaka sugas augsto jutīgumu pret apkārtējās vides faktoriem un saistās ar audžu izplatības nevienmērīgumu. Oša audzes ir sastopamas visā valstī, taču galvenokārt tās koncentrējas Latvijas centrālajā un rietumu daļā (līdz 10.9% no kopējās mežaudžu platības), kur augsnes ir auglīgākas un klimats maigāks. Vismazākais oša audžu īpatsvars ir kontinentālajā Latvijas austrumu daļā, kur ziemas ir skarbākas un pavasaros bieži novērojamas vēlas salnas (Nikodemus et al., 2009; Kenigsvalde et al., 2010). Latvijā osis lielākoties aug jauktos mežos eitrofās augsnēs (97% no kopējās audžu platības) mistrojumā ar citām sugām (piem., apsi *Populus* spp., bērzu *Betula* spp., alksni u.c.) vai parasto egli *Picea abies* (L.) H. Karst., tīraudzes veido reti.

1.2. Parastā oša audžu kalšana

Pirmās defoliācijas pazīmes un vainagu augšējo zaru kalšana novērota Polijas ziemeļaustrumos 1990-to gadu sākumā (Przybył, 2002). Oša kalšanas (ADB) apjomi tiek aprēķināti, izmantojot dažādus lielumus: nokaltušo koku skaitu, ikgadējo mirstības koeficientu, oša mežu platību samazināšanos, nokaltušās koksnes krāju u.c. (Chira et al., 2017; Pliūra et al., 2017; Timmermann et al., 2017). Līdz ar to nav precīzi salīdzināmu datu par oša mežaudžu samazināšanos Eiropā; viens no viennozīmīgajiem ADB raksturlielumiem ir koku mirstība jeb mirušo koku īpatsvars konkrētā laika posmā (Coker et al., 2019), kas izmantota daudzos pētījumos. Kopējā oša mirstība Eiropā svārstās no 6.9 līdz 10.1% gadā (Lenz et al., 2016; Matisone et al., 2018), bet maksimālā stādīto, kā arī dabiski atjaunoto mežaudžu mirstība pārsniedz 70% (Coker et al., 2019). Diemžēl sadrumstalotie publicētie dati par oša mežaudžu stāvokli neļauj izdarīt visaptverošus secinājumus par ADB apjomiem Eiropā, tāpēc standartizēts pārskats būtu ļoti noderīgs.

Tālākā ADB izplatība bija strauja, dažos gados sasniedzot kaimiņos esošo Lietuvu un Latviju, tomēr oficiāli ADB Latvijā apstiprināta tikai 2000. gadā (Timmermann et al., 2011). Vēlāk tā izplatījās visā Centrāleiropā un Ziemeļeiropā; 2012. gadā (iespējams, ar inficētu stādmateriālu) sasniedzot ģeogrāfiski izolēto Lielbritāniju (Orton et al., 2018; 1.1. att.). Detālu hronoloģisko sarakstu ar ADB pirmajiem novērojumiem Eiropas valstīs izstrādāja McKinney et al. (2014).

Vislielākā oša mežaudžu platība Latvijā bija 1998. gadā, sasniedzot 21905.3 ha jeb 0.8% no kopējās mežaudžu platības (Laiviņš et al., 2016), taču, sākot ar 2000. gadu, tā strauji samazinājās (Liepiņš, 2003). Šobrīd oša mežaudžu platība ir tikai 9354 ha (0.3% no kopējās mežaudžu platības), no kuras 5% (470 ha) ir jaunaudzes (≤40 gadi) (Valsts meža dienests, 2019).

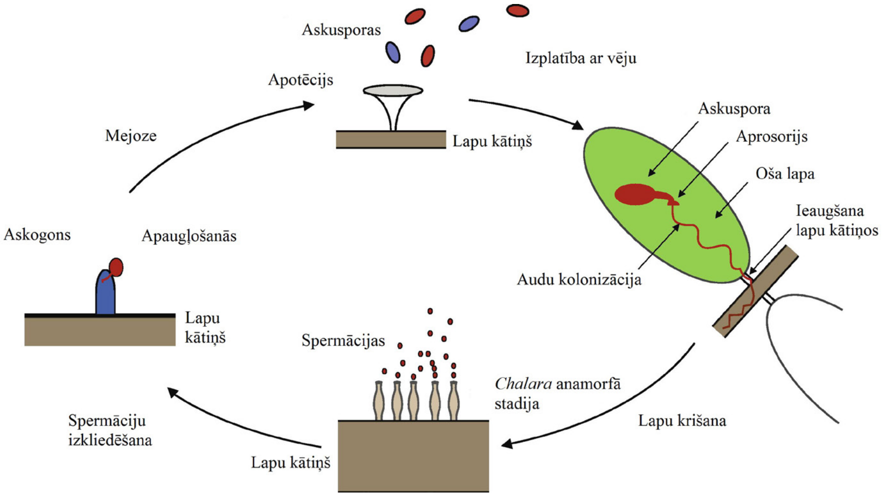
1.3. Parastā oša kalšanas izraisītāji un inficēšanas mehānismi

Balstoties uz pirmajiem novērojumiem, sākotnēji uzskatīja, ka ADB izraisa sals vai sausuma radītais stress (Pukacki & Przybyl, 2005). Bojātajiem kokiem raksturīgi simptomi: nekrotiski plankumi uz lapām vai lapu kātiņiem (Skovsgaard et al., 2010), brūni plankumi uz pumpuriem (Bengtsson et al., 2014), kā arī lapu un galotņu vīšana

(Schumacher et al., 2009). Šiem simptomiem seko nekrotisku bojājumu veidošanās, kas no lapām izplatās uz dzinumiem, zariem un stumbriem, kā rezultātā bojātās koka daļas nokalst (Bakys et al., 2009a; Skovsgaard et al., 2010; Bengtsson et al., 2014). Zaru kalšana veicina ūdenszaru augšanu, līdz ar to – krūmveida vainagu veidošanos (Gross et al., 2014). Ilgāki simptomu novērojumi rosināja izvērtēt arī citus oša kalšanas cēloņus, piemēram, patogēnu izraisītu slimību.

Oša kalšanas cēloņa noteikšana ilga vairākus gadus (Przybył, 2002). 2006. gadā T. Kowalski no inficētiem parastā oša dzinumiem, zariem un stumbriem izolēja un aprakstīja anamorfu sēni *Chalara fraxinea* T. Kowalski, kas tika uzskatīta par ADB izraisītāju (Kowalski, 2006). Sēnes patogenitāti vēlāk savos pētījumos apstiprināja Kowalski & Holdenrieder (2009a) un Bakys et al. (2009a,b). 2009. gadā Kowalski & Holdenrieder (2009b) atklāja ka *C. fraxinea* teleomorfā stadija ir *Hymenoscyphus albidus* (Gillet) W. Phillips, kas agrāk Eiropā bija zināms kā nobiru saprofīts. Queloz et al. (2011) pierādīja, ka ir divas morfoloģiski praktiski identiskas, bet ģenētiski atšķirīgas *Hymenoscyphus* sugas: *H. albidus*, kas bija pazīstama jau iepriekš, un jauna suga – *H. pseudoalbidus* V. Queloz, C.R. Grünig, R. Berndt, T. Kowalski, T.N. Sieber & O. Holdenrieder, kas izraisa ADB. Gadu vēlāk noskaidrots, ka *H. pseudoalbidus* Eiropā ticis introducēts no Āzijas (Zhao et al., 2012). Vēl trīs gadus vēlāk Baral et al. (2014) precizēja sēnes nosaukumu pēc pasaulē pieņemtās nomenklatūras, nomainot to uz *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya ar anamorfās stadijas nosaukumu *Chalara fraxinea* un taksonomisko sinonīmu *Hymenoscyphus pseudoalbidus*.

Balstoties uz literatūras datiem, Gross et al. (2012, 2014) aprakstīja *H.* fraxineus dzīves ciklu (1.2. att.). Viss dzīves cikls ilgst vienu gadu. Askusporas veidojas lapu nobirās no apotēcijiem, kas vasarā veidojas uz iepriekšējā gada nokritušo ošu lapu



1.2. att. ***Hymenoscyphus fraxineus*** **dzīves cikla shematisks attēlojums (pēc Gross et al., 2012)**

Sarkanā un zilā sporu un micēlija krāsa attēlo divus *H. fraxineus* pārošanās veidus.

kātiņiem. Askusporas izplatās ar vēju. Saimniekaugā tās, izmantojot aprosorijas, iekļūst caur lapām. Iekļūstot lapā, sēne izplatās lapu kātiņā (viens lapu kātiņš var būt kolonizēts ar vairākiem *H. fraxineus* genotipiem). Pēc lapu nobiršanas uz lapu kātiņiem veidojas sēnes anamorfā stadija, kura izdala spermācijas. Apaugļošanās notiek caur konīdijām, kas rudenī, kad temperatūra pazeminās, lielā skaitā veidojas uz nobirušo lapu kātiņiem. Jaunie apotēciji uz ošu lapu kātiņiem veidojas nākamās augšanas sezonas vasarā, uzsākot jaunu infekcijas ciklu.

Galvenais slimības izplatītājs ir vējš, kas pārnes askusporas no lapu nobirām uz veselām ošu lapām. Aprēķināts, ka slimību ierosinošo askusporu izplatīšanās ātrums Eiropā ir no 30 līdz 70 km gadā (Luchi et al., 2012; Solheim et al., 2012; Børja et al., 2017).

Patogēns *H. fraxineus* tiek uzskatīts par primāro ADB ierosinātāju, bet slimības novājināto koku bojāeju bieži izraisa sekundārie patogēni, piemēram, celmenes *Armillaria* spp. (Skovsgaard et al., 2010; Bakys et al., 2011; Enderle et al., 2013; Chandelier et al., 2016). Celmenes strauji kolonizē koksni pie sakņu kakla *H. fraxineus* bojājumu vietās (Husson et al., 2012), tāpēc inficētiem ošiem bieži tiek novērota sakņu trupe. Latvijā 2007. gadā ADB izraisošo *H. fraxineus* klātbūtni laboratoriski apstiprināja Austrijas zinātnieks T. Kirisits (Kenigsvalde et al., 2010).

1.4. Parastā oša uzņēmība un rezistence pret *Hymenoscyphus fraxineus*

Ne visiem kokiem, pat augot vietās ar augstu infekcijas fonu, tiek konstatēti slimības simptomi, kas liecina par dabisko noturību pret patogēnu un/vai īpašiem ekoloģiskiem faktoriem, kas ierobežo patogēnu (Stener, 2013; Enderle et al., 2015). Tāpēc rezistence un metodes tās uzlabošanai ir visbiežāk pētītie ADB aspekti (Kjaer et al., 2012; McKinney et al., 2014). Lielākajā daļā pētījumu secināts, ka apmēram 1% no kokiem varētu būt ar paaugstinātu rezistenci (McKinney et al., 2011a; Pliūra et al., 2011; Kjaer et al., 2012; Lobo et al., 2014; Muñoz et al., 2016; Stener, 2018). Tomēr Pliūra et al. (2015) norāda, ka nevienai no pārbaudītajām oša provenieņcēm vai oša pēcnācējiem nav bijusi pilnīga rezistence pret patogēnu. McKinney et al. (2014) secinājusi, ka slimība Eiropā ir pārāk īsu laiku, lai attīstītos dabiskā rezistence pret *H. fraxineus*, kamēr Carlsson-Graner & Thrall (2015) norāda uz risku, ka patogēna evolūcija varētu būt straujāka nekā saimniekaugam. Neskatoties uz to, provenienču eksperimentu un oša selekciju programmu nozīme Eiropā joprojām palielinās (Pliūra et al., 2011; Enderle et al., 2015; Havrdová et al., 2016). Rezistence pret ADB uzrādīja viduvēju iedzimstamību Eiropā (h^2 = 0.20–0.40), norādot uz ierobežotu selekcijas potenciālu nākamo oša paaudžu veselības stāvokļa uzlabošanā (Pliūra et al., 2011; Kjaer et al., 2012; Stener, 2013; Lobo et al., 2014; McKinney et al., 2014; Enderle et al., 2015; Muñoz et al., 2016; Stener, 2018). Vairākos *in vitro* pētījumos ir novērota dažu sēņu endofītu antagonistiska aktivitāte pret *H. fraxineus* (Schlegel et al., 2016; Haňáčková et al., 2017; Kosawang et al., 2018). Tomēr pozitīva endofītisko mikrobiomu ietekme uz oša jutīgumu pret ADB *in situ* nav novērota (Haňáčková et al., 2017; Hietala et al., 2018; Schlegel et al., 2018).

Vairākos pētījumos ir analizēti faktori, kas ietekmē infekcijas pakāpi audzē, piemēram, infekcijas klātbūtnes laiks, oša vecums un dimensijas, augšanas apstākļi, audzes sastāvs u.c. Pētījumu rezultāti lielākoties ir vienprātīgi, taču ir norādītas dažas reģionālās atšķirības. Lielākajā daļā pētījumu konstatēts, ka jaunāko (Enderle et al., 2017; Rozsypálek et al., 2017; Enderle et al., 2018) un mazāko koku (Bengtsson & Senström, 2017; Cleary et al., 2017; Marçais et al., 2017; Pliūra et al., 2017; Enderle et al., 2018) vitalitāte ir zemāka. Tas, iespējams, skaidrojams ar palielinātu *H. fraxineus* askosporu daudzumu nobirās, kas mazākos/zemākos kokus var vieglāk inficēt (Timmermann et al., 2011; Hietala et al., 2013; Chandelier et al., 2014). Papildus tam, Timmermann et al. (2017), secināja, ka atsevišķa nekroze uz stumbra mazam kokam var izraisīt galotnes vai par visa koka ātru nokalšanu. Turklāt paaugas oši ir pakļauti lielākai konkurencei ar pārējo veģetāciju, kas tos var novājināt (Cech, 2008). Vecākos kokos slimība attīstās lēnāk (McKinney et al., 2011a; Lenz et al., 2016), kas saistāms ar lielāku lapotni un līdz ar to pakāpenisku vainaga kalšanu (Timmermann et al., 2017).

Oša kalšana notiek visos biotopos un augšanas apstākļos, tomēr konstatēts, ka mitrākās vietās mirstība ir augstāka (Ghelardini et al., 2017; Heinze et al., 2017; Timmermann et al., 2017). Nosusināšanai var būt pozitīva ietekme uz oša vitalitāti, jo pārāk mitrās vietās koki var būt mazāk izturīgi pret slimībām (Dobrowolska et al., 2011; Schumacher, 2011; Ahlberg, 2014). Arī stress, ko rada konkurence ar citām sugām, it īpaši dabiskajai atjaunošanai plānotajiem jauniem kokiem, novājina ošus un palielina to uzņēmību pret *H. fraxineus* (Keer, 1998; Givnish, 2002; Royo & Carson, 2006; Skovsgaard et al., 2010).

Ošu vitalitāte un nokaltušo koku apjomi starp audzēm atšķiras, retāk sastopamās oša tīraudzes slimo visspēcīgāk (Dobrowolska et al., 2011), kas, iespējams, saistāms ar augstu patogēna un saimniekaugu blīvumu. No slimības mazāk cieš jauktas audzes (Schumacher, 2011; Stener, 2013), kurās ķīmisko, fizisko un bioloģisko barjeru klātbūtne ierobežo primāro un sekundāro patogēnu izplatīšanos (Loreau et al., 2001; Jactel et al., 2005; Pautasso et al., 2005; Kosawang et al., 2018). Marçais et al. (2017) un Coker et al. (2019) aprakstījuši infekcijas cikliskumu, kad 6–8 gadus pēc spēcīgas infekcijas, strauji samazinoties oša biezumam audzē, to mirstības rādītāji uzlabojas (Enderle et al., 2018). Neskatoties uz to, detāli pētījumi par patogēna izplatīšanos norāda, ka atlikušajos dzīvajos kokos slimība lēnām progresē (Bengtsson et al., 2014).

Pieaugot slimības apmēriem, infekcijas izplatības samazināšanai izmēģinātas dažādas apsaimniekošanas metodes. Taču, piemēram, bojāto zaru apgriešana (Marciulyniene et al., 2017), bojāto koku sadedzināšana un celmu apstrāde ar herbicīdiem (McCracken et al., 2017) infekcijas izplatību neierobežoja. Tomēr joprojām turpinās diskusijas par atsevišķu mežkopības aktivitāšu lietderīgumu, piemēram, kopšanu, kuras ietekmē var mainīties mikroklimats un infekcijas apstākļi, tādējādi samazinot slimības ietekmi (Bakys et al., 2013; Ahlberg, 2014; Cleary et al., 2017). Dānijā vienvecuma jaunaudzēs vissliktākā oša vitalitāte konstatēta nekoptos parauglaukumos, taču tā nebija saistīta ar audzes biezumu

(Bakys et al., 2013). Tāpēc dažādi faktori, piemēram, oša un citu sugu biezums, sugu sastāvs, augsnes un mitruma režīms, kā arī mijiedarbība starp šiem faktoriem norāda, ka ADB gaitu nosaka vides faktoru komplekss.

Meteoroloģiskie apstākļi ietekmē gan oša, gan patogēna veiksmīgu augšanu (Wardle, 1961; Kowalski & Bartnik, 2010; Maresi, 2014). Piemēram, zemas gaisa temperatūras, vēlas pavasara salnas, sausums un nelabvēlīgi mitruma apstākļi novājina kokus un rada tiem stresu, tādējādi uzlabojot apstākļus infekcijas izplatībai (Thomsen & Skovsgaard, 2006; La Porta et al., 2008; Pautasso et al., 2010; Tulik et al., 2010). Vairāki zinātnieki ir nonākuši pie secinājuma, ka sausais klimats un sezonāls sausums ir nelabvēlīgi ADB (Chira et al., 2017; Grosdidier et al. 2018). Piemēram, Itālijā *H. fraxineus* izplatība tiek saistīta gan ar augsto gaisa temperatūru, gan bagātīgajiem nokrišņiem visas augšanas sezonas laikā (Maresi, 2014). Tomēr reģionālajām un lokālajām klimatiskajām atšķirībām ir liela ietekme uz oša vitalitāti (Maresi, 2014). Raugoties globāli, klimata pārmaiņas ir ticamākais faktors, kas slimības izplatību nākotnē varētu dabiski ierobežot, jo paaugstināta temperatūra un sausums ir nelabvēlīgi ADB, un tiek uzskatīts, ka Dienvideiropas siltākajos apgabalos slimības risks ir zemāks (Goberville et al., 2016; Grosdidier et al., 2018). Neskatoties uz to, parastā oša eksistenci nākotnē Eiropā var apdraudēt arī smaragdzaļā krāšnvabole *Agrilus planipennis* Fairmare, kas iznīcina oša populācijas Ziemeļamerikas austrumu daļā un šobrīd strauji izplatās Krievijas Eiropas daļā (Liebhold, 2012; Valenta et al., 2015).

1.5. Patogēna *Hymenoscyphus fraxineus* skarto parastā oša audžu transformēšanās

Oša dabiskā atjaunošanās pirms ADB bija plaši pētīta, ieskaitot tādus aspekts kā oša biezums dažādos meža tipos (Sakss, 1958; Tabari & Lust, 1999; Laiviņš & Mangale, 2004), audzes struktūra (Ellenberg, 1996; Tabari et al., 1999; Götmark et al., 2005; Dufour & Piegay, 2008), produktivitāte (Wardle, 1961; Jaworski, 1995; Střeštil & Šammonil, 2006), konkurence ar zemsedzi un citiem kokaugiem (Wardle, 1961; de la Cretaz & Kelty, 2002) u.c. Mitchell (2014) ir modelējusi gaidāmās izmaiņas mežaudžu vaskulāro augu sabiedrībās, kas saistītas ar ADB Lielbritānijā, taču līdzīga veida *in situ* pētījumu pēc ADB trūkst.

Slimības skartās oša audzes Eiropā pastāvīgi transformējas, dažas no tām tiek atjaunotas ar citām koku sugām, jo oša stādīšana ekonomisku apsvērumu dēļ ir pārtraukta (Kirisits et al., 2011; Bakys, 2013). Tomēr daļā no nocirstajām oša audzēm tiek plānota dabiskā atjaunošana, jo šādās audzēs labi atjaunojas citas koku sugas, kas auga mistrojumā ar osi (Lygis et al., 2014). Līdz ar to audžu floristiskais sastāvs saglabājas līdzīgs kā pirms ADB. Piemēram, Lietuvā auglīgās un mitrās vietās galvenokārt atjaunojas sukcesijas sākumstadijas vai pioniersugas, piemēram, baltalksnis *Alnus incana* (L.) Moench., āra bērzs *Betula pendula* Roth un parastā apse *Populus tremula* L. (Lygis et al., 2014).

Dabiski atjaunojušos oša indivīdu skaits pēc ADB krasi samazinās, tomēr atjaunošanās intensitātei novērotas reģionālas atšķirības. Jaunās oša paaugas biezums jaunaudzēs Austrumeiropā ir lielāks nekā Centrāleiropā. Piemēram, Lietuvā 2011. gadā dabiski atjaunojās 599 oši ha^{-1} (Lygis et al., 2014), Igaunijā 2015. gadā – 3500 oši ha^{-1} (Drenkhan et al., 2017), bet Vācijā 2015. gadā atjaunojās 653 oši ha^{-1} (Enderle et al., 2018). Oša atjaunošanās varētu būt saistīta ar sēklu koku vitalitāti konkrētajā audzē vai reģionā. Semizer-Cuming et al. (2019) konstatēja, ka kokiem ar zemu jutību pret ADB ir augstāka reproduktīvā spēja, salīdzinot ar ļoti jutīgiem kokiem, kas var uzlabot dabisko atjaunošanos. Jauno ošu vitalitāte Eiropā ir apmēram līdzīga, jo vitālo koku īpatsvars svārstās no 32 līdz 41% (Lygis et al., 2014; Drenkhan et al., 2017; Giongo et al., 2017; Enderle et al., 2018), norādot uz lokālo apstākļu ietekmi.

Oša stādīšanai tuvākajā nākotnē perspektīvu nav. Vācijā visi iestādītie oši bija inficēti, un to mirstība laikā no 2013. līdz 2014. gadam sasniedza 73% (Enderle et al., 2017). Tāpēc mežu atjaunošanā rekomendē izmantot alternatīvas sugas, kas dabiskos apstākļos aug kopā ar osi. Chira et al. (2017) ierosināja, ka Rumānijā oša mežus varētu aizstāt ar hibrīdo papeli *Populus x canadensis* Moench, dažādām ozolu sugām vai Pensilvānijas osi *Fraxinus pennsylvanica* Marshall, kas šobrīd ir neuzņēmīgs pret ADB. Vācijā slimības skartās oša audzes tiek papildinātas ar apšu hibrīdiem (Enderle et al., 2017). Upmalās un kaļķainās augsnēs tiek rekomendētas citas alternatīvas sugas (vītols *Salix* spp., apse, ozols, kļava *Acer* spp., goba *Ulmus* spp., alksnis, liepa *Tilia* spp., saldais ķirsis *Prunus avium* L., parastais skābardis *Carpinus betulus* L. u.c. (Sioen et al., 2017). Oša kalšana atstāj negatīvu ietekmi uz sugām, kas saistītas ar oša mežiem (putni, zīdītāji, sūnas, sēnes, bezmugurkaulnieki un ķērpji), samazinot to daudzumu vai pat apdraudot to eksistenci (Lõhmus & Runnel, 2014; Mitchell et al., 2014; Mitchell, 2014). Noskaidrots, ka Lielbritānijā tikai 69% no sugām, kas saistītas ar parasto osi var atrast alternatīvu koku sugu (Mitchell et al., 2014). Eksperimentālajos parauglaukumos Igaunijā piecu gadu laikā viena epifītu suga – askomicēte *Pyrenula laevigata* (Pers.) Arnold – izzuda un vēl trīs sugas tuvākajā nākotnē varētu lokāli izzust, ja pilnībā zaudēs savu dzīvotni uz oša. Tikai viens epifītiskais ķērpis *Lobaria pulmonaria* (L.) Hoffm saražoja <20 lapoņus uz alternatīva saimniekauga gobas, kļavas vai liepas (Lõhmus & Runnel, 2014), norādot, ka parastais osis, salīdzinot ar citām Eiropas lapkoku sugām, ar vidi mijiedarbojas unikālā veidā, kas īpaši attiecas uz barības vielu apriti (Mitchell et al., 2014). Latvijā vislielākā epifītu bagātība novērota uz ošiem, lielākā daļā šo sugu sastopamas arī uz parastās gobas *Ulmus glabra* Huds (Gerra-Inohosa, 2018). Tomēr abas šīs koku sugas ir slimību apdraudētas, kas var negatīvi ietekmēt epifītu daudzveidību nākotnē.

1.6. Promocijas darba mērķis

Promocijas darba mērķis bija noskaidrot parastā oša *Fraxinus excelsior* L. audžu kalšanas dinamiku un faktorus, kas ietekmē koku uzņēmību pret patogēnu; novērtēt slimības skarto audžu sukcesiju un atjaunošanās potenciālu.

1.7. Promocijas darba uzdevumi

Promocijas darbā izvirzīti četri uzdevumi:

1. noskaidrot oša audžu kalšanas dinamikas reģionālās atšķirības un tās saistību ar meteoroloģiskajiem faktoriem;
2. noteikt parastā oša ģenētisko daudzveidību un populācijas struktūru;
3. raksturot oša atjaunošanos un sukcesiju slimības skartajās pieaugušajās oša audzēs un jaunaudzēs;
4. novērtēt augšanas apstākļu un saimnieciskās darbības ietekmi uz oša paaugas atjaunošanos un vitalitāti.

1.8. Promocijas darba tēzes

Promocijas darbā izvirzītas trīs tēzes:

1. intensīvas oša audžu destrukcijas apstākļos Latvijā joprojām pastāv augsts sugas saglabāšanās potenciāls, un nākotnē osis būs sastopams jauktās audzēs kā piemistrojuma suga salīdzinoši nelielā daudzumā;
2. oši ir vitālāki intensīvi apsaimniekotās (koptās, nosusinātās utt.) jauktās audzēs, savukārt piemistrojuma sugām var būt atšķirīga ietekme uz oša fitosanitāro stāvokli;
3. oša pieauguma jutībai pret meteoroloģiskajiem faktoriem ir neizteikta saistība ar oša kalšanu, bet būtisko faktoru kopums norāda uz pieaugošu stresu klimata pārmaiņu rezultātā, kas var samazināt koku rezistenci.

1.9. Pētījuma novitāte

Promocijas darbā pirmo reizi Latvijā detalizēti analizēta invazīvā patogēna izraisīta masveida vietējās koku sugas, parastā oša, mežaudžu kalšana. Pirmo reizi vistuvāk oša audžu izplatības dabiskā areāla ziemeļu robežai novērtēta slimības skarto audžu transformācija un oša dabiskā atjaunošanās dažādos meža tipos. Pirmo reizi Baltijas valstīs novērtēta augšanas apstākļu un saimnieciskās darbības ietekme uz oša paaugas atjaunošanās intensitāti un vitalitāti. Novērtēta oša radiālā pieauguma gaitas un jutības pret meteoroloģiskajiem faktoriem saistība ar oša kalšanu.

1.10. Promocijas darba uzbūve

Promocijas darbs sastāv no deviņām publikācijām. Pirmās divas publikācijas (I un II publikācija) raksturo ADB gaitu un apjomus. Patogēna *H. fraxineus* vienas sezonas attīstības dinamika aprakstīta III publikācijā. Oša radiālā pieauguma gaita un jutība pret meteoroloģiskajiem faktoriem saistībā ar ADB pētīta IV publikācijā. Parastā oša ģenētiskā daudzveidība un populāciju struktūra Latvijā raksturota V publikācijā. Nākamajās divās publikācijās (VI un VII) analizēta kokaugu sukcesija ADB skartajās audzēs. Parastā oša dabiskā atjaunošanās jaunaudzēs atkarībā no augšanas apstākļiem un saimnieciskās darbības analizēta VIII un IX publikācijā.

1.11. Promocijas darba aprobācija

Ziņojumi par pētījuma rezultātiem prezentēti 15 starptautiskās konferencēs:

1. Burņeviča N., **Matisone I.**, Zaļuma A., Brūna L., Laiviņš M., Gaitnieks T. (2019). Ash dieback and other actual diseases in Latvia. Consolidating approaches to mitigate the ash dieback disease in Baltic States and Germany, October 16, 2019, Kaunas – October 17, 2019, Vilnius, Lithuania. Referāts.
2. **Matisone I.** (2017). Ash dieback in Latvia: causes, consequences, lessons for forest management. Forestry doctoral school. June 19–22, 2017, Jaunkalsnava, Latvia. Referāts.
3. **Pušpure*I.,** Zaļuma A., Gaitnieks T., Burņeviča N., Matisons R. (2017). Seasonal development of lesions of young common ash in Latvia. Invasive Forest Pathogens & Implications for Biology & Policy, IUFRO Working Party 7.02.02, May 7–11, 2017, Niagara Falls, Ontario. Stenda referāts.
4. **Pušpure*I.**, Matisons R., Laiviņš M., Lībiete Z. (2016). Influence of stand structure on resistance of common ash young stands to ash dieback. Integrating Scientific Knowledge in Mixed Forests EuMIXFOR Final Conference COST Action FP 1206, October 5–7, 2016, Prague, Czech Republic. Stenda referāts.
5. **Pušpure*I.**, Matisons R., Laiviņš M., Gaitnieks T. (2016). Natural regeneration of common ash in young stands in Latvia 3. COST Action FP1103. *Fraxinus* dieback in Europe: elaborating guidelines and strategies for sustainable management, March 1–3, 2016, Bratislava, Slovakia. Referāts.
6. **Pušpure*****I.**, Gerra-Inohosa L., Matisons R., Laiviņš M. (2016). Tree-ring width of European ash differing by crown condition and its relationship with climatic factors in Latvia. Cost action FP1103. *Fraxinus* dieback in Europe: elaborating guidelines and strategies for sustainable management, March 29–April 3, 2016, Riga, Latvia. Referāts.
7. **Pušpure*I.**, Matisons R., Laiviņš M., Burņeviča N., Gaitnieks T. (2016). Natural regeneration of common ash in young stands in Latvia 2. COST Action FP1103. *Fraxinus* dieback in Europe: elaborating guidelines and strategies for sustainable management, March 29–April 3, 2016, Riga, Latvia. Referāts.
8. **Pušpure*I.** (2015). Natural regeneration of common ash *Fraxinus excelsior* L. stands in Latvia 1. COST Action FP1103. *Fraxinus* dieback in Europe: elaborating guidelines and strategies for sustainable management, April 12–16, 2015, Dubrovnik, Croatia. Referāts.
9. **Pušpure*I**. (2015). Quality assessment of European ash *Fraxinus excelsior* L. genetic resource forests in Latvia. 21st Annual International Scientific

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* mainīts uzvārds no Pušpure uz Matisone.

Conference Research for Rural Development 2015, May 13–15, 2015. Jelgava, Latvia. Referāts.

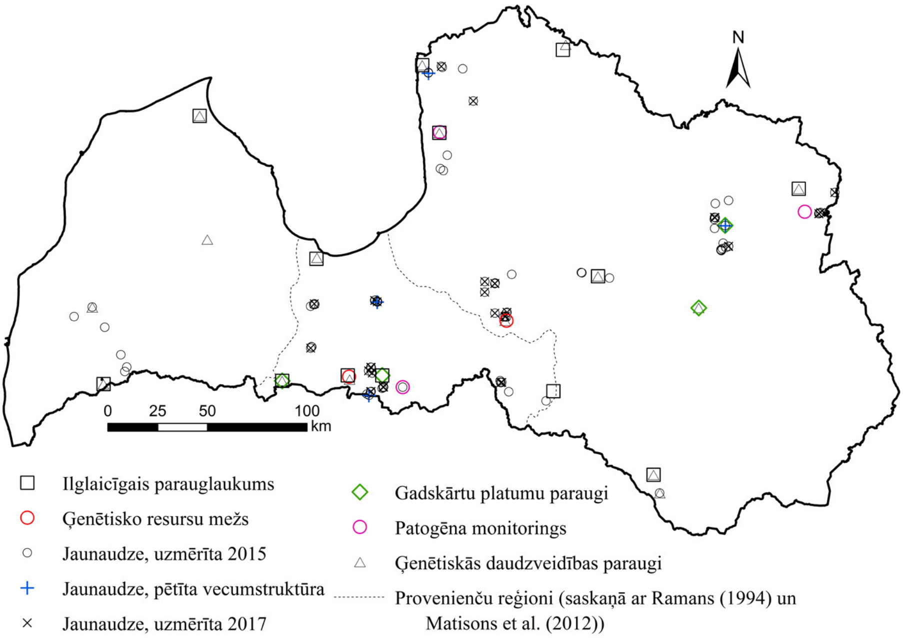
10. **Pušpure*I.** (2015). Comparison of species diversity in two *Fraxinus excelsior* L. woodland key habitat regions of Latvia. 8th International Conference on Biodiversity Research, April 28–30, 2015, Daugavpils, Latvia. Stenda referāts.
11. Laiviņš M., **Pušpure*****I.** (2015). The destruction and transformation of *Fraxinus excelsior* forest stands in Latvia. Scientific Conference of the Forest Sector: Knowledge based forest sector, November 4–6, 2015, Riga, Latvia. Referāts.
12. Laiviņš M., **Pušpure*I.**, Gerra-Inohosa L. (2015). The destruction and transformation of *Fraxinus excelsior* L. forest stands in Latvia. Scientific Conference of the Forest Sector: Knowledge based forest sector, November 4–6, 2015, Riga, Latvia. Stenda referāts.
13. Laiviņš M., Gerra-Inohosa L., **Pušpure*I.** (2015). Monitoring of *Fraxinus excelsior* L. forest stands in Latvia. Scientific Conference of the Forest Sector: Knowledge based forest sector, November 4–6, 2015, Riga, Latvia. Stenda referāts.
14. **Pušpure*****I.,** Laiviņš M., Gerra-Inohosa L. (2015). The dynamic of shrub layer in *Fraxinus excelsior* L. forest stands in response to ash dieback in Latvia. Scientific Conference of the Forest Sector: Knowledge based forest sector, November 4–6, 2015, Riga, Latvia. Stenda referāts.
15. **Pušpure*****I.,** Gerra-Inohosa L., Laiviņš M. (2015). Natural regeneration and vitality of common ash *Fraxinus excelsior* L. forest stands in Latvia. Nordic-Baltic Forest Conference “Wise Use of Improved Forest Reproductive Material”, September 15–16, 2015, Riga, Latvia. Stenda referāts.

2. MATERIĀLI UN METODES

2.1. Pētījuma objekti, lauka un laboratorijas darbi

2.1.1. Oša kalšanas dinamika

Oša audžu kalšanas dinamika pētīta 15 ilglaicīgajos parauglaukumos (PSP), kas 2005. gadā ierīkoti visā Latvijas teritorijā (II publikācija, 2.1. att.). Parauglaukumi ierīkoti dažāda vecuma (51–138 gadi) audzēs, kur vismaz sākotnēji dominējis osis. Parauglaukumi (izņemot divus sezonāli mitros PSP netālu no Ķemeriem un Ainažiem) ierīkoti līdzenās normāla mitruma eitrofās augsnēs, kas atbilst gāršas *Aegopodiosa,* slapjās gāršas *Dryopteriosa,* liekņas *Filipendulosa* un platlapju kūdreņa *Oxalidosa turf. mel*. meža tipiem (cf. Bušs, 1976). Katrā audzē ierīkots viens apļveida parauglaukums (R = 15 m; S = ca. 706 m^2). Katrā PSP uzmērīti visi koki (ieskaitot kritalas un stumbeņus) ar stumbra caurmēru krūšaugstumā (DBH) ≥6 cm, noteikts to stāvoklis (dzīvs vai nokaltis), kā arī uzmērīts DBH, augstums (H) un kritalas garums. Mērījumi katrā PSP veikti 2005., 2010., un 2015. gadā.



2.1. att. **Pētīto parastā oša audžu izvietojums un koku provenienču reģioni Latvijā**

2.1.2. Hymenoscyphus fraxineus izraisīto bojājumu dinamika sezonas laikā

Patogēna *H. fraxineus* radīto bojājumu attīstības dinamika pētīta trīs (2.1. att.) dabiski atjaunojušās parastā oša audzēs vecumā no 5–8 gadiem, kurās iepriekšējās rotācijas periodā dominējis osis (III publikācija). Novērojumi veikti ik mēnesi no 2015. gada jūnija līdz septembrim, kad siltajos vasaras mēnešos sagaidāma visaugstākā patogēna aktivitāte (Timmermann et al., 2011; Bengtsson et al., 2014). Parauglaukumi reprezentē gāršas meža tipu *Aegopodiosa* (Bušs, 1976) un atrodas līdzena reljefa labi drenētās auglīgās minerālaugsnēs. Bauskas, Aizpurves un Limbažu audžu biezums atbilstoši bija 5000, 1500 un 1500 koki ha^{-1}. Visās audzēs osis bija valdošā suga ar parastās apses *Populus tremula* L., blīgznas *Salix caprea* L. un parastās kļavas *Acer platanoides* L. (līdz 1500 koki ha^{-1}) piemistrojumu.

Katrā audzē izraudzīti 10 dominantie oši (augstumā 2.5–3.0 m) ar vienu līdz trīs nelieliem stumbra bojājumiem – nekrozēm (maksimālais bojājumu laukums kokam bija 223.4 cm^2). Reizi mēnesī – no jūnija līdz septembrim – katram izvēlētajam kokam izmērīts H, DBH un atbilstoši piecu klašu skalai raksturota koka vitalitāte (2.1. tabula). Jūnijā uz caurspīdīga papīra apvilkta kontūra visiem redzamajiem bojājumiem uz stumbriem, zariem un galotnēm. Kontūra (virs iepriekšējā mērījuma) pārzīmēta katru mēnesi, pievienojot arī jaunos bojājumus. Pēc pēdējās apsekošanas septembrī ievākti bojāto koku daļu paraugi (stumbriem un lielākajiem zariem), kas laboratorijā nomizoti, pēc tām uz caurspīdīga papīra uzzīmēta slimības rezultātā iekrāsotās koksnes kontūra. Vēlāk šie koksnes paraugi izmantoti patogēna *H. fraxineus* izdalīšanai. No papīra pēc iezīmētajām kontūrām, izmantojot TAMAYA digitālo planimetru PLANIX 10S “Marble”, uzmērīts katra bojājuma ikmēneša laukums.

2.1. tabula

Oša vitalitātes vērtējums un tā skaidrojums

Klase	*H. fraxineus* bojājumu pakāpe (%)	Koka vizuālais raksturojums
1	0–10	Koks izskatās vesels vai nedaudz bojātas atsevišķas lapas
2	11–25	Bojātas vairākas lapas, atsevišķas nekrozes uz mizas
3	26–60	Pilnībā bojāts/atmiris atsevišķs zars, bojāta daļa lapojuma, nekroze uz mizas lielos laukumos
4	61–99	Pilnībā bojāta līdz atmirusi daļa vainaga, dzīvi atsevišķi ūdenszari
5	100	Koks pilnīgi nokaltis

Patogēna *H. fraxineus* izdalīšanai no 17 iepriekš ievāktiem paraugiem no koksnes nekrozes robežas (mizas kopā ar veselo/bojāto koksni) ar skalpeli izgrieza nelielus ca. 8×8 mm gabaliņus (Schumacher et al., 2009). Katru iegūto paraugu sterilizēja 30 sekundes, turot 35% ūdeņraža peroksīdā, pēc tam nomazgājot divas reizes vienu minūti sterilizētā destilētā ūdenī. Pēc izžāvēšanas paraugus ievietoja iesala agara barotnē un četras nedēļas inkubēja tumsā 20°C temperatūrā. Visas citas no paraugiem izaugušās sēnes vienreiz trīs dienās mehāniski atdalītas. Patogēns *H. fraxineus* mikroskopiski identificēts, pamatojoties uz Kowalski (2006).

2.1.3. Oša vitalitāte un jutība pret meteoroloģiskajiem faktoriem

Lai raksturotu oša vitalitātes saistību ar pieauguma jutību pret meteoroloģiskajiem faktoriem (IV publikācija), analizētas četras pieaugušas oša audzes, divas Latvijas austrumu un divas rietumu daļā (cf. Baumanis et al., 2001; Matisons et al., 2012; 2.1. att.). Koku vainagu stāvoklis audzēs bija atšķirīgs (no veseliem līdz stipri bojātiem). Katrā audzē izvēlēti 10 dominantie oši ar vizuāli veseliem (vainaga atmirums ≤10%) un 10 oši ar bojātiem vainagiem (vainaga atmirums 30–60%). Ar Preslera svārpstu katram kokam no pretējām stumbra pusēm krūšu augstumā ievākti divi koksnes paraugi. Ievāktie koksnes paraugi laboratorijā izžāvēti līdz gaissausam stāvoklim, ielīmēti fiksācijas planšetēs un slīpēti ar dažādu frakciju smilšpapīru (no 120 līdz 400 graudi uz collu). Lai labāk atpazītu gadskārtas un uzlabotu kontrastu starp agrīno un vēlīno koksni, noslīpēto paraugu virsmas apstrādātas ar baltu krītu. Gadskārtu platumi (TRW) uzmērīti, izmantojot mērīšanas sistēmu Lintab 5 (RinnTECH, Heidelberg, Germany).

2.1.4. Parastā oša populāciju struktūra un ģenētiskā daudzveidība

Parastā oša populāciju struktūra un ģenētiskā daudzveidība analizēta 16 audzēs visā Latvijā (2.1. att.) (V publikācija). Divas no šīm audzēm izraudzītas kā oša ģenētisko resursu meži (GRF). Katrā audzē (apmēram 2500 m^2 platībā) nejauši izvēlēti 24 dabiski atjaunojušies oši, no kuriem 1–2 m augstumā ievāktas vairākas lapas. Kopā analizēti 372 oša indivīdi.

Izmantojot uz CTAB balstītu metodi, no lapām izdalīta DNS (Porebski et al., 1997). Genotipēšana veikta, izmantojot sešus kodola SSR marķierus (Femsatl4, Femsatl10, Femsatl11, Femsatl16, Femsatl19) (Lefort et al., 1999) un M2-30 (Brachet et al., 1999), kā arī sešus hloroplastu marķierus ccmp2, ccmp4, ccmp6, ccmp10, ccmp7 un ccmp3 (Weising et al., 1999). Polimerāzes ķēdes reakcija (PCR) veikta Eppendorf Mastercycler ep gradienta termālajā cilindrā. Amplifikācijas fragmenti tika izdalīti ar ABI Prism 3130xl Genetic Analyzer (Applied Biosystems) iekārtu.

2.1.5. Parastā oša audžu sukcesija

Oša mežaudžu sukcesija analizēta 15 PSP (2.1. att.). Katrā PSP paaugas un pameža raksturošanai (VI publikācija) septiņu metru attālumā no parauglaukuma centra ierīkoti trīs mazāki apļveida parauglaukumi ($R = 5$ m, $S =$ ca. 78.5 m^2). Parauglaukumos uzmērīts visu pameža un paaugas indivīdu (kuriem DBH ≤ 6 cm) augstums. Mērījumi veikti 2005., 2010. un 2015. gadā. Papildus tam oša audžu sukcesija novērtēta divos GRF netālu no Skrīveriem un Bērvircavas (2.1. att.) (VII publikācija). Kopumā analizētas 28 GRF audzes (nogabali) Skrīveros un 73 GRF audzes Bērvircavā. Audžu vecums svārstījās no 20–126 gadiem; osis tajās bija (joprojām vai pirms ADB) dominējošā suga ar parastās gobas, parastās apses, melnalkšņa *Alnus glutinosa* L., āra bērza u.c. sugu piemistrojumu. Audzēm raksturīgi auglīgi augšanas apstākļi (gāršas, slapjās gāršas vai platlapju āreņa *Mercurialiosa mel.* meža tipos). Katrā audzē pēc nejaušības principa ierīkots viens 20×20 m parauglaukums, kurā noteikts katras I stāva sugas projektīvais segums (%). Pa parauglaukuma vienu diagonāli ierīkots viens 25×1 m parauglaukums, kur uzskaitītas visas paaugas un pameža sugas (H ≤ 7.0 m), ošu vitalitāte novērtēta piecu klašu skalā (2.1. tabula).

2.1.6. Oša dabiskā atjaunošanās un vitalitāte jaunaudzēs

Oša dabiskā atjaunošanās analizēta 90 jaunaudzēs visā Latvijas teritorijā (2.1. att.) (VIII publikācija). Pētīto audžu vecums bija no 5–40 gadiem, nogabalu platība pārsniedza vienu ha, un osis iepriekšējā rotācijā veidoja ≥40% no audzes krājas. Parauglaukumi lielākoties ierīkoti gāršas, slapjās gāršas, platlapju kūdreņa un platlapju āreņa *Mercurialiosa mel.* meža tipos (cf. Bušs, 1976). Katrā audzē (nogabalā) pa garāko diagonāli ierīkots viens 100×2 m parauglaukums, kur uzskaitīti visi paaugas un pameža indivīdi līdz 7 m augstumam un nomērīts to augstums. Vitalitāte katram osim raksturota piecu klašu skalā (2.1. tabula). Ošu vecuma noteikšanai četrās no šīm 90 audzēm (divas sešus gadus un divas astoņus gadus vecas; 2.1. att.), nozāģējot visus parauglaukumā esošos ošus, virs sakņu kakla ievākti stumbra paraugi (ripas). Nozāģētajiem ošiem uzmērīts augstums un stumbra caurmērs virs sakņu kakla, kā arī noteikta vitalitāte. Augstuma analīzei no visiem ošiem katrā audzē nejauši izvēlēti 10 koki katrā augstumklasē (7 klases; koka augstums 0–3 m ar 0.5 m intervālu). Ievāktie paraugi laboratorijā noslīpēti, mikroskopā uzskaitītas gadskārtas. Audzes uzmērītas 2015. gadā.

Oša vitalitātes vērtējumam atkarībā no jaunaudzes sastāva (IX publikācija) no 90 audzēm (VIII publikācija) izvēlētas 35 dažādu dominējošo sugu jaunaudzes vecumā no 26 līdz 36 gadiem. Atlase veidota tā, lai audzes, kurās dominē melnalksnis, baltalksnis, āra bērzs, osis un parastā egle, Latvijas rietumu un austrumu daļā būtu vienādās proporcijās (2.1. att.). Audzes apsekotas 2017. gadā, 100×2 m parauglaukumā uzmērīts paaugas un pameža augstums (atbilstoši 2015. gada metodikai, tikai bez stumbra paraugu ievākšanas).

2.2. Datu analīze un papilduS datu avoti

Jaunaudžu (<41 gads) platību dinamika (relatīvās izmaiņas) Latvijā analizēta trīs gadu periodos no 2000.–2009. gadam (līdz 2015. gadam kopējai platībai) (I publikācija), balstoties uz Valsts meža dienesta datu bāzes datiem (Valsts meža dienests, 2019). Rajons izmantots kā statistikas vienība, analīzē rajoni sagrupēti Ramana (1994) definētajos fizioģeogrāfiskajos dabas rajonos. Dabas rajoni, kuros oša audžu platība 2000. gadā bija <200 ha, no analīzes izslēgti. Telpiskā analīze veikta, izmantojot Arc View 9.1 programmatūru (ESRI, 2006).

Dažādi audžu raksturlielumi – meža tipi, vecums, kopšanas reižu skaits, audzes krāja, iepriekšējās rotācijas un pašreizējais audzes sastāvs (formula) – kas izmantoti

II, III, IV, VII, VIII un IX publikācijā, iegūti no Valsts meža dienesta datu bāzes (Valsts meža dienests, 2019).

Pāru *t*-tests (izmantojot Bonferroni transformāciju *p*-vērtībām) veikts, lai novērtētu bojājumu laukuma pieaugumu starp secīgajiem novērojumiem (III publikācija). Dispersijas analīze (ANOVA) lietota, lai noteiktu paaugas oša biezumu atkarībā no I stāvā dominējošās sugas (VI un VII publikācija), kā arī, lai noteiktu kopējo un katras sugas atjaunošanās biezumu dažādos novērojumu periodos (VI publikācija). Šī pati analīze izmantota, lai novērtētu ošu diametra, augstuma un vecuma atšķirības starp audzēm un vitalitātes klasēm (VIII publikācija), kā arī atšķirību noteikšanai starp bojājumu lielumu un to izplešanās ātrumu (kokam) saistībā ar to izvietojumu uz koka un vecumu (eksistējošais (EL) vai jaunais (NL) bojājums); oša vitalitāti starp audzēm (III publikācija). Saistības starp ošu diametru, vecumu un augstumu novērtētas ar lineāro modeli (VIII publikācija). Ar šo modeli analizēta arī oša vitalitāte atkarībā no I stāva projektīvā seguma un I stāvā dominējošas sugas (VII publikācija). Ar χ^2 testu salīdzināts paaugas un pameža (kopā un atsevišķi) sastāvs starp novērojumu periodiem (VI publikācija).

Ar Pīrsona korelācijas analīzi novērtētas sakarības starp oša paaugas biezumu un kokaugu skaitu pamežā/paaugā (VII un VIII publikācija), kā arī saistība starp paaugas/pameža (un abu kopā) un nokaltušo ošu biezumu I stāvā (VI publikācija). Šī analīze izmantota, lai novērtētu oša vitalitāti atkarībā no koka augstuma, vecuma (VIII publikācija), kā arī starp bojājumu laukumu zem un virs mizas (III publikācija). Neparametriskā Kendala korelācijas analīze izmantota lineārās sakarības starp oša atjaunošanās biezumu un sugu bagātību audzē noteikšanai (VI publikācija), kā arī koku morfometrisko parametru (H, D) ietekmes uz to vitalitāti novērtēšanai (III publikācija).

Vispārinātais lineārais modelis, izmantojot Gausa regresiju, lietots, lai analizētu reģionu un sugu kompozīcijas (paaugā un I stāvā dominējošā suga) ietekmi uz oša atjaunošanos (VIII publikācija). Ar vispārināto lineāro modeli, izmantojot Puasona regresiju, analizētas atšķirības starp aktīvo/neaktīvo/latento bojājumu skaitu uz koka, kā arī atšķirības starp NL un EL skaitu atkarībā no to izvietojuma uz koka, apsekošanas datuma, oša vitalitātes un parauglaukuma (III publikācija). Atšķirības starp paaugas oša biezumu atkarībā no meža tipa, audzes vecuma (klasēs), sugu sastāva (paaugā dominējošā suga) analizētas ar vispārināto lineāro jauktā efekta modeli (VIII publikācija). Jaukto modeļu būtiskums novērtēts, izmantojot *Likelihood ratio* testu (West et al., 2006). Datu kopu un modeļu atlikumu atbilstība normālajam sadalījumam pārbaudīta, izmantojot grafisko analīzi (Elferts, 2013).

Jaukta tipa ranžētā binomiālā regresija izmantota, lai novērtētu paaugas augstuma un pameža biezuma ietekmi uz oša vitalitāti starp reģioniem (VIII publikācija). Ar šo metodi analizēts arī oša vitalitātes (klašu) izmaiņas saistībā ar audzes struktūru un sastāvu (IX publikācija). Koks izmantots kā novērojums, bet parauglaukums kā nejaušības efekts (*random intercept*). Modeļa izstrādes laikā, balstoties uz arbitrārās atlases principiem, pārbaudīti dažādi regresori un to kombinācijas. Vienlaicīgi kopā testēti līdz pat astoņiem regresoriem. Labākais modelis atlasīts, balstoties uz Akaikes informācijas kritēriju (AIC), kā arī modeļu atbilstību priekšnosacījumiem. Būtiskajiem regresoriem pārbaudīta kolinearitāte (Fox & Weisberg, 2011).

Detrendētā korespondentanalīze lietota, lai noteiktu sugu kompozīciju izmaiņas laikā (VI publikācija).

Augšanas gaita un tās jutība pret meteoroloģiskajiem faktoriem ošiem ar dažādu vitalitāti novērtēta ar dendrohronoloģijas metodēm (IV publikācija). Visas nomērītās gadskārtu platumu rindas šķērsdatētas, un pārbaudīta to kvalitāte. Datu kopu raksturošanai aprēķināts populācijas signāla izpausmes indekss (EPS), trokšņa līmenis (Wigley et al., 1984), sinhronitāte (*Gleichläufigkeit*), starprindu korelācijas un pirmās kārtas autokorelācijas koeficients. Katrai audzei/grupai (bojāti un veseli) no šķērsdatētajām koku gadskārtu platumu rindām izveidotas atlikumu hronoloģijas. Izmantota dubultā detrendēšana ar negatīvo eksponenciālo un trešās pakāpes līknēm. Meteoroloģisko signālu noteikšanai hronoloģijās izmantota būtstrepa Pīrsona korelācijas analīze. Meteoroloģiskajiem faktoriem, kas uzrādīja būtisku korelāciju ar TRW, pārbaudīta kolinearitāte. Tīkloti meteoroloģiskie dati – vidējā gaisa temperatūra, nokrišņu summa un standartizēti nokrišņu-iztvaikošanas indeksi pa mēnešiem – iegūti no Austrumanglijas Universitātes tiešsaistes datu krātuves (Harris et al., 2014).

Oša populāciju struktūra noteikta, izmantojot molekulārās ģenētikas analīzes (V publikācija). Amplificētie DNA fragmenti vizualizēti, izmantojot GeneMapper 3.5. Hloroplastu marķieru dati apvienoti haplotipos. Kodolu SSR dati analizēti ar Fstat 2.9.3.2 (Goudet, 2001) un GenAlEx 6.5 (Peakall & Smouse, 2012) programmatūrām. Oša ģenētiskā daudzveidība analizēta ar dendrogrammu palīdzību, kas veidotas programmatūrā MEGA 5.2 (Tamura et al., 2011). Baja klāsteranalīze ar STRUCTURE 2.1 (Pritchard et al., 2000) izmantota, lai novērtētu iespējamo nSSR genotipu klāsteru skaitu. Iespējamais klāsteru skaits raksturots ar delta K kritēriju (Evanno et al., 2005), izmantojot STRUCTURE HARVESTER 0.6.93 programmatūru (Earl & vonHoldt, 2012).

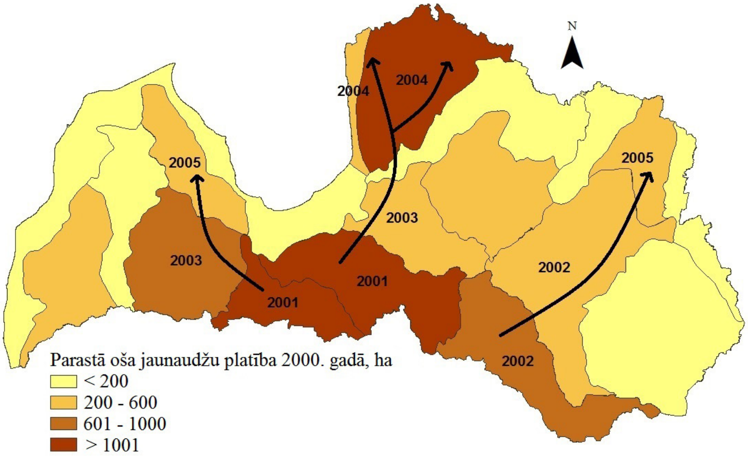
Datu apstrāde veikta pie būtiskuma līmeņa $\alpha = 0.05$ programmā R v. 3.6.1 (R Core Team, 2019), izmantojot paketes “lme4” (Bates et al., 2015), “ordinal” (Christensen, 2018), “car” (Fox & Weisberg, 2011), “lmerTest” (Kuznetsova et al., 2015), “dplR” (Bunn, 2008) un “multcomp” (Hothorn et al., 2008).

3. REZULTĀTI UN DISKUSIJA

3.1. Parastā oša audžu kalšanas raksturojums

Oša kalšana izraisīja straužu oša audžu platību un krājas samazināšanos (II publikācija), kas bija izteikts tieši jaunaudzēs (I publikācija). Oša jaunaudžu platība laika posmā no 2000.–2015. gadam samazinājās 4.4 reizes. Tas ietekmēja arī oša audžu vecumstruktūru Latvijā, jo jaunaudžu (<40 gadi) proporcija no kopējās oša mežaudžu platības samazinājās no 43% 2000. gadā uz 17% 2015. gadā. Novērojumu sākumposmā no 2000.–2006. gadam platību samazināšanās bija straujāka (vidēji 805 ha gadā), taču vēlāk (2007.–2015. gads) tā samazinājās lēnāk (vidēji 279 ha gadā), norādot uz procesiem, kas atkarīgi no biezības. Līdzīgi rezultāti publicēti arī citās valstīs. Piemēram, Lietuvā laika posmā no 2001.–2015. gadam oša audžu platība samazinājās par 2043 ha gadā (Pliūra et al., 2017), bet Rumānijā no 2001.–2010. gadam par 415 ha gadā (Chira et al., 2017).

Oša jaunaudžu kalšana sākotnēji – 2000. gadā – konstatēta Latvijas dienvidu daļā (Zemgales līdzenumā), kas robežojas ar Lietuvu, kur ADB jau bija konstatēta (Juodvalkis & Vasilauskas, 2002). No 2001. gada, iespējams, kā migrācijas koridorus izmantojot zemienes un upju ielejas, ADB strauji izplatījās ziemeļu virzienā (3.1. att.). Centrāleiropā tieši upes tiek uzskatītas par galveno ADB izplatības ceļu (Chira et al., 2017). Līdz 2006. gadam ADB bija masveidīgi izplatījusies pa visu Latvijas teritoriju. Patogēna *H. fraxineus* izplatības ātrums bija apm. 40 km gadā, kamēr Itālijas ziemeļu daļā tas bija 50–60 km gadā (Luchi et al., 2012), bet Norvēģijā svārstījās no



3.1. att. **Hipotētiskie *Hymenoscyphus fraxineus* izplatības ceļi Latvijā**
Ar gadiem norādīta patogēna izplatība Latvijā.

25–78 km gadā (Børja et al., 2017; Solheim & Hietala., 2017). Latvijā ADB izplatība bija lēnāka augstieņu rajonos, kur oša audzes izvietotas mozaīkas veidā, līdz ar to oša audžu blīvums un konektivitāte ir zemāka nekā, piemēram, Vācijā (Enderle et al., 2018).

Oša kalšanas apjomi Latvijā, balstoties uz meža inventarizācijas (FI) un PSP datiem, analizēti II publikācijā. Atbilstoši FI datiem, audžu platība, kurās dominēja osis, no 1998. līdz 2015. gadam samazinājās gandrīz divkārt. Šī samazināšanās notika ātrāk (par ca. 6%) valsts rietumu daļā, kur bija lielāka audžu platība un savienojamība (Liepiņš et al., 2016), kas varēja veicināt slimības izplatīšanos (Enderle et al., 2018).

Starp datu avotiem novērota neatbilstība attiecībā uz ADB skarto audžu pieauguma dinamiku. Oficiālā (FI) statistika uzrādīja pakāpenisku krājas palielināšanos oša dominējošajās audzēs no 2005.–2015. gadam (3.1. tabula). Savukārt pēc PSP datiem konstatēta krājas samazināšanās no ca. 320 m^3 ha^{-1} 2005. gadā uz 151 m^3 ha^{-1} 2015. gadā. Attiecīgi mirušās koksnes krāja palielinājās no 18 m^3 ha^{-1} līdz 212 m^3 ha^{-1} (3.1. tabula). Koksnes krājas izmaiņas bija līdzīgas Latvijas rietumu un austrumu daļā.

3.1. tabula

Oša audžu statistika Latvijā saskaņā ar Latvijas Valsts meža dienesta un ilglaicīgo parauglaukumu datiem laika posmā no 2005.–2015. gadam

Izkliede raksturota ar ticamības intervālu 95%

Statistiskais rādītājs	Gads		
	2005.	2010.	2015.
Latvijas Valsts meža dienesta dati			
Kopējā audžu platība, ha	18315.70	16263.50	13673.71
Kopējā koksnes krāja, m^3	3737303	3698663	3410624
Vidējā koksnes krāja, m^3 ha^{-1}	204.05	227.42	249.43
Ilglaicīgo parauglaukumu dati			
Dzīvi			
Audzes biezums, indivīdi ha^{-1}	256 ±55	136 ±45	77 ±32
Koksnes krāja, m^3 ha^{-1}	322 ±89	246 ±77	151 ±59
Nokaltuši			
Audzes biezums, indivīdi ha^{-1}	74 ±50	178 ±82	233 ±79
Koksnes krāja, m^3 ha^{-1}	18 ±14	119 ±54	212 ±66

Koku izdzīvošanas rādītāji Latvijas rietumu un austrumu daļā bija līdzīgi; attiecīgi no 2005.–2009. gadam un 2010.–2015. gadam mirstības koeficients (*r%*) bija 9.6% un 8.2% gadā. Oša kopējā mirstība Latvijā bija zemāka nekā Lietuvā un Vācijā (attiecīgi, 6.9% pret 8.7% un 10.1% gadā; cf. Pliūra et al., 2017; Enderle et al., 2017), norādot uz iespējami augstāku Latvijas oša audžu noturību. Taču tā bija augstāka nekā vidēja vecuma (*r%* = ca. 6% gadā) un pāraugušiem kokiem (ca. 3% gadā) Norvēģijā (Timmermann et al., 2017), briestaudzes vecuma kokiem Austrijā (0.3% gadā, Keßler et al., 2012) vai "ilgdzīvojošajiem" kokiem Zviedrijā (ca. 1% gadā; Bengtsson & Senström, 2017), norādot uz vecāko koku labāku noturību pret patogēnu. Novērots, ka koku krāja samazinājās (*r%* = 5.3% gadā; 3.1. tabula) lēnāk nekā biezums, kas varētu būt saistīts ar "ilgdzīvojošo" koku klātbūtni pētītajās oša populācijās. Paaugstinātā "ilgdzīvojošo koku" rezistence, kas novērota arī citos pētījumos (Skovsgaard et al., 2010; McKinney et al., 2011a), visdrīzāk izskaidro *r%* atšķirības atkarībā no audzes biezuma un koksnes krājas (3.1. tabula).

Paaugstinātā mirstība, kas aprēķināta, balstoties uz oša biezuma izmaiņām ADB sākumposmā (3.1. tabula), norāda, ka lielākā daļa oša populācijas bija ļoti uzņēmīga pret patogēnu (McKinney et al., 2014). Šādas mirstības tendences saskan ar Marçais et al. (2017) un Coker et al. (2019) pētījumiem, kur novērots, ka 6–8 gadus pēc spēcīgas infekcijas mirstības rādītājiem ir tendence samazināties.

Oša kalšanu izraisošais patogēns *H. fraxineus* izolēts 6 no 17 ievāktajiem paraugiem (35%), kas apstiprina konkrētā patogēna saistību ar oša bojāeju Latvijā (III publikācija). Pozitīvo paraugu proporcija bija zemāka nekā Zviedrijā (Bengtsson et al., 2014) un Vācijā (Schumacher et al., 2009), kas, iespējams, liecina par sekundāro patogēnu iesaistīšanos (Husson et al., 2012), kas šajā pētījumā netika analizēti. Zemā *H. fraxineus* klātbūtne var būt saistīta ar sezonālo (McKinney et al., 2011b; Bengtsson et al., 2014), kā arī individuālo (Schumacher et al., 2009) vairķermeņu daudzuma variācijām, un intensīvo paraugu virsmas sterilizēšanu pirms inkubācijas. Nevienmērīgā dažāda vecuma audžu, kā arī dažādu koku inficēšanās vienā audzē un nevienmērīgi mirstības rādītāji Latvijas un Eiropas kontekstā norāda uz dažādiem lokāliem faktoriem, kas varētu ietekmē oša rezistenci.

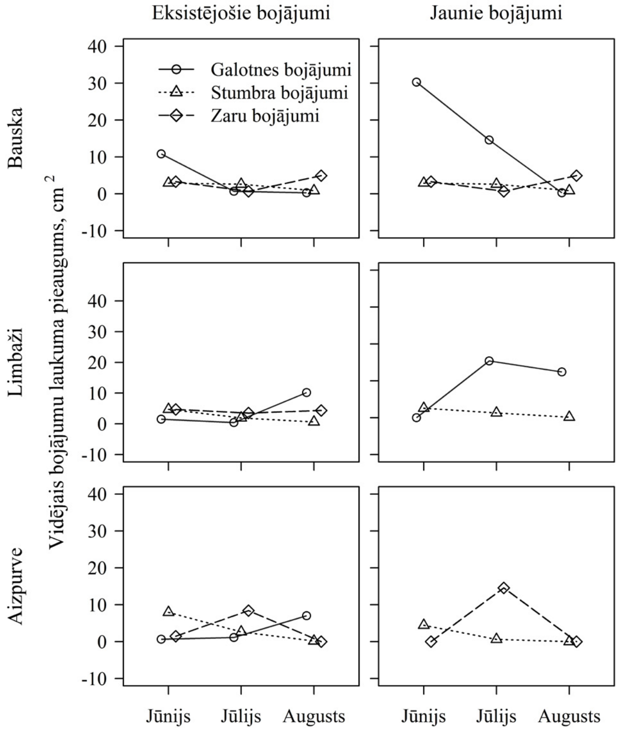
3.2. Patogēna *Hymenoscyphus fraxineus* bojājumu vienas sezonas attīstības dinamika

Oša kalšana lielākoties norit strauji; koki nokalst dažu gadu laikā pēc pirmo simptomu parādīšanās (atsevišķos gadījumos simptomi var nebūt redzami), bet dažiem kokiem slimība var būt hroniska, un to vitalitāte samazinās pakāpeniski (Bakys et al., 2009b; Timmermann et al., 2011; Enderle et al., 2013; Landolt et al., 2016). Analizējot bojājumu sezonālās attīstības dinamiku, var prognozēt, vai inficētie koki nokaltīs ātri, vai tiem attīstīsies hroniski bojājumi (Bengtsson et al., 2014). Patogēna *H. fraxineus* izraisīto stumbra, zaru un galotņu bojājumu attīstība pētīta trīs jaunaudzēs (III publikācija). Lielākā daļa analizēto bojājumu (67%) bija EL, lielākoties uz stumbriem (50%). Jaunie bojājumi, kas veidoja 33%, visbiežāk parādījās novērojumu sākumperiodā – jūnijā un jūlijā, norādot uz bojājumu aktivitātes sezonālo raksturu, ko var skaidrot ar lapu un dzinumu nobriešanu (Schumacher et al., 2009; Timmermann et al., 2011). Ņemot vērā, ka *H. fraxineus* saimniekaugu inficē caur dzinumiem un lapām (Kirisits & Cech, 2009; Kirisits et al., 2009; Cleary et al., 2013), lielākā daļa NL parādījās uz zariem (55%), kas neietekmē augstuma pieaugumu; tomēr jāņem vērā, ka kokam zaru ir vairāk nekā galotņu.

Bojājumu aktivitāte un izplešanās (laukuma pieaugums) ir saistīta ar meteoroloģiskajiem faktoriem (temperatūru) – visaugstākā tā bija jūnijā un jūlijā, kad novērota arī augstākā gaisa temperatūra (ca. 15–18°C), kas ir tuvu patogēna *H. fraxineus* optimālajai augšanas temperatūrai – 20°C (Kowalski & Bartnik, 2010;

Timmermann et al., 2011). Bojājumu izplešanās būtiski atšķīrās starp EL un NL, kā arī starp dažādām EL atrašanās vietām uz koka (3.2. att.). Bojājumu laukums palielinājās vidēji no 52.5 ±11.3 cm^2 (vidējais ±standartkļūda) vienam kokam jūnijā līdz 92.1 ±14.7 cm^2 novērojumu beigās septembrī (3.2. att.). Visātrāk palielinājās galotņu un zaru bojājumu laukums, jo tie ir tuvu koka stumbram, pa kuru arī notiek patogēna izplatība saimniekaugā (Schumacher et al., 2009), turklāt šajās vietās kokam ir plānāka miza, kas atvieglo infekcijas simptomu attīstību (Husson et al., 2012). Jaunie bojājumi attīstās trīs reizes ātrāk nekā EL, norādot, ka pirmajos mēnešos pēc parādīšanās bojājums strauji palielinās līdz noteiktam izmēram. Līdz ar to var secināt, ka saimniekaugam ir vajadzīgs apmēram mēnesis, lai vismaz daļēji ierobežotu patogēna attīstību. No otras puses, tas varētu būt saistīts ar jauno sēņu indivīdu augstāku aktivitāti (Lygis et al., 2016). Iegūtie dati liecina par koka spēju izdzīvot un augt arī pie augsta infekcijas fona.

Sezonas laikā 24% zaru un 22% galotņu bojājumu pilnīgi apņēma inficēto zaru/galotni, izraisot to nokalšanu. Tomēr novērota arī saimniekauga spēja ierobežot infekcijas izplatību (Bengtsson et al., 2014). No EL 22% bija neaktīvi, 13% zaru un 7% galotņu bojājumu vairs neizplatījās tālāk, tiem sasniedzot zaru pamatu vai stumbru. Tas varētu būt saistīts ar koksnes anatomiskajām īpašībām, piemēram,



3.2. att. **Eksistējošo (pirms novērojumu uzsākšanas) un jauno (parādās novērojumu laikā) bojājumu laukumu vidējais pieaugums mēnesī pētītajās audzēs atkarībā no bojājumu atrašanās vietas uz koka** atšķirībām vadaudu lielumā un savienojamībā, kā arī ar serdes diametra atšķirībām starp ikgadējiem augstuma pieaugumiem (Schweingruber, 2007). Taču novērota arī bojājumu pāreja no koku galotnēm un zariem uz stumbriem, jo 18% galotņu bojājumu turpināja izplatīties uz leju pa stumbru, kas liecina par šo bojājumu turpmāku attīstību nākamajā sezonā.

Oša vidējā vitalitāte (klasēs) pasliktinājās no 1.6 aktīvās veģetācijas sezonas sākumā uz 2.9 tās beigās. Tomēr vidējā NL proporcija, zemā koku mirstība (3.3%, cf. Lygis et al., 2014) norāda uz hronisku simptomu veidošanos. Neaktīvo bojājumu klātbūtne norāda, ka koks spēj ierobežot patogēna izplatību (Pallardy, 2008).

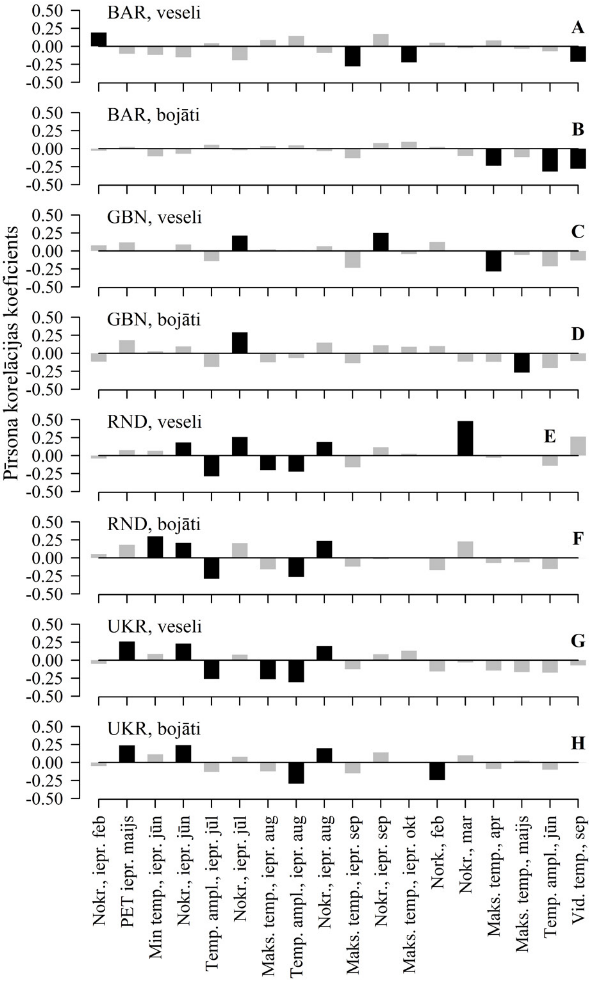
Pētījumā nav konstatēta būtiska sakarība starp koka vitalitāti novērojumu perioda sākumā un EL/NL attīstību, kā arī koka vitalitāti un bojājumu skaitu, kas norāda uz to, ka infekcijas fons visiem kokiem neatkarīgi no iepriekšējās infekcijas ir līdzīgs. Nav konstatētas būtiskas sakarības starp EL un NL skaitu, norādot uz līdzīgu bojājuma rašanās varbūtību neatkarīgi no iepriekšējās infekcijas, līdz ar to lielāka ietekme ir nejaušajiem procesiem vai mikroklimatam. Ošu izdzīvošanas iespējas ir individuālas, tomēr lielākajai daļai koku konstatēti hroniski bojājumi, kas labvēlīgos apstākļos, iespējams, varētu strauji progresēt. Apmēram ⅕ koku konstatētas nokaltušas galotnes, tādējādi samazinot to konkurētspēju.

3.3. Parastā oša jutība pret meteoroloģiskajiem faktoriem

Augšanas gaita un koku jutība pret meteoroloģiskajiem faktoriem var ietekmēt koku uzņēmību pret patogēniem (Helama et al., 2009; Tulik et al., 2018), tāpēc šāda informācija var būt vērtīga, novērtējot ADB saistību ar meteoroloģiskajām izmaiņām. Lai arī Latvijā osis atrodas tuvu tā dabiskā izplatības areāla ziemeļu robežai (BFW, 2020), augšanas individualitāte bija diezgan izteikta, līdzīgi kā tas ir kokiem optimālos apstākļos (Fritts, 2001). Tomēr novērotas kopējas tendences (IV publikācija). Oša uzņēmība pret patogēnu varētu būt saistīta ar koku novecošanos (Enderle et al., 2013), jo veselie koki bija jaunāki, tiem bija lielāks vidējais TRW un mazāka autokorelācija (0.77 vs. 0.82) nekā bojātajiem. Atšķirīgais pieaugums starp grupām (veseli un bojāti oši) liecināja par saistību ar koku sociālo stāvokli (t.i., Krafta klasēm) (Timmermann et al., 2011; Martin-Bento et al., 2008). Bojātajiem kokiem pēdējās desmitgadēs novērota nomākta augšana un paaugstināta atkarība no barības vielu rezervēm (autokorelācija).

Latvijas centrālajā daļā, TRW novērota jutība pret meteoroloģiskajiem faktoriem iepriekšējajā veģetācijas sezonā un miera periodā, kas liecina par vides faktoru ietekmes pārnesi laikā (Zweifel, 2020). Sugām ar aplocēs izkārtotiem vadaudiem barības vielu rezerves galvenokārt tiek izmantotas agrīnai augšanai (Barbaroux & Breda, 2002), kas ietekmē pieaugumu turpmākajā sezonā (Tyree & Zimmermann, 2002). Attiecīgi, vasaras nokrišņi atstāja pozitīvu ietekmi uz TRW (3.3. att.), norādot uz ūdens deficītu. Temperatūru maiņa var apgrūtināt fizioloģiskos procesus (Pallardy, 2008; Berry & Downton, 1982), izskaidrojot diennakts temperatūru amplitūdu negatīvo ietekmi uz TRW (3.3. att.). Nokrišņu un temperatūras ietekme iepriekšējā augustā bija vērojama visos parauglaukumos (3.3. att.), jo tas ir laiks, kad sākas barības vielu rezervju veidošanās (Barbaroux & Breda, 2002).

Latvijas austrumu daļā, galvenokārt uzrādot negatīvas korelācijas, ošu pieaugums bija jutīgs pret meteoroloģiskajiem faktoriem iepriekšējā rudenī (septembrī, oktobrī) un pašreizējā veģetācijas sezonā (aprīlis–septembris) (3.3. att.). Pieaugoša temperatūra rudenī var palielināt koku elpošanu, tādējādi zaudējot uzkrātās barības vielas (Ögren et al., 1997). Paaugstināta temperatūra septembrī var izraisīt ūdens deficītu (Traykovic, 2005), jo novērota pozitīva korelācija ar nokrišņiem (3.3. att.). Temperatūras negatīvo ietekmi pašreizējā pavasarī var izskaidrot ar aktīvā



3.3. att. **Būtstrepa Pīrsona korelācijas koeficienta vērtības starp meteoroloģiskajiem faktoriem un gadskārtu platumu atlikumu hronoloģijām ošiem ar vitālu (A, C, E, G) un bojātu (B, D, F, H) vainagu audzēs pie Barkavas (BAR), Gulbenes (GBN), Rundāles (RND) un Ukriem (UKR)**

Analizēts laika posms no 1934. (1948 veselajiem kokiem RND) līdz 2010. gadam. Būtiskās korelācijas (pie α = 0.05) norādītas ar tumšāku krāsu. Parādīti tikai būtiskie faktori. PET – potenciālā evapotranspirācija.

perioda agrāku sākumu (agrāku lapu plaukšanu), pakļaujot kokus vēlu salnu riskam (Gu et al., 2008).

Veseliem un bojātiem kokiem atšķirības jutībā pret meteoroloģiskajiem faktoriem nebija izteiktas (3.3. att.), kas norāda uz nelielu meteoroloģiskā jutīguma ietekmi uz oša uzņēmību pret slimību. Bojātie koki Latvijas centrālajā daļā bija jutīgāki pret ūdens deficītu un temperatūras režīmu iepriekšējā augustā (3.3. att.), norādot, ka nelabvēlīgos apstākļos, kokiem ir lielāks stress, un līdz ar to uzņēmība pret patogēnu (Timmermann et al., 2011). Tomēr veselajiem kokiem papildus novērota jutība pret maksimālo temperatūru iepriekšējā augustā un nokrišņiem martā. Martā nokrišņi parasti ir sniega veidā, un to iedarbību var izskaidrot ar sniega kārtas izolējošajām īpašībām, kas ietekmē saknes (Hardy et al., 2001; Tierney et al., 2001) un gruntsūdens līmeni (Tyree & Zimmermann, 2002). Šādu attiecību nekonstatēšana var nozīmēt, ka bojātajiem kokiem jau pirms inficēšanās sakņu sistēma bijusi mazāk jutīga. Austrumlatvijā bojātos kokus vairāk ietekmēja jūnija un jūlija temperatūra. Vecāko koku TRW (GBN parauglaukumā) uzrādīja augstāku jutīgumu pret nokrišņiem, it īpaši bojātiem kokiem (3.3. att.), iezīmējot ar vecumu saistītās jutības izmaiņas (Carrer & Urbinati, 2004), un atspoguļojot ūdens stresa ietekmi uz koku uzņēmību pret šo slimību (Chira et al., 2017; Kowalski et al., 2010). Tieša sakarība starp oša vitalitāti un koku jutību pret meteoroloģiskajiem faktoriem netika nevērota, tomēr saistība starp pieaugumu un meteoroloģiskajiem faktoriem liecināja par pieaugošu stresu klimata pārmaiņu rezultātā, un attiecīgi paaugstinātu uzņēmību pret patogēnu.

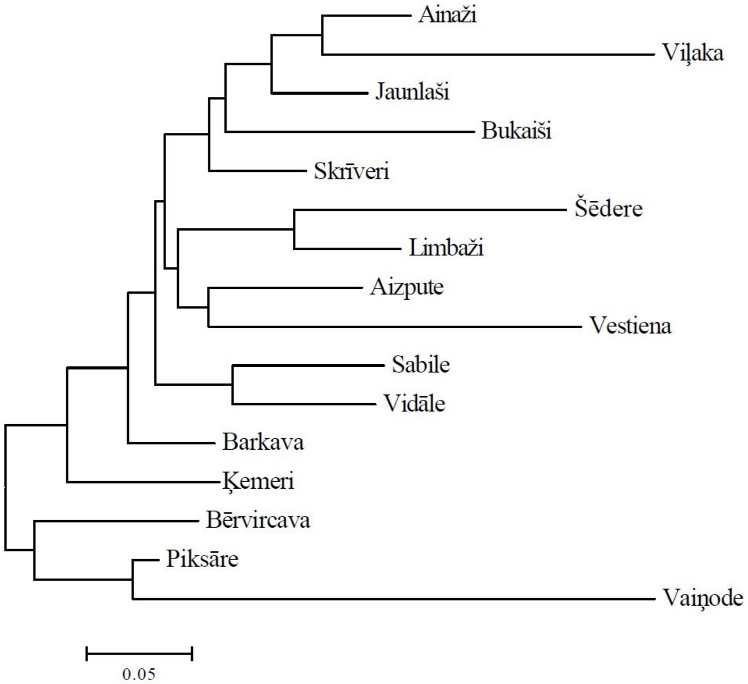
3.4. Parastā oša ģenētiskā daudzveidība Latvijā

Populācijas ģenētiskā daudzveidība ir viens no galvenajiem faktoriem, kas nosaka tās adaptāciju jaunai un strauji mainīgai videi, tai skaitā pēc dažādiem traucējumiem (Aitken & Bemmels, 2016; Tiffin & Ross-Ibarra, 2017). Parastā oša ģenētiskā daudzveidība un populāciju struktūra pētīta, izmantojot hloroplastu un kodolu DNA marķierus. Trīs no sešiem pētītajiem hloroplastu SSR marķieriem bija polimorfiski (V publikācija). Šo trīs lokusu genotipi apvienoti divos haplotipos: H01 bija sastopams visās audzēs, izņemot Ķemerus, kas liecina par atšķirīgu populācijas izcelsmi. Šis haplotips ir plaši izplatīts Austrumeiropā un Skandināvijā. Ķemeros identificētais haplotips H02 ir visizplatītākais Centrāleiropā (ieskaitot Poliju) (Heuertz et al., 2004a). Iespējams, ka oši, kuriem konstatēts H02, cēlušies no tuvumā esošā parka ošu sēklu kokiem, kuri parkā kā stādmateriāls ievesti no Rietumeiropas (Dambis et al., 2007). Eiropā visbiežāk ir sastopami haplotipi H01 un H02, kas kopā veido 68% no visiem indivīdiem (Heuertz et al., 2004a). Interesanti, ka Ķemeru audze kodolu SSR marķieru analīzē neatšķīrās no pārējām audzēm (V publikācija), atspoguļojot vietējo populāciju hibridizāciju. Tas norāda uz Latvijas oša populāciju lokālu specializāciju, kas pastāv arī pie intensīvas gēnu plūsmas (Aitken & Bemmels, 2016; Moran et al., 2017), kas, iespējams, veidojas augstas fragmentācijas dēļ (Liepiņš et al., 2016).

Novērotā heterozigotāte (vidējā) bija zemāka nekā sagaidīts (vidējās vērtības attiecīgi 0.64 vs. 0.82), kas liecina par samazinātu populācijas pielāgošanās spēju ekstremāliem apstākļiem (Aitken & Bemmels, 2016; Tiffin & Ross-Ibarra, 2017), tai skaitā patogēnu invāzijām. Attiecīgi, fiksācijas indekss bija pozitīvs visiem lokusiem (vidējais 0.23). Kopumā identificētas 14 privātas alēles, no kurām attiecīgajās audzēs tikai trim bija frekvence >0.05, tomēr vairums no tām nebija efektīvas, norādot uz zemas frekvences alēļu lielo īpatsvaru.

Kodolu SSR marķieru analīze AMOVA parādīja zemu, bet nozīmīgu (*Fst* = 0.045, *p* < 0.001) populāciju diferenciācijas līmeni, kas norāda uz vietējo specializāciju (Aitken & Bemmels, 2016). Ģenētiski visatšķirīgākās bija Viļakas un Vaiņodes audzes (pāru *Fst* = 0.151), kā arī Šēderes un Vestienas audzes (3.4. att.). Taču populāciju grupēšanai nebija ģeogrāfisks raksturs. To pašu apstiprināja ar delta K kritērijs.

Oša ģenētiskā daudzveidība Latvijā ir zemāka nekā lielākajā daļā dienvidu populāciju (3.2. tabula). Alēļu skaits gandrīz visos lokusos ir augstāks citās valstīs, izņemot Skotiju. Oša populācijām, kas atrodas tuvu sugas izplatības areāla ziemeļu malai, ir zemāka ģenētiskā daudzveidība, ko var skaidrot ar lokālo specializāciju pret skarbākiem apstākļiem pēcleduslaikmeta rekolonizācijas gaitā (Wallander, 2008). Inbrīdinga koeficients bija pozitīvs gandrīz visiem analizētajiem lokusiem (3.2. tabula), netieši norādot, ka tā varētu būt parastā oša vispārīga iezīme, kas saistīta ar sugas poligāmiju (Wallander, 2008). Oša ģenētiskā struktūra liecināja par



3.4. att. **Analizēto audžu pāru Nei ģenētiskās distances tuvākā kaimiņa dendrogramma** zemu kodola ģenētisko daudzveidību ar nelielu lokālo specializāciju, kas var noteikt vietējās populācijas ierobežoto spēju pielāgoties dažādiem apstākļiem. Tomēr genofonds Ķemeros, kas uzrādīja saistību ar citu populāciju, var tikt uzskatīts par papildu ģenētiskās daudzveidības avotu.

3.2. tabula

Alēļu skaita un inbrīdinga koeficienta salīdzinājums starp dažādām parastā oša populācijām

Populācija / Lokusi	Latvija[1]		Skotija[2]		Francija[3]		Itālija[4]		Bulgārija[5]		Bosnija un Hercegovina[6]		Rumānija[7]	
-	Na	F	Na	F	Na	F	Na	F	Na	F	Na	F	Na	F
Femsatl4	25	0.46	nd	nd	37	0.10	32	0.27	50	0.08	20	0.39	37	0.01
Femsatl10	34	0.42	nd	nd	nd	nd	76	0.33	nd	nd	55	0.37	nd	nd
Femsatl11	17	0.09	nd	nd	40	0.03	42	0.31	32	0.08	24	0.33	32	0.01
Femsatl16	10	0.27	6	0.03	nd	nd	9	0.19	10	0.12	12	0.16	10	0.17
Femsatl19	21	0.08	19	0.33	36	0.13	55	0.08	33	0.13	26	0.17	27	–0.07
M2-30	37	0.14	30	0.19	56	0.16	nd	nd	59	0.11	nd	nd	42	0.04

a – alēļu skaits; F – inbrīdinga koeficients; nd – nav datu. 1 – šis pētījums; 2 – Bacles et al., 2005; 3 – Morand et al., 2002; 4 – Ferrazzini et al., 2007; 5 – Heuertz et al., 2001; 6 – Ballian et al., 2008; 7 – Heuertz at al., 2003.

3.5. Kokaugu sukcesija parastā oša destrukcijas skartajās audzēs

Pēc dažāda veida traucējumiem, tai skaitā slimībām, mežaudzes atjaunošanās un tās dinamika izteikti mainās, tādējādi ietekmējot turpmāko audzes sastāvu un koku vitalitāti (Lygis et al., 2014; Thomas et al., 2018). Dolan & Kilgore (2018) novēroja, ka pēc oša smaragdzaļās krāšņvaboles bojājumiem strauji pieaug ēncietīgo paaugas un pameža sugu biezums; samazinoties ošu skaitam, palielinās dažādu svešzemju un vietējo pameža sugu biezums.

Balstoties uz apvienotajiem rezultātiem no VI un VII publikācijas, vidējais paaugas un pamežā biezums vidēja līdz pieauguša vecuma audzēs laikā no 2005.–2015. gadam attiecīgi bija 6520 ±1401 un 11193 ±1751 indivīdi ha^{-1}. Pārsvarā atjaunojas platlapji un pioniersugas; lielākais biezums konstatēts parastajai gobai, osim, parastajai kļavai, un parastajai liepai *Tilia cordata* Mill. Līdzīgās proporcijās atjaunojas divas līdz četras sugas, norādot uz mistrotu audžu veidošanos nākotnē. Tomēr audzēs, kur I stāvā dominē parastā kļava, veidojas bieza kļavas paauga, kas var veicināt kļavu tīraudžu veidošanos. Lielākoties paaugā atjaunojas sugas, kas dominē I stāvā, bet audzēs, kurās dominēja osis, sākusies intensīva pārkrūmošanās (1. tabula VI publikācijā). Lai gan parastā lazda *Corylus avellana* L. tiek uzskatīta par vērtīgu sugu oša mežos (Loidi, 2004), kopā ar parasto ievu *Padus avium* Mill. tā veido 53% no pameža (VI un VII publikācija), kas ierobežo paaugas atjaunošanos (Runkle, 1990; Gillman et al., 2003; Royo & Carson, 2006). Turklāt oša kalšana sekmē traucējumus mīlošo adventīvo sugu ieviešanos (piem., Kaukāza plūme *Prunus cerasifera* var.

divaricata Bailey., pīlādžlapu sorbārija *Sorbaria sorbifolia* (L.) A. Braun un jāņogas *Ribes* spp.; Gonzales et al., 2002) (1. tabula VI publikācijā).

2015. gada uzskaitē konstatēta strauja paaugas un pameža biezuma palielināšanās (gandrīz dubultošanās), taču, pretstatā citām publikācijām (de la Cretaz & Kelty, 2002; Coomes et al., 2003; Royo & Carson, 2006), lielākajā daļā audžu biezuma palielināšanās nebija saistīta ar ADB apjomiem. To varētu skaidrot ar paaugas un pameža aizkavētu reakciju pret ADB. Neskatoties uz sugu skaita pieaugumu, paaugas un pameža biezums visos trīs periodos saglabājas līdzīgs (attiecīgi, 36 un 64%), mainoties tikai 2% robežās.

Pamatojoties uz zemo paaugas oša biezumu (1987 ±397 oši ha^{-1}), var secināt, ka oša tīraudzes dabiskā veidā neveidosies, taču osis saglabāsies kā piemistrojuma suga. Lai gan 81% no uzskaitītās oša paaugas bija veseli, tikai 10% pārsniedza 0.5 m augstumu, norādot uz intensīvu pašizretināšanos (līdzīgus rezultātus ieguvuši Sakss (1958) un Giongo et al. (2017)), un, iespējams, arī ADB ietekmi uz to. Kokiem augot, veselības stāvoklis pasliktinās, piemēram, no ošiem, kas pārsnieguši 2 m augstumu, 32% bija veseli, bet 17% – nokaltuši. Līdz ar to ir sagaidāms, ka tikai neliela daļa (<5%) no tiem sasniegs I stāva līmeni, un, līdzīgi kā prognozēja Laiviņš & Mangale (2004), osis nākotnē būs piemistrojuma suga.

Oša paaugas biezumu būtiski ietekmē I stāvā dominējošā suga. Novērots, ka šobrīd oša paaugas atjaunošanās zem mātes audzēm praktiski nenotiek, lai gan pirms ADB oša paaugas skaits pozitīvi korelēja ar mātes koku skaitu un tuvumu (Harmer et al., 2005). Baltalkšņu un melnalkšņu īpatsvara pieaugums I stāvā negatīvi ietekmē oša paaugas atjaunošanās biezumu un vitalitāti, taču bērzam ir pozitīva ietekme. Novērota pozitīva korelācija starp paaugas oša biezumu un kopējo sugu skaitu paaugā un pamežā ($\tau = 0.184$, $p = 0.03$), kas liecina par sugu daudzveidības pozitīvo ietekmi (Forrester & Bauhus, 2016). Lai gan ADB skartajās audzēs osis paaugā atjaunojas, tas ir inficēts ar *H. fraxineus*, kas samazina konkurētspēju un palielina koku mirstību. Sagaidāms, ka nākotnē citas koku un īpaši krūmu sugas konkurēs ar osi un, audzēm pakāpeniski pārveidojoties, osis saglabāsies tikai kā piemistrojuma suga.

3.6. Parastā oša dabiskā atjaunošanās jaunaudzēs

3.6.1. Sugu sastāvs un oša atjaunošanās

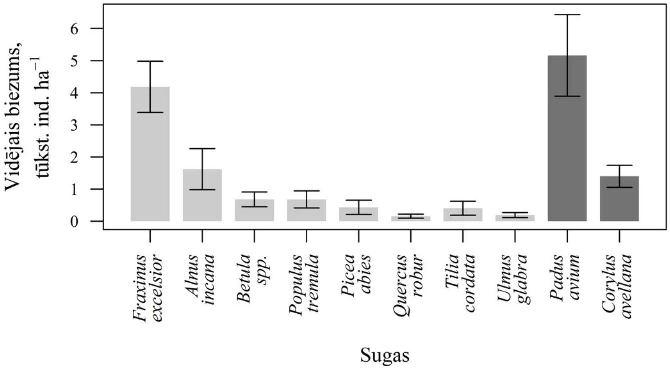
Eiropā, tai skaitā Latvijā, slimības skartās oša audzes tiek nozāģētas un vēlāk aizstātas ar citām koku sugām, savukārt daļu no šīm teritorijām paredz dabiskai atjaunošanai, jaunos ošus pakļaujot lielai konkurencei (Lygis et al., 2014; McKinney et al., 2014). Oša jaunaudzēs vidējais pameža un paaugas biezums attiecīgi bija 9497 ±1454 un 7150 ±558 indivīdi ha^{-1} (VIII publikācija). Paaugā vislielākais biezums ir osim (4185 ±401 oši ha^{-1}), baltalksnim, bērzam un parastajai apsei, bet pamežā dominē divas sugas – ieva un lazda (3.5. att.). Oša kalšana nav ietekmējusi jaunās kokaudzes sastāvu, jo paaugā atjaunojas tās pašas sugas (3.5. att.; Lygis et al., 2014), kas pirms ADB (Sakss, 1958). Taču sugu proporcijas ir izmainītas. Oša paaugas biezums Latvijā ir lielāks, nekā novērots Lietuvā (599 oši ha^{-1}), Igaunijā (3500 oši ha^{-1}) un Vācijā (653 oši ha^{-1}) (Lygis et al., 2014; Drenkhan et al., 2017; Enderle et al., 2018), bet zemāks nekā Eiropā tas bija pirms ADB (15–30 10^3 oši ha^{-1}; Sakss, 1958; Harmer et al., 1997; Tabari & Lust, 1999; Lygis et al., 2014). Paaugas un pameža attiecība ir 48.4 vs. 51.6; palielinātais pameža sugu īpatsvars (3.5. att.) norāda uz progresējošām izmaiņām audžu sastāvā.

Oša kalšana ir veicinājusi pameža sugu attīstību; lielais to biezums izkonkurē osi, jo novērota negatīva korelācija starp oša un pameža, kā arī paaugas biezumu (attiecīgi $r = 0.24$ un 0.23). Līdzīgas starpsugu attiecības slimību skartās audzēs novērotas arī citos pētījumos (Keer, 1998; Givnish, 2002; Royo &Carson, 2006; Skovsgaard et al., 2010).

3.6.2. Hymenoscyphus fraxineus sastopamība oša paaugā

No uzskaitītās oša paaugas 10.0% bija nokaltuši (VIII publikācija), kas ir pielīdzināms dabiskai oša paaugas mirstībai (Sakss, 1958; Harmer et al., 2005). Diemžēl, pamatojoties uz pieejamajiem datiem, ir grūti noteikt ADB komplementāro efektu uz mirstību. Piemēram, Sakss (1958) konstatējis, ka oša paauga kailciršu izcirtumos nīkuļo un aiziet bojā, līdzīgi arī Anglijā pirms ADB pēc pameža izvākšanas un I, II stāva ošu retināšanas oša paaugas biezums ik gadu samazinājās par 40–50% (Harmer et al., 2005). Salīdzinājumam – dabiski atjaunojušos oša paaugas mirstība Igaunijā, Lietuvā, Vācija un Itālijā bija attiecīgi 7%, 17%, 16.2% un 17.6% (Drenkhan et al., 2017; Enderle et al. 2017; Giongo et al., 2017), kas norāda uz oša vitalitātes reģionālām izmaiņām. Būtiskas reģionālas izmaiņas novērotas arī Latvijas mērogā, jo oša vitalitāte Latvijas rietumu daļā bija sliktāka nekā centrālajā un austrumu daļā.

Novērota sakarība starp oša paaugas vitalitāti un augstumu ($r = 0.28$, $p < 0.001$), kā arī vecumu ($p < 0.001$, 3.6. att.). Veselo un nokaltušo paaugas ošu proporcija līdz un virs 3 m, attiecīgi bija 81 vs. 4% un 54 vs. 33%, līdzīga sakarība līdz un virs 2 m novērota arī Itālijas Alpos (70 vs. 14% un 38 vs. 20%; Giongo et al., 2017). Tas nozīmē, ka ir nepieciešams konkrēts laiks, lai oša paauga tiktu inficēta, kā arī to, ka slimības spiediens uz vecākiem kokiem pieaug.



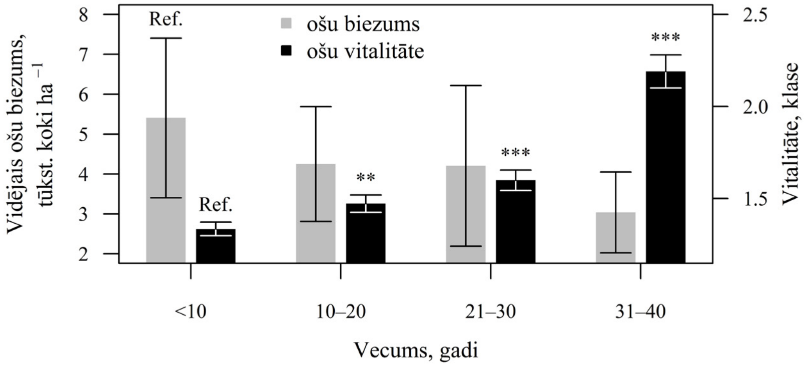
3.5. att. **Oša jaunaudžu (≤40 gadi) paaugā un pamežā dominējošo sugu vidējais biezums**

Oša pieauguma analīzē novērota augsta vietas specifika, kas norāda uz sugas plastiskumu. Konstatēta būtiska lineāra sakarība starp ošu diametru, kā arī vecumu un augstumu (VIII publikācija). Pirmos 5–8 gadus ošu augstums palielinājās neregulāri un individuāli, ko varēja ietekmēt atšķirīgie augšanas apstākļi. Ošu augstumu un diametru negatīvi ietekmēja slimība, kas liecina par patogēna kumulatīvo iedarbību. Zemāko un mazākā caurmēra koku vitalitāte bija labāka, bet ar laiku tā būtiski pasliktinājās. Kopumā paaugas oša vitalitāte Latvijā jaunaudzēs bija labāka nekā vecākās audzēs, kas, iezīmējot reģionālas atšķirības, ir pretstatā Drenkhan et al. (2017) novērotajam Igaunijā. Salīdzinot ar Centrāleiropu, Latvijā oši atjaunojas labāk, un to mirstība ir zemāka. Tomēr atklātos apstākļos (t.i. pēc vienlaidus atjaunošanas cirtes) ošu augstuma pieaugumi intensīvā konkurencē ir samazināti, kas ierobežo to kopējo konkurētspēju, tādējādi veicinot jauktu audžu veidošanos ar osi piemistrojumā.

3.7. Oša paaugas atjaunošanos un vitalitāti ietekmējošie faktori

Oša kalšanas ierobežošana pieaugušās audzēs, pielietojot jebkādas mežkopības metodes, ir praktiski neiespējama (Gross et al., 2014). Taču, veidojot jaunaudzes, gan oša populācijas saglābšanai, gan komerciāliem nolūkiem ir iespējams ņemt vērā vides aspektus un izmantot dažādas metodes, kas varētu pozitīvi ietekmēt oša vitalitāti lokālā mērogā (Havrdová et al., 2017; Skovsgaard et al., 2017).

Oša paaugas biezums bija līdzīgs visā Latvijas teritorijā (VIII publikācija), dažāda vecuma audzēs (3.6. att.), meža tipos, un dažāda iepriekšējā sastāva audzēs. Lai gan lielāka sugu daudzveidībai pozitīvi ietekmē oša paaugas vitalitāti (Fraxigen, 2005; Dobrowolska et al., 2011; Ahlberg, 2014), paaugas sastāvam nebija būtiskas ietekmes uz oša biezumu Latvijā. Lielākais oša paaugas biezums jaunaudzēs konstatēts, augot kopā ar baltalksni (labākā palīgsuga osim arī pirms ADB; Sakss, 1958) un bērzu, kas daļēji ir pretstatā rezultātiem pieaugušās audzēs (VII publikācija). Parastās egles klātbūtnei atkarībā no sugu proporcijām novērota mainīga ietekme uz oša paaugas biezumu. Ja audzi veido parastā egle un osis, nelielam egles biezumam

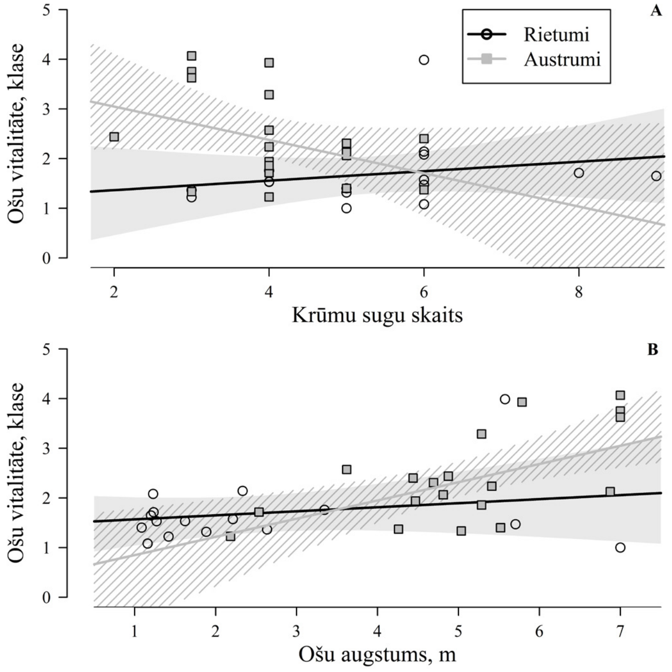


3.6. att. **Vidējais oša paaugas biezums un vitalitāte dažāda vecuma jaunaudzēs**
Zvaigznītes norāda atšķirību no jaunākajām audzēm, kas pieņemtas par atsauces līmeni (Ref.), būtiskumu. Būtiskuma līmeņi: * – $p \leq 0.05$, ** – $p < 0.01$, *** – $p \leq 0.001$.

(<30%) ir negatīva ietekme uz oša biezumu, bet augstāks egles biezums (60–80%) uzrāda pretēju efektu. Taču iegūtie rezultāti varētu būt arī nejauši.

Oša vitalitāti ietekmēja audzes struktūra. Izmantojot jaukta tipa regresiju, noteikts, ka paaugas augstums un pameža sugu skaits vislabāk raksturoja oša vitalitāti, taču abu šo rādītāju ietekmei bija izteiktas reģionālas atšķirības (mijiedarbība; IX publikācija).

Izteiktās reģionālās atšķirības starp pameža sugu daudzveidību un oša vitalitāti (3.7. att.) varētu būt saistītas ar meteoroloģiskajiem apstākļiem (Papic et al., 2018). Maigāks klimats Latvijas rietumu daļā acīmredzot veicina *H. fraxineus* izplatību (Kowalski & Bartnik, 2010; Dvorak et al., 2016), kas limitē oša augstuma pieaugumu veidošanos (3.3. tabula, 3.7. B att.). Iespējams, ka šādos apstākļos piemistrojuma sugām vieglāk izkonkurēt osi, pasliktinot tā vitalitāti (3.7. A att.). Latvijas austrumu daļā, kur klimats ir skarbāks, tādējādi mazāk piemērots *H. fraxineus* attīstībai (Kowalski & Bartnik, 2010), pameža sugu daudzveidībai bija pozitīva ietekme uz oša vitalitāti (3.7. A att.), jo pameža sugas, iespējams, darbojas kā bioloģiskā barjera (Kosawang et al., 2018) un kavē patogēna attīstību (Jactel et al., 2005; Pautasso et al., 2005). Paaugas augstums šajā reģionā bija lielāks, kas līdzīgi kā Papić et al. (2018) pētījumā liecina par labākiem augšanas apstākļiem kontinentālā klimatā.



3.7. att. **Oša vitalitātes (klases) un krūmu sugu skaita (A), kā arī oša paaugas augstuma (B) attiecības mistrotās oša jaunaudzēs Latvijas rietumu un austrumu daļā (provenienču reģionos)**
Poligoni (iesvītrojums) parāda 95% ticamības intervālus.

3.3. tabula

Jaukta tipa ranžētās binomiālās regresijas, kas apraksta oša vitalitāti jaunaudzēs atkarībā no audzes un koku īpašībām, apraksts

Modeļa fiksētās daļas dispersijas tabula, II tipa tests

Mainīgais	χ^2	Brīvības pakāpju skaits	*p*-vērtība
Krūmu sugu skaits	0.14	1	0.71
Reģions	0.48	1	0.49
Ošu augstums	245.04	1	<0.001
Krūmu sugu skaits × reģions	9.12	1	<0.01
Reģions × ošu augstums	9.43	1	<0.01

Lai gan pētījumos nav vienprātības par augšanas apstākļu ietekmi uz *H. fraxineus* bojājumu apjomu (Bakys et al., 2013; Timmermann et al., 2017), Latvijā oša vitalitāte būtiski atšķīrās starp dažādiem augšanas apstākļiem (VIII publikācija). Līdzīgi kā Gross et al (2014) pētījumā, oša vitalitāte bija sliktāka mitros augšanas apstākļos, labākā oša vitalitāte bija sausās un labi drenētās minerālaugsnēs (>76% ošu bija veseli vai ar minimāliem slimības simptomiem), bet sliktākā – slapjās minerālaugsnēs, kur 27% ošu bija nokaltuši. Nosusināšanas pozitīvā ietekme uz oša vitalitāti konstatēta arī citās Eiropas valstīs (Dobrowolska et al., 2011; Schumacher, 2011; Ahlberg, 2014).

Līdzīgi kā Centrāleiropā un Rietumeiropā (Dobrowolska et al., 2011; Schumacher, 2011; Stener, 2013), Latvijā labāka oša atjaunošanās un vitalitāte konstatēta jauktās audzēs (82–95% ošu veseli, 1% nokaltuši), nevis tīraudzēs (66% vs. 20%; VIII publikācija). Taču būtiska nozīme ir arī paaugas sugu sastāvam (dominējošajai sugai). Lielākie *H. fraxineus* bojājumi novēroti jaunaudzēs, kur dominē parastā egle vai kļava (egles/oša audzēs – 40% ošu bija nokaltuši, egles audzēs – 12%, kļavas – 14%). Tas skaidrojams ar vainagu un sakņu konkurenci, kā arī ar augsnes paskābināšanos (Lei et al., 2012) un neizteiktām patogēna izplatības barjerām (Pautasso et al., 2005). Lai arī osim un parastajai kļavai ir līdzīga augšanas stratēģija (Petritan et al., 2009), oša konkurētspēja ADB ietekmē ir samazināta (Urbinati & Cillia, 1995), tādējādi uzlabojot parastās kļavas dominanci. Līdzīgi kā Givnish (2002) pētījumā, labākā oša vitalitāte konstatēta audzēs, kuru sastāvā ir bērzs (bērza un bērza/oša audzēs 87% ošu bija veseli) un parastā apse (89%), norādot uz pioniersugu klātbūtnes pozitīvo ietekmi.

Oša paaugas vitalitāti var uzlabot ar apsaimniekošanu – regulāru kopšanu (Niemelä et al., 1992; Guzman & Dirzo, 2001; Fraxigen, 2005), tādējādi ošiem samazinot biotisko (konkurence) un abiotisko (paaugstināts mitrums) faktoru radīto stresu (Cech & Hoyer-Tomiczek, 2007; Skovsgaard et al., 2010; Bakys et al., 2013). Būtiski labāka (VIII publikācija) oša vitalitāte novērota jaunaudzēs, kas bija koptas visbiežāk – četras reizes, taču, samazinoties kopšanu skaitam, vitalitāte pasliktinājās. Dabiski atjaunojušos oša jaunaudžu atlase un saglabāšana atbilstoši vides apstākļiem un piemērota apsaimniekošana veicinās oša saglabāšanos nākotnē.

SECINĀJUMI

1. Laika posmā no 2005. līdz 2015. gadam visā Latvijas teritorijā novērota strauja oša kalšana (mirstība 6.9% gadā), krājai samazinoties attiecīgi no 322 uz 151 m^3 ha^{-1}. Oša mirstība visā Latvijā bija līdzīga, bet vitalitātei novērotas lokālas atšķirības, kas norāda uz nevienmērīgu oša audžu atjaunošanās potenciālu nākotnē.
2. Ošu bojājumu attīstībai ir sezonāls raksturs. Strauja bojājumu attīstība notiek siltākajos vasaras mēnešos, bet tā nav saistīta ar koka vitalitāti un iepriekšējo bojājumu daudzumu uz koka. Bojājumu attīstības dinamika norāda uz oša spēju lokalizēt slimības izplatību primāri skartajās vasas daļās. Hroniski bojājumi konstatēti 78% analizēto koku; oša konkurētspēju būtiski samazina galotņu kalšana, kas novērota aptuveni piektajai daļai inficēto koku.
3. Ierobežotā oša audžu ģenētiskā daudzveidība, uz ko norāda kopumā vienveidīgā izcelsme un zemā kodola ģenētiskā daudzveidība (augsts inbrīdinga koeficients), liecina par izteiktu ģenētisko specializāciju un ierobežotām spējām uzlabot rezistenci pret patogēnu, izmantojot vietējo reproduktīvo materiālu.
4. Novērotās korelācijas starp pieaugumu un meteoroloģiskajiem faktoriem liecina par pieaugošu stresu klimata pārmaiņu rezultātā, un attiecīgi paaugstinātu uzņēmību pret patogēnu. Bojātie un veselie koki uzrādīja kopumā līdzīgu augšanas jutību pret meteoroloģiskajiem faktoriem.
5. Osis dabiski atjaunojas gan jaunaudzēs (4185 ±401 oši ha^{-1}), gan slimības skartajās mežaudzēs (1987 ±397 oši ha^{-1}), taču jaunaudzēs vitālās paaugas īpatsvars ir apmēram ⅓ no kopējā īpatņu skaita, un, kokiem augot, tas turpina samazināties. Mežaudzēs 90% oša paaugas iznīkst jau sējeņu stadijā. Oša konkurētspēju samazina citu platlapu sugu piemistrojums. Oša mirstības rādītāji Latvijā ir zemāki nekā Centrāleiropā, kas norāda uz reģionālām rezistences atšķirībām.
6. Apsaimniekošanas un augšanas apstākļiem ir reģionāla ietekme uz oša vitalitāti, bet ne uz atjaunošanās biezumu (koku skaitu uz hektāra). Intensīva kopšana uzlabo oša audžu fitosanitāro stāvokli; augstāka koku vitalitāte konstatēta sausieņu un meliorētajos mežos. Audzes sastāvam ir izteikta reģionāla ietekme uz oša vitalitāti – kokaudzes sugu daudzveidībai ir pozitīva ietekme Latvijas austrumu daļā, tomēr piemistrojuma sugām var būt atšķirīgs efekts.

PRIEKŠLIKUMI

1. Ņemot vērā atšķirīgo oša vitalitāti un pozitīvo saimnieciskās darbības ietekmi, svarīgi veikt oša veselības stāvokļa monitoringu, lai apzinātu vitalitāti ietekmējošos faktorus. Monitorings veicams veģetācijas sezonā, kad vislabāk identificējami slimības simptomi.
2. Primāri oša audzes kā vērtīgs ģenētiskais materiāls jāsaglabā Latvijas austrumu daļā, kur oši ir visvitālākie. Tomēr vitālākās oša audzes ģenētiskās daudzveidības uzturēšanai vēlams saudzēt visā Latvijā. Ieteicama ģenētiski atšķirīgāko populāciju identifikācija selekcijas potenciāla nodrošināšanai.
3. Meža apsaimniekošana veicama atšķirīgi jaunaudzēm un citu vecumgrupu audzēm: oša saglabāšanai vēlams veidot mistrotas jaunaudzes ar nelielu oša īpatsvaru. Tajās identificējami vitālākie oši, kuriem veicināma ilgtermiņa izdzīvošana, nodrošinot intensīvu kopšanu (atēnošanu). Citu vecumgrupu audzēs vitālie dominantie koki atstājami kā sēklu avots. Audzes ar zemu vitalitāti ilgtermiņā nav saglabājamas.

PATEICĪBAS

Pirmkārt, es pateicos vadītājiem Natālijai Burņevičai un Tālim Gaitniekam par atbalstu, konsultācijām un iedrošinājumu visa promocijas darba izstrādes laikā. Īpašs paldies Robertam Matisonam, gan kā darba vadītājam, gan kā vīram, par pirmo pieredzi zinātnē (publikāciju rakstīšanā), atbalstu ar datu statistisko apstrādi, vispārīgiem padomiem, nenovērtējamu palīdzību novest iesākto līdz galam, kā arī motivāciju publikāciju un promocijas darba sagatavošanas laikā.

Es esmu pateicīga vadošajam pētniekam Dr. habil. geogr. Mārim Laiviņam par promocijas darba ideju un savu ar ošiem saistīto darba rezultātu uzticēšanu. Liels paldies maniem kolēģiem no meža ekoloģijas un mežkopības grupas par palīdzību lauku darbos, kā arī kolēģiem no meža patoloģijas un mikoloģijas grupas par laboratorijas darbiem un padomiem saistībā ar patogēnu. Pateicos Dr. Rimvydas Vasaitis par pirmo starptautisko zinātnisko pieredzi un iespēju pievienoties starptautiskai ADB pētnieku komandai (FRAXBACK).

Liels paldies manai ģimenei, īpaši mammai un tētim, kas mani jau bērnībā iepazīstināja ar mežu un tajā notiekošo.

1. INTRODUCTION

1.1. General description of common ash

Common ash *Fraxinus excelsior* L. (Oleaceae) is one of four species of ash native to Europe (Thomas, 2016). Its range covers 64% of the European territory (Grime et al., 2007), from southern Fennoscandia to northern Greece and from northern Spain to the Volga River basin in the western Russia (Thomas, 2016) (Fig. 1.1). Its altitudinal distribution reaches 2200 m a.s.l. in mountainous areas (Pyrenees, Alps) (Beck et al., 2016). The natural range of ash coincides with that of pedunculate oak *Quercus robur* L., a characteristic species of the nemoral forest zone in Europe (Dobrowolska et al., 2011). In Europe, common ash occurs in vast areas of diverse age classes of primary and secondary woodlands. Typical habitats are deciduous forests, valley forests, river banks, meadows (overgrown), as well as other open habitats. Common ash forests contribute 1–2% of the forest area and standing volume stock in most European countries (Fischer & Lorenz, 2011; Rozsypálek et al., 2017). The proportion of common ash forests in the Northern area of its distribution range is smaller than in Central Europe and particularly England (Cleary et al., 2017; Drenkhan et al., 2017; Heinze et al., 2017).

In Sweden (Dobrowolska et al., 2011) and the UK (Wardle, 1961), ash has been considered as a pioneer species, while in Denmark it is considered as an intermediate between a pioneer and climax species (Ahlberg, 2014). In Central and Northern Europe, ash has been associated with invasive species (Wagner, 1990; Fraxigen, 2005) and the term 'fraxinisation', which is defined by highly successful self-regeneration, has been used (Fraxigen, 2005).

Common ash is an ecologically plastic tree species, which occurs across a wide range of soil types (except on acid soils) and growth conditions (Wardle, 1961). In the eastern part of its natural range, ash can dominate in floodplain forests and on moist clay-loam lowland sites (Dufour & Piegay, 2008), and in forests on relatively dry sites on stony mountain slopes, and in ravine forests (Weber-Blaschke et al., 2008). Therefore, historically two different ecotypes of ash have been described: the so-called 'water ash' adapted to moist site conditions and 'chalk ash' adapted to dry calcareous sites (Münch & Dietrich, 1925). Common ash in Europe dominates mainly on sites that are less favourable for beech *Fagus* spp., oak *Quercus* spp. and, to some extent, alder *Alnus* spp. (Jahn, 1991; Ellenberg, 1996), although it mostly occurs in admixture in various forest communities (mostly with several broad-leaved tree species), and rarely forms pure stands (Dobrowolska et al., 2011; Pautasso et al., 2013).

The genetic diversity and population structure of common ash have been studied using nuclear and chloroplast markers (e.g., SSR; Brachet et al., 1999; Lefort et al., 1999). In Europe, analysis of common ash populations showed a relatively low level of polymorphism, as 12 haplotypes at four polymorphic microsatellites in a set of 201 populations and two PCR-RFLP haplotypes in a subset of 62 populations were detected (Heuertz et al., 2004a). In comparison, in investigation of European broadleaved tree species, a mean of 15 haplotypes in 22 populations were observed, including seven haplotypes in 24 ash *Fraxinus* spp. populations (Petit et al., 2003). The postglacial re-colonization routes resulted in genetically distinct lineages of common ash, as indicated by maternally inherited chloroplast (plastid) DNA in Europe (Heuertz et al., 2004a). Based on the post-glacial recolonization of Europe, the geographical distribution of haplotypes of common ash is uneven. Most of the haplotypes were found in Central and Southern Europe, while Northern and Eastern Europe is predominantly occupied by a single haplotype (H01) (Tollefsrud et al., 2016), indicating genetic variation of common ash due to the spatial pattern of haplotype distribution. In Western and Central Europe, relatively high homogeneity, allelic richness, and genetic diversity between populations were observed (Heuertz et al., 2004b), while at the northern part of the range of common ash, population fragmentation and low-allelic richness has been described (Heuertz et al., 2004a; Tollefsrud et al., 2016; Børja et al., 2017; Cleary et al., 2017). Ash is a wind-pollinated species, and population fragmentation in the northern range of common ash could be counteracted by pollen flow between populations (Bacles et al., 2005).

Prior to dieback, ash was an economically important tree species (Dobrowolska et al., 2011; Pratt, 2017; Enderle et al., 2019 and references therein). It is a fast-growing tree with hard and elastic hardwood suitable for production of high-quality timber for manufacture of furniture, veneer, flooring, composite wood, sports equipment and other products (Fraxigen, 2005; Enderle et al., 2017; Rozsypálek

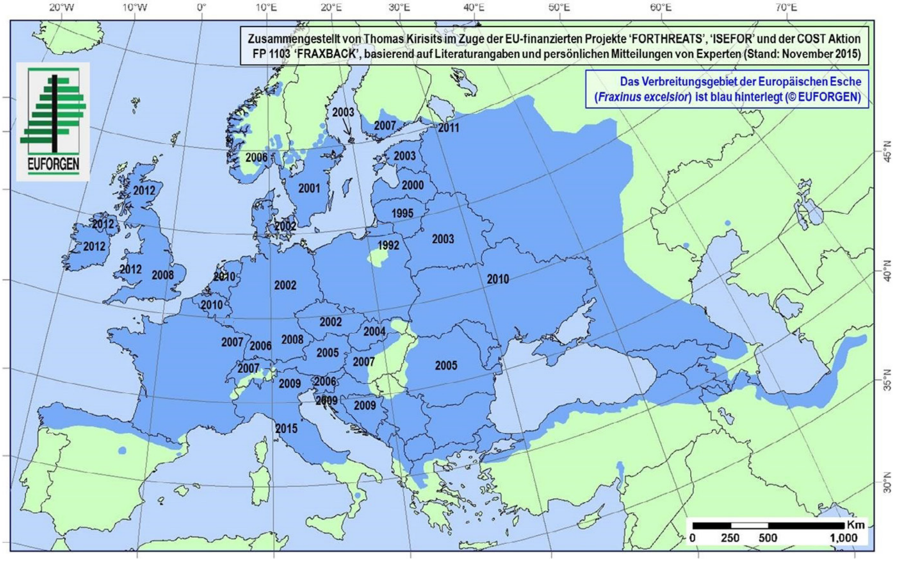


Fig. 1.1. **Distribution range of *Fraxinus excelsior* (blue)**
Dates indicate the spread of *Hymenoscyphus fraxineus* infection across Europe (BFW, 2020).

et al., 2017). In several European countries, common ash plays a central role in landscape as a major component of gardens, parks, and hedges (Pautasso et al., 2013; McCracken et al., 2017). It is an ecologically highly important species, and is associated with high habitat diversity (Lõhmus & Runnel, 2014; Mitchell et al., 2014). For example, Mitchell et al. (2014) identified 953 species tightly associated with common ash in the UK, of which 44 species fully depend on ash.

In Latvia, ash occurs close to its northern distribution limit, which causes high sensitivity of the species to environmental factors and uneven spatial distribution of stands. Ash stands are quite frequent, but they mainly occur in central and the western parts of Latvia (up to 10.9% of total forest area), where soils are more fertile and the climate is milder. The smallest proportion of ash stands is found in the most continental eastern part of Latvia, due to colder winters and late spring frosts (Nikodemus et al., 2009; Kenigsvalde et al., 2010). In Latvia, ash mainly grows in mixed forests on eutrophic soils (97% of the total stand area), together with other deciduous trees (e.g., aspen *Populus* spp., birch *Betula* spp., alder etc.) and Norway spruce *Picea abies* (L.) H. Karst; pure stands are rare.

1.2. Dieback of common ash stands

The first signs of defoliation and shoot dieback in the upper crowns of common ashes were observed in North-Eastern Poland at the beginning of the 1990s (Przybył, 2002). The extent of ash dieback (ADB) was monitored using diverse proxies such as the amount of dead trees, annual mortality rates, reduction of area of ash forests, standing volume of dead trees, etc. (Chira et al., 2017; Pliūra et al., 2017; Timmermann et al., 2017). Accordingly, there are no precise comparable data on the decrease of ash forest area in Europe, and therefore one of unambiguous characteristics of this disease is tree mortality (proportion of trees that have died during a given period; Coker et al., 2019), which has been documented in many studies. The overall mortality of the affected ash stands in Europe ranged from 6.9 to 10.1% per year (Lenz et al., 2016; Matisone et al., 2018), and the maximum recorded mortality of planted forests, woodlands, and naturally regenerated saplings exceeded 70% (Coker et al., 2019). Unfortunately, such fragmented data do not allow to draw comprehensive conclusions on ADB in Europe, and hence a standardized review would be helpful.

Further spread of the ADB from Poland was rapid, as it reached neighbouring Lithuania and Latvia in the few following years, although its presence in Latvia was published only in 2000 (Timmermann et al., 2011). Later it spread throughout Central and Northern Europe; in 2012, it reached geographically isolated UK, likely due to import of infected plant material (Orton et al., 2018) (Fig. 1.1). A detailed chronological list of the first observations of ADB in European countries was compliled by McKinney et al. (2014).

In Latvia, the proportion of ash was the highest in 1998 when the total area of ash stands comprised 21905.3 ha or 0.8% of the total forest area (Laiviņš et al., 2016). Decrease of area of ash stands in forests in Latvia was reported by Liepiņš

(2003), who observed a sharp reduction after 2000. At present, the area of common ash stands comprises only 9354 ha (0.3%) of the total forest area, of which only 5% (470 ha) are young ash stands (≤40 years), and there is a clear tendency to further decrease (Valsts meža dienests, 2019).

1.3. Causal agents and infection mechanisms of dieback of common ash

According to the observed symptoms, initially it was hypothesized that frost or drought stress may have caused the ash dieback (Pukacki & Przybyl, 2005). The affected trees often showed symptoms like macroscopic cankers on leaves and leafstalks (Skovsgaard et al., 2010), brown spots on buds (Bengtsson et al., 2014), and wilting of leaves and top shoots (Schumacher et al., 2009). These symptoms were followed by the formation of necrotic lesions spreading along rachii onto shoots, branches, and stems, resulting in dieback of the affected parts of trees (Bakys et al., 2009a; Skovsgaard et al., 2010; Bengtsson et al., 2014). Branch dieback causes trees to induce formation of epicormic shoots, resulting in a bushy appearance of tree crowns (Gross et al., 2014). Such symptoms pointed to the involvement of another causal agent such as biological pathogen.

Identification of the causal agent of ADB took several years (Przybył, 2002). In 2006, from shoots, branches and stems of symptomatic *F. excelsior*, Tadeusz Kowalski isolated and described an anamorphic fungus *Chalara fraxinea* T. Kowalski, which was then proposed as the causal agent of ADB (Kowalski, 2006). The pathogenicity of the fungus was later confirmed by Kowalski & Holdenrieder (2009a) and Bakys et al. (2009a,b). In 2009, Kowalski & Holdenrieder (2009b) identified a teleomorph of *C. fraxinea* as *Hymenoscyphus albidus* (Gillet) W. Phillips, which previously was known as a harmless saprotroph and decomposer of ash litter in Europe. Queloz et al. (2011), from putative *H. albidus* isolates, identified two morphologically virtually identical, but genetically differing *Hymenoscyphus* species named *H. albidus*, which was known previously, and a new species *H. pseudoalbidus* V. Queloz, C.R. Grünig, R. Berndt, T. Kowalski, T.N. Sieber & O. Holdenrieder, which, however, was later identified as the sexual stage of the ash dieback pathogen. *Hymenoscyphus pseudoalbidus* was later proven to be introduced to Europe from Asia (Zhao et al., 2012). Three years later Baral et al. (2014) announced the nomenclaturally correct scientific name for the fungus causing ADB in Europe – *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya with the basionym *Chalara fraxinea*, and *Hymenoscyphus pseudoalbidus* as the taxonomic synonym of *H. fraxineus*.

Based on a literature review, Gross et al. (2012, 2014) described the life cycle of *H.* fraxineus (Fig. 1.2). The whole life cycle takes one year. Ascospores are produced in the leaf litter by apothecia formed during summer on fallen leaf petioles of the previous year, indicating complex legacy controls of the infection pressure. Ascospores are dispersed by wind and can penetrate a host's leaflets via appressoria. After the colonization phase, the fungus advances into the leaf petiole (petioles can be colonized by multiple *H. fraxineus* genotypes resulting from multiple infections). After leaf fall, an anamorph is formed on petioles and spermatia are released. Fertilization occurs by conidia, which are readily produced on petioles in autumn when temperature is falling. New apothecia are produced during summer of the next growing season, initiating a new cycle of infection.

The most important vector of disease spread is wind dispersal of airborne ascospores. The dispersal rate in Europe was estimated to be 30 to 70 km per year (Luchi et al., 2012; Solheim et al., 2012; Børja et al., 2017).

Pathogen *H. fraxineus* has been considered to be the primary agent of ADB, but the death of the weakened trees is often caused by secondary pathogens, e.g. *Armillaria* spp. (Skovsgaard et al., 2010; Bakys et al., 2011; Enderle et al., 2013; Chandelier et al., 2016). *Armillaria* quickly colonizes lesions formed by *H. fraxineus* in the inner bark of the collar area (Husson et al., 2012); hence root rot has been often recorded on symptomatic ash trees. In Latvia, presence of the *H. fraxineus* as the causal agent of ADB was proved in the laboratory by T. Kirisits in 2007 (Kenigsvalde et al., 2010).

1.4. Susceptibility and resistance of common ash to *Hymenoscyphus fraxineus*

Not all trees show symptoms, even under high infection pressure, which points towards natural resistance against the pathogen and/or specific ecological factors limiting the virulence of the pathogen (Stener, 2013; Enderle et al., 2015). Therefore, resistance and methods to improve resistance have been the most often studied issues (Kjaer et al., 2012; McKinney et al., 2014). Many studies have shown that ca. 1% of trees might be considered resistant (McKinney et al., 2011a; Pliūra et al., 2011; Kjaer et al., 2012; Lobo et al., 2014; Muñoz et al., 2016; Stener, 2018). Still, Pliūra et al. (2015) indicated that none of the tested provenances or progenies of ash had complete resistance to the pathogen. McKinney et al. (2014) concluded that presence of the disease in Europe is too short for the development of natural resistance against *H. fraxineus*, while Carlsson-Graner & Thrall (2015) indicated the risk that co-evolution of the pathogen is more rapid than that of the host. Nevertheless, experiments with provenance trials and ash breeding programs have been showing increasing importance in Europe (Pliūra et al., 2011; Enderle et al., 2015; Havrdová et al., 2016). Heritability (proportion of genetic effects from the total variance of a trait) of resistance to ADB in clonal trials in Europe ranged from 0.20–0.40, indicating limited potential for improvement of health condition of the next generations of ash trees by breeding (Pliūra et al., 2011; Kjaer et al., 2012; Stener, 2013; Lobo et al., 2014; McKinney et al., 2014; Enderle et al., 2015; Muñoz et al., 2016; Stener, 2018). In several *in vitro* studies, antagonistic activity of some fungal endophytes against *H. fraxineus* was observed (Schlegel et al., 2016; Haňáčková et al., 2017; Kosawang et al., 2018). Nevertheless, explicit positive effects of endophytic microbiomes on ash susceptibility to ADB have not been observed (Hietala et al., 2018; Haňáčková et al., 2017; Schlegel et al., 2018).

Numerous studies have investigated factors affecting the severity of ash dieback within a stand, such as timing of the infection, the age and dimension of ash trees, site conditions, stand composition etc. The results of these studies are

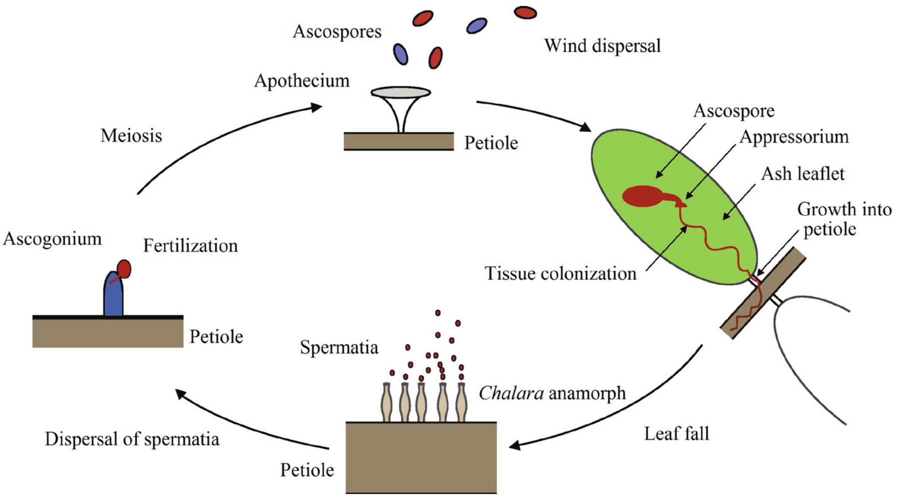


Fig. 1.2. **Schematic representation of life cycle of the *Hymenoscyphus fraxineus* (Gross et al., 2012)**
Red and blue colours of spores and mycelia represent the two mating types of *H. fraxineus.*

largely similar, but some regional differences are indicated. Most studies indicate that younger (Enderle et al., 2017; Rozsypálek et al., 2017; Enderle et al., 2018) and smaller trees (Bengtsson & Senström, 2017; Cleary et al., 2017; Marçais et al., 2017; Pliūra et al., 2017; Enderle et al., 2018) have been more affected. A possible reason for this could be the increased amounts of ascospores of *H. fraxineus* on ash leaf litter, which can easily infect smaller/lower trees (Timmermann et al., 2011; Hietala et al., 2013). Timmermann et al. (2017) highlighted faster spread of the disease on smaller trees, as a single necrosis on the stem may lead to dieback of the top or even the whole tree. Smaller ash trees are exposed to higher competition with other vegetation, which weakens them (Cech, 2008). In mature trees, the disease progresses slower (McKinney et al., 2011a; Lenz et al., 2016), probably due to more extensive foliage and gradual decline in crown condition (Timmermann et al., 2017), as well as lower density of *H. fraxineus* ascospores at crown height (Chandelier et al., 2014).

Ash dieback occurs in diverse habitats (lowlands, floodplain or mountain forests) and site conditions, but dieback is more severe in more humid sites (Ghelardini et al., 2017; Heinze et al., 2017; Timmermann et al., 2017). Drainage systems can have positive effects on ash health condition, as in the over-moist sites, trees can be less resistant to disease because of water stress (Dobrowolska et al., 2011; Schumacher, 2011; Ahlberg, 2014). In addition, the stress caused by competition with other species, especially, for young trees left for the natural regeneration, weakens trees and increases their susceptibility to *H. fraxineus* (Keer, 1998; Givnish, 2002; Royo & Carson, 2006; Skovsgaard et al., 2010).

The severity of ADB varies among stands with different composition; the less common pure stands have been the most affected (Dobrowolska et al., 2011), probably because of high pathogen and host densities. In mixed stands, ash suffers less damage (Schumacher, 2011; Stener, 2013), likely due to the presence of chemical, physical, and biological barriers, which limit spread of the primary and secondary pathogens (Loreau et al., 2001; Jactel et al., 2005; Pautasso et al., 2005; Kosawang et al., 2018). Marçais et al. (2017) and Coker et al. (2019) described 6- to 8-year cyclicity of the infection after strong infection pressure; the mortality rates tend to decrease most likely due to severe decrease of population density of ash (Enderle et al., 2018). Nevertheless, detailed studies on the expansion of the pathogen indicated that the disease still progresses slowly in the surviving trees (Bengtsson et al., 2014).

With the expansion of dieback, different management activities have been applied to reduce the spread of the infection. However, pruning of the affected branches (Marciulyniene et al., 2017), whole tree burning, and application of herbicides on the stumps of the cut trees (McCracken et al., 2017) have not stopped the spread of the infection. However, some silvicultural activities, for example, thinning, have been discussed, as they can change the microclimate and hence alter the infection conditions, thus reducing disease severity (Bakys et al., 2013; Ahlberg, 2014; Cleary et al., 2017). In Denmark, in young even-aged ash stands, severity of the disease was the highest in unthinned plots, even though it was not related to stand density (Bakys et al., 2013). Therefore, different factors such as density of ash and other species, species composition, soil and moisture regime, as well as the interactions between them, have complex controls on the spread of the pathogen and progression of ADB.

Climatic and meteorological conditions affect the successful growth of both ash and pathogen (Wardle, 1961; Kowalski & Bartnik, 2010; Maresi, 2014). For example, low air temperatures, late spring frosts, drought and unfavourable moisture conditions weaken and stress trees, thus facilitating the infection by the pathogen (Thomsen & Skovsgaard, 2006; La Porta et al., 2008; Pautasso et al., 2010; Tulik et al., 2010). A consensus has been proposed that dry climate and seasonal drought are unfavourable for ADB (Chira et al., 2017; Grosdidier et al., 2018). In Italy, the spread of *H. fraxineus* has been associated with both high temperature and abundant precipitation during the entire growing season (Maresi, 2014). Still, regional and local climatic differences have major effect on the health condition of ash (Maresi, 2014). On a global level, climate change is a plausible factor that could alleviate the disease in the future, as increased temperature and drought are unfavourable for ADB, and the disease risk is considered to be low in the warmer areas of the Southern Europe (Goberville et al., 2016; Grosdidier et al., 2018). Nonetheless, the existence of common ash in Europe is threatened by the jewel beetle – emerald ash borer *Agrilus planipennis* Fairmare, which has been destroying ash populations in the eastern North America, and now is expanding in the European part of Russia (Liebhold, 2012; Valenta et al., 2015).

1.5. Transformation of dieback affected common ash stands

Natural regeneration of ash before ADB has been widely studied, including factors such as ash density in diverse site types (Sakss, 1958; Tabari & Lust, 1999; Laiviņš & Mangale, 2004), stand structure (Ellenberg, 1996; Tabari et al., 1999; Dufour & Piegay, 2008), stand composition (Götmark et al., 2005), productivity (Wardle, 1961; Jaworski, 1995; Střeštil & Šammonil, 2006), and competition with herbaceous and other species (Wardle, 1961; de la Cretaz & Kelty, 2002). Mitchell (2014) has modelled expected changes in woodland vascular plant communities caused by loss of ash in UK, but such data and studies *in situ* after ADB are still lacking.

In Europe, the affected ash stands are transforming every year; some of them have been replanted with other tree species, as in many countries planting of ash has been stopped for economical reasons (Kirisits et al., 2011; Bakys, 2013). Still, a part of clear-cut ash stands have been left for the natural regeneration, due to successful natural regeneration of other tree species that grow in admixture with ash (Lygis et al., 2014). Accordingly, the floristic composition of ADB-affected ash stands might remain similar to the pre-dieback conditions. For example, in Lithuania, fertile and moist sites mostly regenerate with early successional or pioneer species such as grey alder *Alnus incana* (L.) Moench., silver birch *Betula pendula* Roth and, in some cases, common aspen *Populus tremula* L. (Lygis et al., 2014).

Natural regeneration of ash has sharply decreased after ADB; however, regional differences in density of seedlings has been observed. In Eastern Europe, the density of ash sapling is higher than in Central Europe. For example, the density of ash saplings in naturally regenerated stands in 2011 was estimated to be 599 ashes ha^{-1} in Lithuania (Lygis et al., 2014), 3500 ashes ha^{-1} in Estonia in 2015 (Drenkhan et al., 2017), and 653 ashes ha^{-1} in Germany in 2015 (Enderle et al., 2018). The regeneration of ash might be related to the health condition of ash trees in the particular stand or region. Semizer-Cuming et al. (2019) showed that trees with low susceptibility to ADB have higher reproductive fitness in comparison to highly susceptible trees, which can facilitate development of natural resistance. The health condition of young ash trees in Europe appears similar, as the proportion of healthy saplings ranges from 32 to 41% (Lygis et al., 2014; Drenkhan et al., 2017; Giongo et al., 2017; Enderle et al., 2018), suggesting effects of local (site) conditions.

The perspectives for planting of ash are low; in Germany all planted ash trees were found to be infected and their mortality reached 73% in 2013 to 2014 (Enderle et al., 2017). Therefore, alternatives for reforestation by forming admixtures with ash in natural regenetation are recommended. Chira et al. (2017) suggested that ash forests in Romania might be replaced by the hybrid poplar *Populus x canadensis* Moench; mixed oak forests, or green ash *Fraxinus pennsylvanica* Marshall, which appears tolerant to ADB. In Germany, poplar hybrids have been used for filling gaps in affected ash stands (Enderle et al., 2017). Other species (willow *Salix* spp., aspen, oak, maple *Acer* spp., elms *Ulmus* spp., alder, lime *Tilia* spp., wild cherry *Prunus avium* L., European hornbeam *Carpinus betulus* L. etc.), which are tolerant to flooding, have been recommended as alternatives to ash in riverine and calcerous sites (Sioen et al., 2017). Ash dieback has effect on the diversity of species associated with ash forests (birds, mammals, bryophytes, fungi, invertebrates and lichens), causing decline of species abundance and threat of complete loss (Lõhmus & Runnel, 2014; Mitchell et al., 2014; Mitchell, 2014). In UK, only 69% of species associated with common ash can use alternative tree species as hosts (Mitchell et al., 2014). In experimental plots in Estonia during five years, one epiphyte species – ascomycete *Pyrenula laevigata* (Pers.) Arnold disappeared and three more species were predicted to become locally extinct in the near future due to loss of its habitat. Only one epiphytic lichen, *Lobaria pulmonaria* (L.) Hoffm, produced <20 new thalli on alternative hosts elm, maple and lime (Lõhmus & Runnel, 2014), indicating that common ash, compared to other European deciduous tree species, interacts with the environment in a unique way, particularly in relation to nutrient cycling (Mitchell et al., 2014). In Latvia, among tree species, the highest richness of epiphytes was observed on ash, and most of those species were found also on wych elm *Ulmus glabra* Huds (Gerra-Inohosa, 2018). The existence of both of these tree species is threatened, indicating negative effects on epiphyte diversity in the future.

1.6. The aim of the thesis

The aim of the thesis was to assess the dieback dynamics of the common ash *Fraxinus excelsior* L. stands and determine the main factors affecting susceptibility of trees, as well as future succession and potential regeneration of the affected common ash stands in Latvia.

1.7. Thesis objectives

Four objectives were set in the doctoral thesis:

1. to assess regional differences in dynamics of ash dieback and their relationship with meteorological factors;
2. to determine the genetic diversity and population structure of common ash;
3. to characterise natural regeneration and succession of the affected ash stands of different age;
4. to identify site properties and management practices that affect regeneration and health condition of ash.

1.8. Thesis

1. In Latvia, under intensive ADB, there is a high potential for continuation of the species, as ash will continue to occur in mixed stands as an admixture species with a relatively small proportion in the future.
2. The health condition of ash is better in intensively managed (thinning, drainage system, etc.) and mixed stands, while admixture species might have contrasting effects.

3. The sensitivity of ash growth to meteorological factors has a weak relationship with ADB, but a combination of significant factors increase stress as a result of climate change, which may reduce tree resistance.

1.9. Scientific novelty

The thesis investigates large-scale dieback of common ash focusing on local details of the host-pathogen interactions in Latvia. Transformation and regeneration of the affected stands under near-marginal conditions close to northern distribution limit of common ash were assessed under diverse stand types, and described in relation to stand composition and management regimes. The linkages between climate-growth sensitivity and susceptibility and health status of trees were analysed.

1.10. Thesis structure

The doctoral thesis consists of nine research papers. Two papers (Paper I and II) describe the rate and extent of ADB. The seasonal dynamics of *H. fraxineus* is reported in Paper III. Pattern and sensitivity of radial growth to meteorological factors in relation to susceptibility to ADB is investigated in Paper IV. Genetic diversity and population structure of common ash in Latvia is described in Paper V. Another two papers (VI and VII) are addressed to the succession of woody plants in common ash stands affected by ADB. Natural regeneration of common ash in young stands in relation to site properties and management is analysed in Papers VIII and IX.

1.11. Approbation of research results (conferences)

The results of the research have been presented in 15 scientific conferences:

1. Burņeviča N., **Matisone I.**, Zaļuma A., Brūna L., Laiviņš M., Gaitnieks T. (2019). Ash dieback and other actual diseases in Latvia. Consolidating approaches to mitigate the ash dieback disease in Baltic States and Germany, October 16, 2019, Kaunas – October 17, 2019, Vilnius, Lithuania. Oral presentation.
2. **Matisone I.** (2017). Ash dieback in Latvia: causes, consequences, lessons for forest management. Forestry doctoral school, June 19–22, 2017, Jaunkalsnava, Latvia. Oral presentation.
3. **Pušpure***I.,[2]Zaļuma A., Gaitnieks T., Burņeviča N., Matisons R. (2017). Seasonal development of lesions of young common ash in Latvia. Invasive Forest Pathogens & Implications for Biology & Policy, IUFRO Working Party 7.02.02, May 7–11, 2017, Niagara Falls, Ontario. Poster.
4. **Pušpure***I., Matisons R., Laiviņš M., Lībiete Z. (2016). Influence of stand structure on resistance of common ash young stands to ash dieback. Integrating Scientific Knowledge in Mixed Forests EuMIXFOR Final Conference COST Action FP1206, October 5–7, 2016, Prague, Czech Republic. Poster.

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[*] last name changed from Pušpure to Matisone.

5. **Pušpure***I., Matisons R., Laiviņš M., Gaitnieks T. (2016). Natural regeneration of common ash in young stands in Latvia 3. COST Action FP1103. *Fraxinus* dieback in Europe: elaborating guidelines and strategies for sustainable management, March 1–3, 2016, Bratislava, Slovakia. Oral presentation.
6. **Pušpure***I., Gerra-Inohosa L., Matisons R., Laiviņš M. (2016). Tree-ring width of European ash differing by crown condition and its relationship with climatic factors in Latvia. Cost action FP1103. *Fraxinus* dieback in Europe: elaborating guidelines and strategies for sustainable management, March 29–April 3, 2016, Riga, Latvia. Oral presentation.
7. **Pušpure***I., Matisons R., Laiviņš M., Burņeviča N., Gaitnieks T. (2016). Natural regeneration of common ash in young stands in Latvia 2. COST Action FP1103. *Fraxinus* dieback in Europe: elaborating guidelines and strategies for sustainable management, March 29–April 3, 2016, Riga, Latvia. Oral presentation.
8. **Pušpure***I. (2015). Natural regeneration of common ash *Fraxinus excelsior* L. stands in Latvia 1. COST Action FP1103. *Fraxinus* dieback in Europe: elaborating guidelines and strategies for sustainable management, April 12–16, 2015, Dubrovnik, Croatia. Oral presentation.
9. **Pušpure***I. (2015). Quality assessment of European ash *Fraxinus excelsior* L. genetic resource forests in Latvia. 21st Annual International Scientific Conference Research for Rural Development 2015, May 13–15, 2015, Jelgava, Latvia. Oral presentation.
10. **Pušpure***I. (2015). Comparison of species diversity in two *Fraxinus excelsior* L. woodland key habitat regions of Latvia. 8th International Conference on Biodiversity Research, April 28–30, 2015, Daugavpils, Latvia. Poster.
12. Laiviņš M., **Pušpure***I. (2015). The destruction and transformation of *Fraxinus excelsior* forest stands in Latvia. Scientific Conference of the Forest Sector: Knowledge based forest sector, November 4–6, 2015, Riga, Latvia. Oral presentation.
13. Laiviņš M., **Pušpure***I., Gerra-Inohosa L. (2015). The destruction and transformation of *Fraxinus excelsior* L. forest stands in Latvia. Scientific Conference of the Forest Sector: Knowledge based forest sector, November 4–6, 2015, Riga, Latvia. Poster.
14. Laiviņš M., Gerra-Inohosa L., **Pušpure***I. (2015). Monitoring of *Fraxinus excelsior* L. forest stands in Latvia. Scientific Conference of the Forest Sector: Knowledge based forest sector, November 4–6, 2015, Riga, Latvia. Poster.
15. **Pušpure***I., Laiviņš M., Gerra-Inohosa L. (2015). The dynamic of shrub layer in *Fraxinus excelsior* L. forest stands in response to ash dieback in Latvia. Scientific Conference of the Forest Sector: Knowledge based forest sector, November 4–6, 2015, Riga, Latvia. Poster.
15. **Pušpure***I., Gerra-Inohosa L., Laiviņš M. (2015). Natural regeneration and vitality of common ash *Fraxinus excelsior* L. forest stands in Latvia. Nordic-Baltic Forest Conference "Wise Use of Improved Forest Reproductive Material", September 15–16, 2015, Riga, Latvia. Poster.

2. MATERIALS AND METHODS

2.1. Study sites, field and laboratory work

2.1.1. Dynamics of ADB

Dieback of ash stands was studied in 15 permanent sampling plots (PSP) established in 2005 across the territory of Latvia (Paper II, Fig. 2.1). The PSP were established in uneven-aged (51–138 years) stands initially dominated by common ash, which had suffered different degree of dieback. The stands (except two seasonally waterlogged stands near Ķemeri and Ainaži) were located in eutrophic conditions with normal moisture regime on a flat topography corresponding to *Aegopodiosa, Dryopteriosa, Oxalidosa turf. mel*, and *Filipendulosa* site types according to Bušs (1976). In each stand, a circular plot (R = 15 m; S = ca. 706 m^2) was established. Within each PSP, all trees (incl. logs/snags) with stem diameter at breast height (DBH) ≥6 cm were measured, recording their health condition (living or dead), as well as DBH and height/length (H). The PSP were measured in 2005, 2010, and 2015.

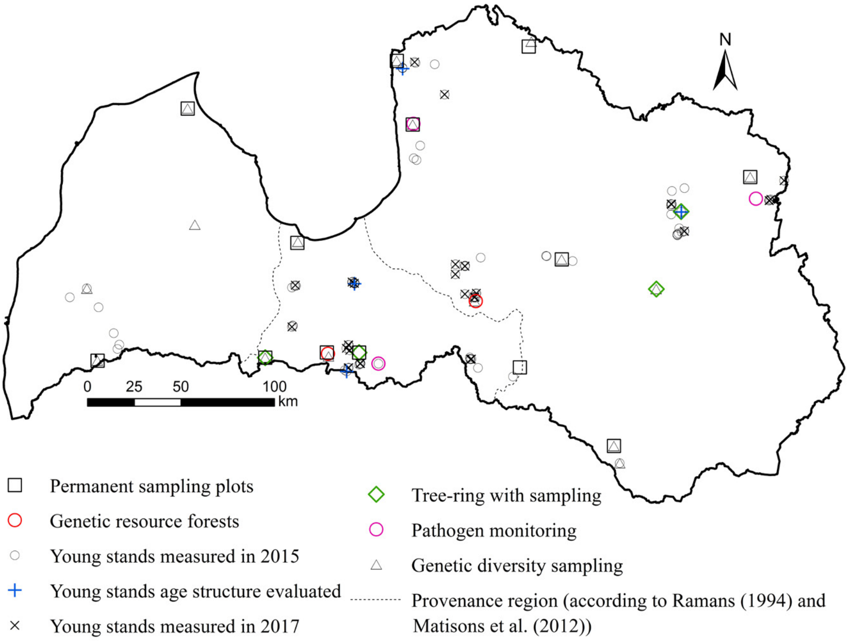

Fig. 2.1. **The locations of the studied stands of common ash and tree provenance regions of Latvia**

2.1.2. Intra-seasonal dynamic of the pathogen Hymenoscyphus fraxineus

Three (Fig. 2.1) 5–8 years old naturally regenerated post-clear-cut stands, which were dominated by ash in the previous rotation, were surveyed from June to September 2015, when the highest fungal activity was expected (Timmermann et al., 2011; Bengtsson et al., 2014) (Paper III). The stands corresponded to the eutrophic *Aegopodiosa* site type (Bušs, 1976) and grew on a flat terrain on a well drained fertile loamy soil. The density of ash in Bauska, Aizpurve and Limbaži stands was 5000, 1500 and 1500 trees ha^{-1}, respectively. In all stands ash was the dominant species with an admixture of common aspen, goat willow *Salix caprea* L. and Norway maple *Acer platanoides* L. (up to 1500 trees ha^{-1}).

In each stand, 10 dominant unsheltered ash saplings (in height range 2.5–3.0 m) with one to three lesions (necrosis) on tree stem with a small initial area (the maximum area of the lesions per tree was 223.4 cm^2) were selected. From June to September (on the 10th to the 15th date of each month), H and DBH of the sampled trees were measured, and health condition according to five classes was recorded (Table 2.1). In June, all visible lesions on branches, stems, and tops were marked on a transparent film. Each month the area of the lesion extension since the last measurement, as well as the emergence of new lesions was marked on the film. After the final survey in September 2015, the damaged parts of the saplings (stems and the largest branches) were sampled: bark was removed, and the area of the discoloured wood was marked on the film. These wood samples were used later for verification of the presence of pathogen *H. fraxineus*. The area of lesions from each tree and month were measured on the films using a TAMAYA digital planimeter PLANIX 10S 'Marble'.

Table 2.1

Classes of ash tree health condition and their description

Class	Degree of *H. fraxineus* damage, %	Visual characteristics
1	0–10	Healthy or individual leaves are slightly damaged
2	11–25	Several leaves are damaged; some necrosis on the bark
3	26–60	Individual branches are completely damaged/dead; part of the foliage damaged; necroses on the bark in large areas
4	61–99	Part of the crown is completely damaged or dead; separate branches (or water sprouts) are alive
5	100	Dead

For isolation of *H. fraxineus*, 17 samples from symptomatic material, i.e., from the inner bark or wood where the quantity of *H. fraxineus* mycelium presumably was the highest (Schumacher et al., 2009), were collected. In the laboratory, each sample was surface sterilized by submersion in 35% hydrogen peroxide for 30 seconds and washed twice in distilled water for one minute. After draining, the samples were placed on malt agar media and incubated in the dark at

20°C for four weeks. Every three days any other emerging fungi were mechanically removed. Identification of *H. fraxineus* was confirmed microscopically according to Kowalski (2006).

2.1.3. Climate-growth sensitivity of ash with different crown health condition

To analyse climate-growth sensitivity and its relation to health condition of ash (Paper IV), four mature stands were selected (two in the western and two in the eastern part of Latvia, cf. Baumanis et al., 2001; Matisons et al., 2012; Fig. 2.1). The stands contained trees with different crown condition (from healthy to strongly affected). In each stand, 10 dominant ash trees that were visually healthy (crown reduction ≤10%) and 10 ash trees with damaged crowns (crown reduction 30–60%) were sampled. From each tree, two increment cores from opposite sides of stem were collected with a Pressler increment corer at breast height. In the laboratory, the increment cores were air dried, glued into wooden mounts, and smoothed with sandpaper of different roughness (from 120 to 400 grains per inch). For better identification of tree rings, and to enhance contrast between early and latewood, the surface of the sanded samples was rubbed with white chalk. Tree-ring widths (TRW) were measured manually using a Lintab 5 measurement system (RinnTECH, Heidelberg, Germany).

2.1.4. Genetic diversity and population structure of common ash stands

Genetic diversity and population structure of common ash was analysed in 16 naturally regenerated ash stands located throughout the territory of Latvia (Fig. 2.1) (Paper V). Two of them, Skrīveri and Bērvircava, were designated as genetic resource forests (GRF). In each stand (in ca. 2500 m^2 area) several leaves from 24 randomly selected young ash individuals were collected at 1–2 m height. In total, 372 individuals were sampled.

The DNA from leaves was isolated using a CTAB-based method (Porebski et al., 1997). The genotyping was done using six nuclear SSR markers (Femsatl4, Femsatl10, Femsatl11, Femsatl16, Femsatl19) (Lefort et al., 1999) and M2-30 (Brachet et al., 1999), as well as six angiosperm chloroplast markers ccmp2, ccmp4, ccmp6, ccmp10, ccmp7 and ccmp3 (Weising et al., 1999). All polymerase chain reactions (PCR) were carried out in an Eppendorf Mastercycler ep gradient thermal cycler. Amplified fragments were separated on an ABI Prism 3130xl Genetic Analyser (Applied Biosystems).

2.1.5. Succession of ash stands

Succession of ash stands was studied in 15 PSP (Fig. 2.1). Within each PSP, three smaller circular plots (R = 5 m, S = ca. 78.5 m^2) were established for the description of understory (Paper VI) at seven-meter distance from the centre of the PSP. In these subplots, height of all understrorey shrub (UG) and advance growth (AG) individuals with DBH ≤ 6 cm was measured. The survey was conducted in 2005, 2010 and 2015. In addition, succession of ash stands was evaluated in two genetic resource forests (GRF) near Skrīveri and Bērvircava (Paper VII, Fig. 2.1). In total 28 GRF stands (units) in Skrīveri and 73 GRF stands in Bērvircava were analysed. The age of these stands ranged from 20–126 years, and ash was the dominant species with an admixture of wych elm, common aspen, black alder *Alnus glutinosa* L., silver birch, and other species. The GRF stands were growing in eutrophic conditions (*Aegopodiosa*, *Dryopteriosa,* or *Mercurialiosa mel.* site types). Within each stand, a 20×20 m plot was established, where the projective cover (%) of each canopy tree species was determined. Along one diagonal of the plot, a 25×1 m transect was established, where all AG and UG species (H ≤ 7.0 m) were counted and health condition of ash was recorded in five classes (Table 2.1).

2.1.6. Regeneration and health condition of young ash stands

Natural regeneration of ash in young stands, where ash contributed ≥40% of standing volume in the previous rotation (Paper VIII), was studied in 90 stands distributed across the territory of Latvia (Fig. 2.1). The age of the studied stands ranged between 5–40 years, and size exceeded one ha. The stands occurred on dry mineral, drained mineral, drained peat, and moist mineral soils. Within each stand, a 100×2 m sampling plot was established along the longest diagonal of the forest compartment. In each plot, all AG and UG individuals up to 7 m height were counted and their height measured. For each ash tree, degree of *H. fraxineus* damage was recorded in five classes (Table 2.1). In four of these 90 stands (two six-year old and two eight-year old; Fig. 2.1), all ash trees within the sample plots were sampled (stem discs at stem base taken) for estimation of age. The height and diameter at stem base of the sampled trees was measured and degree of *H. fraxineus* damage was recorded. From the sampled trees in each stand, 10 ashes for each height class (7 classes; tree height 0–3 m with 0.5 m section) were randomly selected for determination of age. In the laboratory, stem discs were sanded and tree-rings were counted under a microscope. The stands were surveyed in 2015.

For the assessment of health condition of ash in young stands of diverse composition (Paper IX), 35 stands with age 26–36 years and dominated by different species were selected from the 90 stands used for Paper VIII. The selection was stratified to represent young stands dominated by black alder, grey alder, silver birch, common ash and Norway spruce equally for western and eastern parts of Latvia (Fig. 2.1). These stands were revisited and height of trees within 100×2 m sampling plots was measured in 2017 (as in 2015, but without sampling).

2.2. Data analysis and additional data sources

The dynamics (relative changes) of area of young ash stand (<41 years) in Latvia for the three year periods during 2000–2009 (2015 for total area) (Paper I) were analysed according to spatial data from the State Forest Service database (Valsts meža dienests, 2019). Counties were used as statistical units, which were grouped by local physiogeographical nature region as defined by Ramans (1994). Nature regions, where the area of young ash stands in 2000 was <200 ha, were excluded from the analysis. The spatial analysis was applied using the Arc View 9.1 software (ESRI, 2006).

Data on several stand parameters like site type, soil type, age, tending history, standing volume, the current composition of species and composition of species in the previous rotation (used in Papers II, III, IV, VII, VIII, IX) were obtained from the Latvia State Forest Service database (Valsts meža dienests, 2019).

The paired *t*-test (applying Bonferroni transformation of *p*-values) was performed to assess differences in the extension of the lesions between consecutive observations (Paper III). Analysis of variance (ANOVA) was used to determine effect of ash density in AG in relation to dominant canopy tree species (Paper VII and VI), as well as to assess the differences in total and individual regeneration density between observation periods (Paper VI). The same analysis was also applied to assess differences in ash diameter, height and age among sites and health classes (Paper VIII), as well as for differences in size and rate of extension of the lesions (per tree) according to location on a tree and its age (existing (EL) or new (NL)) and for differences in overall health condition of the saplings between sites (Paper III). The relationships between ash diameter, age and height were evaluated using linear models (Paper VIII). Linear models were also used to analyse health condition of ash in relation to projective cover of ash in the canopy, as well as dominant species in the canopy (Paper VII). The Chi-squared test (χ^2) was used to compare composition of the understory and separately for AG and UG species between observation periods (Paper VI).

Pearson correlation analysis was used for the evaluation of the association between ash AG density and the number of woody plants in AG/UG (Paper VII and VIII), as well as to determine the relationships between understory density and the density of dead canopy *F. excelsior* (Paper VI). This analysis was used to assess the relationships between ash health condition and height and age (Paper VIII), as well as between the lesion area above and below bark (Paper III). Non-parametric Kendall correlation analysis was applied to determine linear relationships between ash regeneration density and species richness of stands (Paper VI), as well as to assess the effect of tree morphometric parameters (H, D) on health condition (Paper III).

A generalized mixed model applying Gaussian distribution of residuals was used to assess the effect of region and species composition (dominant species in canopy, AG) on ash regeneration (Paper VIII). A generalized mixed model using a Poisson distribution of residuals, was applied to analyse differences in the number of active/inactive/latent lesions per tree, as well as NL and EL, according to their location, date of observation, health condition of the sapling, and site (Paper III). Generalized linear mixed models were applied to determine effects of soil type, stand age (classes), and species composition (dominant species in AG) on ash density (Paper VIII). The mixed models were fit using the restricted Likelihood ratio test (West et al., 2006). The normality of datasets and model residuals was assessed using graphical analysis (Elferts, 2013).

Mixed ordinal regression was used to assess the effect of AG height and number of UG species in a stand on the health condition of ash among regions (Paper VIII). Mixed ordinal regression also was used to analyse changes in health classes of ash in relation to structure and composition of a stand (Paper IX). Tree was considered as an observation and sampling plots were used as random intercepts. In the models, different parameters and their combinations were tested as linear predictors of ash health conditions. The best combination of predictors was determined by arbitrarily selection; up to seven factors were tested simultaneously. The performance of the models was assessed using the Akaike information criterion (AIC), and conformity with biological realism was evaluated. The significant predictors were tested for multicolinearity (Fox & Weisberg, 2011).

Detrended Correspondence Analysis was used to analyse temporal changes in species composition (Paper VI).

Dendrochronological techniques were used to assess growth patterns and their sensitivity for ash trees differing in health status (Paper IV). The measured time series were crossdated and their quality was checked. For the description of the datasets, the expressed population signal, signal to noise ratio (Wigley et al., 1984), *Gleichläufigkeit*, interseries correlation and the first order autocorrelation coefficients were calculated. Residual chronologies based on the crossdated datasets were produced for each stand/group (damaged and healthy). Double detrending, firstly by the negative exponential curve and, secondly, by the cubic spline was applied. Bootstrapped Pearson correlation analysis was used for the detection of climatic signals in the chronologies. The climatic factors showing significant correlations with TRW were tested for collinearity. Gridded climatic data (mean air temperature, precipitation sum and standardized precipitation-evapotranspiration indices for months) were obtained from the online repository of the Climatic Research Unit of the University of East Anglia (Harris et al., 2014).

Molecular genetic statistics were applied for description of ash population structure (Paper V). The amplified fragments of DNA were visualized using GeneMapper 3.5. The chloroplast marker data were combined into haplotypes. Analysis of nuclear SSR data was done using Fstat 2.9.3.2 (Goudet, 2001) and GenAlEx 6.5 (Peakall & Smouse, 2012). Genetic diversity of common ash was examined using dendrograms constructed using software MEGA 5.2 (Tamura et al., 2011). A Bayesian clustering approach, implemented using software STRUCTURE 2.1 (Pritchard et al., 2000), was used to estimate the most likely number of clusters into which the nSSR genotypes were assigned with a certain likelihood. The most likely number of clusters was identified by the delta K criterion (Evanno et al., 2005) with STRUCTURE HARVESTER 0.6.93 software (Earl & vonHoldt, 2012).

Data analysis was conducted at the significance level $\alpha = 0.05$ in program R v. 3.6.1 (R Core Team, 2019), using the ‘lme4’ (Bates et al., 2015), ‘ordinal’ (Christensen, 2018), ‘car’ (Fox & Weisberg, 2011), ‘lmerTest’ (Kuznetsova et al., 2015), ‘dplR’ (Bunn, 2008) and ‘multcomp’ (Hothorn et al., 2008) libraries.

3. RESULTS AND DISCUSSION

3.1. Dieback of common ash stands

Remarkable reduction in ash stand area distribution (Paper II), which was particularly explicit for young stands, was coused by dieback (Paper I). The total area of young ash stands in Latvia decreased 4.4 times during the period 2000– 2015. This also affected age structure of ash forests in Latvia, as the proportion of young (<40 years) stands from the total area of ash forest decreased from 43% to 17% in 2000 and 2015, respectively. The rate of the decrease in the area of young stands was temporally differed. During 2000–2006, the decline was rapid (on average by 805 ha year^{-1}), while in the following years (2007–2015) the decline slowed down (on average by 279 ha year^{-1}), indicating density-dependence of the process. A similar pattern was observed in Europe; for example, in Lithuania decline of all ash stands between 2001 and 2015 was 2043 ha year^{-1} (Pliūra et al., 2017), but in Romania during 2001–2010, 415 ha year^{-1} (Chira et al., 2017).

In 2000 in Latvia, dieback of young stands of ash was initially observed in the southern part of the country (Semigallia plain), which borders with Lithuania, where ADB had already been confirmed (Juodvalkis & Vasilauskas, 2002). In 2001, ADB rapidly spread northwards, likely using lowlands and rivers as migration corridors (Fig. 3.1). Rivers have been considered as the most important pathways for the pathogen in Central Europe (Chira et al., 2017). Until 2006, massive dieback had spread throughout the territory of Latvia. The dispersal rate of *H. fraxineus* in

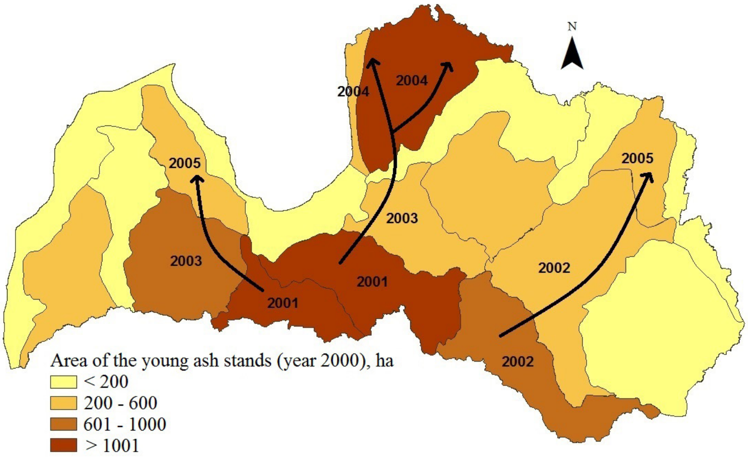

Fig. 3.1. **Hypothetical migration routes of *Hymenoscyphus fraxineus* in Latvia**
Dates indicate the spread of *H. fraxineus* infection across Latvia.

Latvia was estimated to be ca. 40 km year^{-1}, while in the northeastern Italy it was 50–60 km year^{-1} (Luchi et al., 2012), and in Norway ranged from 25–78 km year^{-1} (Børja et al., 2017; Solheim & Hietala, 2017). However, in Latvia, ADB was slower in the upland areas where ash stands were more scattered, and hence population density and connectivity of stands lower, than observed in Germany (Enderle et al., 2018).

The extent of ADB was analysed in Paper II based on forest inventory (FI) and PSP. According to FI data, ADB decreased the area of ash-dominated stands in Latvia nearly twice during the period 1998–2015. The decrease was slightly faster (by ca. 6%) in the western part of Latvia, where the area and connectivity of ash stands was higher (Liepiņš et al., 2016), which might have facilitated the spread of the disease (Enderle et al., 2018).

An inconsistency was observed between data sources regarding increment dynamics of stands affected by ADB. The official (FI) statistics have been showing a gradual increase in the standing volume of the surviving ash-dominated stands in Latvia during 2005–2015 (Table 3.1). In contrast, the data from PSP highlighted considerable decrease in standing volume of ash from ca. 320 m^3 ha^{-1} in 2005 to 151 m^3 ha^{-1} in 2015. Accordingly, the volume of deadwood increased from 18 m^3 ha^{-1} to 212 m^3 ha^{-1} (Table 3.1). The changes in standing volume of ash were similar in the western and eastern parts of Latvia.

Table 3.1

Statistics of ash stands in Latvia according to data from Latvia State Forest Service and permanent sampling plots for the period 2005–2015

The 95% confidence intervals are shown for the permanent sampling plot data

Statistical indicator	Year		
	2005	2010	2015
Latvia State Forest Service data			
Total stand area, ha	18315.70	16263.50	13673.71
Total standing stock m^3	3737303	3698663	3410624
Mean standing volume, m^3 ha^{-1}	204.05	227.42	249.43
Permanent sampling plot data			
Living			
Stand density, trees ha^{-1}	256 ±55	136 ±45	77 ±32
Standing volume, m^3 ha^{-1}	322 ±89	246 ±77	151 ±59
Deadwood			
Stand density, trees ha^{-1}	74 ±50	178 ±82	233 ±79
Standing volume, m^3 ha^{-1}	18 ±14	119 ±54	212 ±66

The changes in survival of trees were also similar in the western and eastern parts of Latvia. In 2005–2009 and 2010–2015, the mortality rate (r%) was 9.6 and 8.2% year^{-1}, respectively. The overall mortality of ash in Latvia was lower than estimated in Lithuania and Germany (6.9 vs. 8.7 and 10.1% year^{-1}, respectively; cf. Pliūra et al., 2017; Enderle et al., 2017), suggesting higher resistance of ash stands from Latvia against the pathogens. Still, it was considerably higher than observed for intermediate-sized (*r%* = ca. 6% year^{-1}) and the largest (ca. 3% year^{-1}) trees in Norway (Timmermann et al., 2017), mature ash in Austria (0.3% year^{-1}, Keßler et al., 2012) and 'veteran' (i.e., long lived; ca. 1% year^{-1}) trees in Sweden (Bengtsson & Senström, 2017), thus indicating higher resistance of long-lived individuals. The decrease in standing volume (*r%* = 5.3% year^{-1}; Table 3.1) was slower compared to that of stand density, which might be explained by the presence of 'veteran' trees in the studied population. The increased resistance of 'veteran' trees, as observed in previous studies (Skovsgaard et al., 2010; McKinney et al., 2011a), likely explained the trade-offs in *r%* calculated based on density and standing volume of stands (Table 3.1).

The mortality of the affected ash, as indicated by changes in ash density, was higher at the beginning of dieback period (Table 3.1), indicating that most of the population was highly sensitive to the pathogen (McKinney et al., 2014). The remaining trees apparently had higher resistance (Bengtsson et al., 2014), explaining the decreased mortality (Table 3.1). Such trends of mortality are consistent with observations of Marçais et al. (2017) and Coker et al. (2019), who observed that 6–8 years after strong infection, the mortality rates tended to stagnate.

The causal agent of ADB *H. fraxineus* was isolated in 35% of the collected 17 samples, confirming its involvement in the formation of lesions on ash saplings and ADB in general (Paper III). The proportion of the positive samples was lower than that found in Sweden (Bengtsson et al., 2014) and Germany (Schumacher et al., 2009), likely suggesting involvement of other agents (Husson et al., 2012), which, however, were not quantified. The low occurrence of the pathogen could be explained by seasonal (McKinney et al., 2011b; Bengtsson et al., 2014) and inter-tree (Schumacher et al., 2009) variation in the number of propagules, as well as intense surface sterilisation of samples prior to incubation. The uneven infection of stands of different age, uneven damage of ash trees in each stand and uneven mortality rates, as well as regional differences (in the European context) in mortality of ash, indicated that local factors affected resistance of ash.

3.2. Intra-seasonal development of *Hymenoscyphus fraxineus*

Mostly the dieback of common ash is a rapid process, as the infected trees can decline within a few years after the first symptoms appear (in some cases, symptoms might not be even visible), but some trees can have chronic symptoms and are weakened gradually (Bakys et al., 2009b; Timmermann et al., 2011; Enderle et al., 2013; Landolt et al., 2016). Accordingly, intra-seasonal dynamics can provide insight into the fate of affected tries – whether trees will die or develop chronic symptoms (Bengtsson et al., 2014). The intra-seasonal development of lesions on stems, branches and shoots caused by *H. fraxineus* in relation to increased mortality was monitored in three young stands (Paper III). Most of the observed lesions (67%) were already previously observed (EL) on stems (50%). The newly emerged lesions (NL) comprised 33% of all observed, and mostly appeared at the beginning of the observation period in June and July, implying a seasonal pattern, which might be explained by the maturation of leaves and shoots (Schumacher et al., 2009; Timmermann et al., 2011). Considering that propagules of *H. fraxineus* (Schumacher et al., 2009) infect their host through leaves and shoots (Kirisits & Cech, 2009; Kirisits et al., 2009; Cleary et al., 2013), the majority of NL appeared on branches (55%), suggesting limited effect on height growth. However, the amount of branches is higher than that of the apical shoots.

The activity and extension of lesions appeared to be related to meteorological differences (temperature), and were the highest in June–July, when the temperature was the highest (ca. 15–18°C) and closest to the growth optimum of *H. fraxineus* (20°C, Kowalski & Bartnik, 2010; Timmermann et al., 2011) (Fig. 3.2). The extension of lesions differed between EL and NL ($p < 0.01$), as well as amongst the affected parts of trees for EL (Fig. 3.2). During the season, the area of lesions increased from 52.5 ±11.3 cm^2 (mean ±standard error) per tree in June to 92.1 ±14.7 cm^2 per tree at the end of the observation period (Fig. 3.2). The area of lesions increased most rapidly on tree tops and branches, which are close to the stem pith, through which the pathogen spreads within its host (Schumacher et al., 2009), and where the bark is thinner, facilitating extension of infection

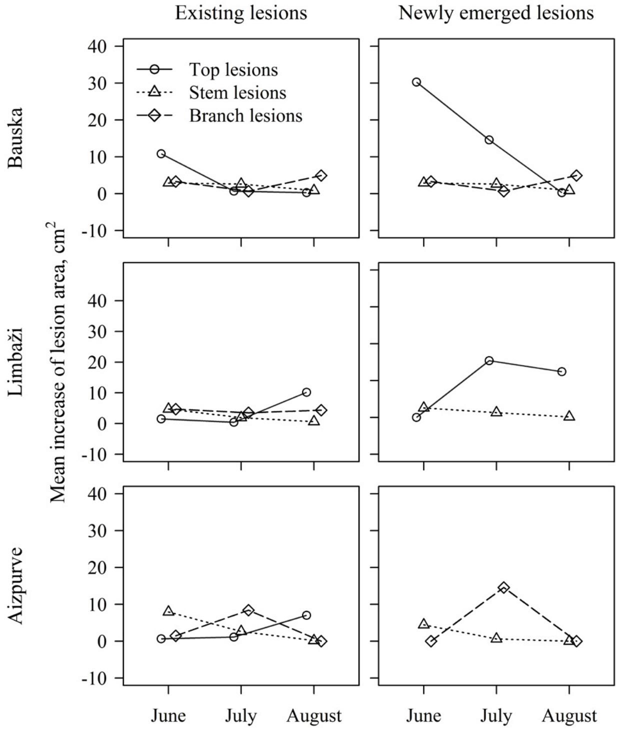

Fig. 3.2. **The mean monthly increase in area of lesions existing before the observation period and newly emerging lesions in the studied stands according to the location on trees**

(Husson et al., 2012). The NL extended more than three times faster compared to EL, indicating that after emergence, lesions rapidly grow to a certain optimum size within the first month. This indicated that ca. one month was needed to partially compartmentalise the development of the pathogen. Alternatively, this might be associated with a higher activity of younger strains of fungus (Lygis et al., 2016). This suggest ability of trees to survive and grow under a high disease intensity background.

During the growing season, 24% and 22% of lesions completely affected branches and tops, respectively, causing their complete death. Nevertheless, some biological limitations (e.g., inactive physical defence) for lesion growth (Bengtsson et al., 2014) were observed; 22% of EL were latent and 13% of branch and 7% of apical branch lesions stopped expansion when reaching the shoot base or the main stem. This might be related to the anatomical properties of wood, such as differences in vessel size and lateral connectivity, or pith diameter between the transitions of height increments of consecutive years (Schweingruber, 2007). Still, some transitions of lesions from tree tops and branches to stems were also observed, as 18% of tree top lesions expanded into the stem, suggesting further development in the following season.

With the expansion of lesions, the overall health condition (class) of saplings decreased from 1.6 to 2.9 over the observation period. Still, the intermediate proportion of NL, and low mortality of trees (3.3%, cf. Lygis et al., 2014), indicated chronic formation of lesions. The presence of latent lesions suggested that trees were able to sufficiently compartmentalise the pathogen (Pallardy, 2008).

There were no significant relationships between the health class at the beginning of the observation period and the expansion rate of EL or NL, nor between health class and the number of lesions, suggesting comparable infection pressure for all saplings, regardless of previous infection. There was no significant relationship between the number of EL and NL, suggesting similar probability of a lesion emerging, irrespective of preceding infection and leading to influence of stochastic processes or microclimate. The chances of ash to survive were individual, but most trees showed chronic symptoms, which could progress rapidly under favourable conditions. Yet, ca. one fifth of trees completely lost their tops and thus reduced that competitiveness.

3.3. Sensitivity of common ash to meteorological factors

The growth pattern and sensitivity to meteorological factors have been identified as factors affecting susceptibility of trees to pathogens (Helama et al., 2009; Tulik et al., 2018), therefore, such information can be valuable when assessing linkage of ADB with climatic changes. Although in Latvia ash occurs near its northern distribution limit (BFW, 2020), the individuality of growth was rather pronounced, similarly as observed for trees under optimum conditions (Fritts, 2001). However, some common tendencies in radial growth were also detected (Paper IV). The susceptibility of ash to fungal infection appeared to be age-related

(Enderle et al., 2013), as the healthy trees were generally younger, had higher mean TRW and contained less autocorrelation (0.77 vs. 0.82) than the damaged ones. The differences in growth rates between the groups suggested linkage with the social status (i.e., crown classes) of trees (Timmermann et al., 2011; Martin-Bento et al., 2008). The damaged trees showed growth suppression and autocorrelation during a few recent decades.

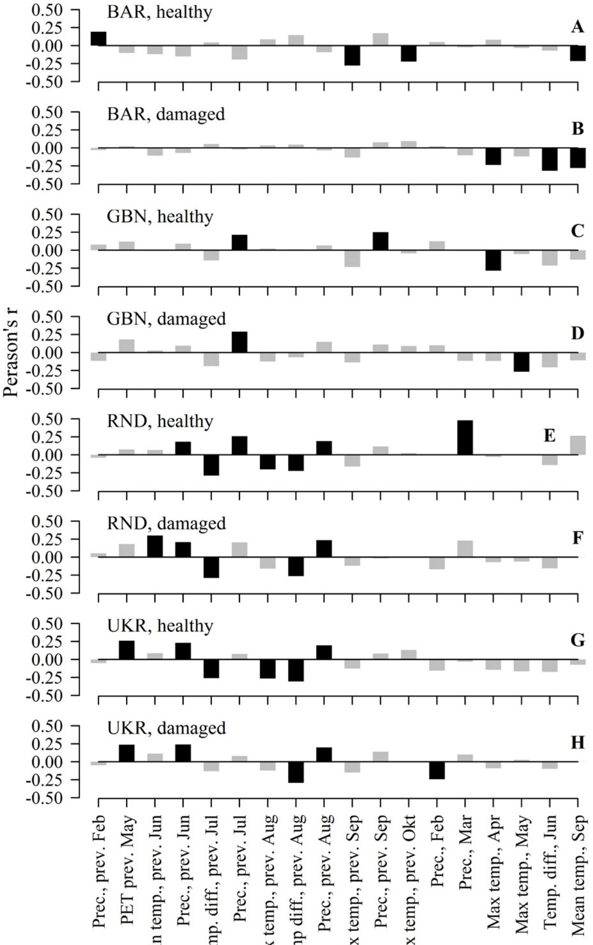

Fig. 3.3. **Bootstrapped Pearson's correlation coefficients calculated between climatic factors and residual chronologies of tree-ring width of ash with healthy (A, C, E, G) and damaged crowns (B, D, F, H) for sites near Barkava (BAR), Gulbene (GBN), Rundāle (RND) and Ukri (UKR), respectively**

The period from 1934 (1948 for healthy trees in RND site) to 2010 was analysed.
Significant correlations (at α = 0.05) are shown in black.
Only the significant factors are plotted. PET – potential evapotranspiration.

In the central part of Latvia, TRW was sensitive to climatic factors related to the previous vegetation season and the dormant period, suggesting legacy effects on xylogenesis (Zweifel, 2020). In the ring porous species, nutrient reserves are mainly deployed for early growth (Barbaroux & Breda, 2002), which affects water relations and increment in the following season (Tyree & Zimmermann, 2002). Accordingly, summer precipitation showed positive affect on TRW (Fig. 3.3), suggesting effect of water deficit. Shifting temperature conditions can burden physiological processes (Pallardy, 2008; Berry & Downton, 1982), explaining negative effect of daily temperature amplitudes on TRW (Fig. 3.3). The effect of precipitation and temperature in the preceding August was significant at all sites (Fig. 3.3), as the formation of nutrient reserves initiates at this time (Barbaroux & Breda, 2002).

In the eastern part of Latvia, TRW appeared sensitive to temperature in the previous autumn (September and October) and in the current vegetation season (April–September), mainly showing negative correlations (Fig. 3.3). In autumn, raised temperature can increase respiration, causing losses of stored nutrients (Ögren et al., 1997). In September, increased temperature, apparently, might also cause water deficit (Traykovic, 2005), as positive correlation with precipitation was observed (Fig. 3.3). The negative effect of temperature in the current spring may be explained by earlier onset of the active period or earlier leaf flush, subjecting trees to late frost (Gu et al., 2008).

Only a few pronounced differences in the sets of the significant climatic factors were observed between the healthy and damaged trees (Fig. 3.3), suggesting slight effect of climatic sensitivity on susceptibility to the disease. The damaged trees in the central part of Latvia were more sensitive to water deficit and temperature regime in the preceding August (Fig. 3.3), suggesting that under unfavourable conditions, trees are more stressed and hence predisposed to the infection (Timmermann et al., 2011). Still, healthy trees showed additional sensitivity (maximum temperature in previous August and precipitation in March). Precipitation in March is usually in the form of snow, and its effect might be explained by the insulating properties of snow layer, affecting roots (Hardy et al., 2001; Tierney et al., 2001), as well as the water table, hence water relations (Tyree & Zimmermann, 2002). The absence of such a relationship might suggest that the damaged trees had a less sensitive root system before the infection. In the eastern part of Latvia, the damaged trees were more affected by temperature in July and September. TRW of the oldest trees (from GBN site) was more sensitive to precipitation, particularly that of the damaged ones (Fig. 3.3), indicating age-related changes in sensitivity (Carrer & Urbinati, 2004), reflecting effect of water stress on susceptibility to the disease (Chira et al., 2017; Kowalski et al., 2010). Direct relationship between health condition and climatic sensitivity of ash was not observed, but the association between growth and meteorological factors indicated the likelihood of increasing stress as a result of climate change, and consequently increased susceptibility to the pathogen.

3.4. Genetic diversity of common ash in Latvia

Genetic diversity of a population is one of the key aspects determining its adaptability to novel and rapidly changing environments (Aitken & Bemmels, 2016; Tiffin & Ross-Ibarra, 2017). Genetic diversity and population structure of common ash in Latvia was studied using chloroplast and nuclear DNA markers. Three of the six analysed chloroplast SSR markers were polymorphic (Paper V). The genotypes of these three loci were combined into two haplotypes: H01 was present in all except the Ķemeri stands, suggesting different origin of the populations. This haplotype is widespread and common in Eastern Europe and Scandinavia. In Ķemeri, H02 was identified, which is the most common haplotype in Central Europe, reaching Poland (Heuertz et al., 2004a). Apparently, trees belonging to this haplotype originated from parental trees from nearby parks, where greening was done using planting material from Western Europe (Dambis et al., 2007). The haplotypes H01 and H02 were found to be the most common alleles in European common ash populations, together representing 68% of the individuals (Heuertz et al., 2004a). Interestingly, the nuclear SSR marker, however, did not differentiate the Ķemeri stand from the others (Paper V), suggesting hybridization with the local population. This indicated local specialization of the Latvian ash population, which persisted under high gene flow (Aitken & Bemmels, 2016; Moran et al., 2017), probably due to growth range fragmentation (Liepiņš et al., 2016).

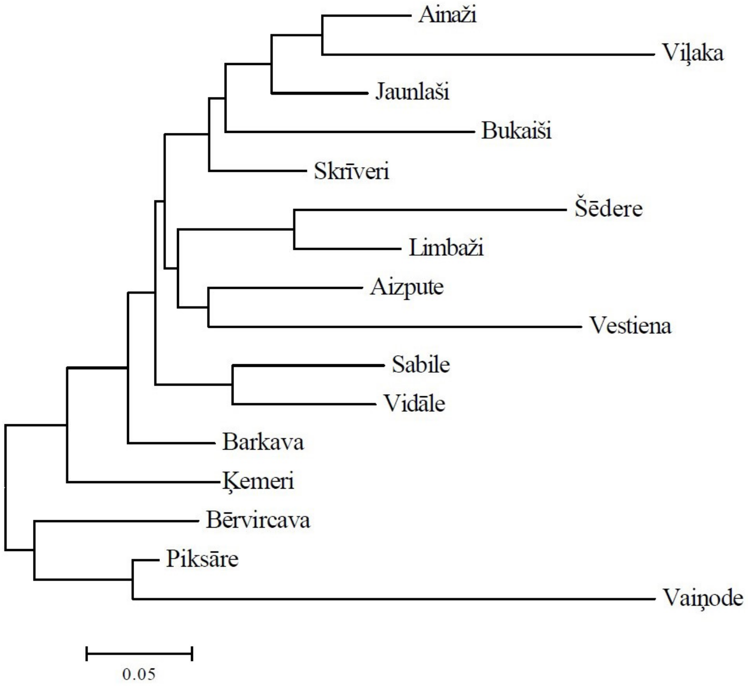

Fig. 3.4. **Neighbour-joining dendrogram of pairwise Nei genetic distances between the analysed common ash stands**

The observed heterozygosity in the studied population (mean of) was lower than the expected (mean values 0.64 vs. 0.82, respectively), suggesting reduced adaptability of the population to extreme conditions (Aitken & Bemmels, 2016; Tiffin & Ross-Ibarra, 2017), including pathogen outbreaks. Accordingly, the fixation index was positive for all loci (mean 0.23). In total, 14 private alleles were identified, of which only three had a frequency >0.05 in the respective stands; however, most of them were non-effective, indicating a high proportion of low frequency alleles.

The AMOVA analysis of nuclear SSR markers showed a low, but significant ($Fst = 0.045$, $p < 0.001$) level of population differentiation, implying local specialization (Aitken & Bemmels, 2016). The most genetically differentiated stands were Viļaka and Vaiņode (pairwise $Fst = 0.151$), as well as Šēdere and Vestiena (Fig. 3.4). However, the clustering of the populations did not reveal an explicit geographical pattern. The same was confirmed by the delta K criterion.

The genetic diversity of common ash in Latvia was lower than identified previously in the more southern populations (Table 3.2). The number of alleles at almost all loci was higher in other countries, with the exception of Scotland. Populations at the northern limit of the species have low genetic diversity, likely due to local specialisation to hostile environment during the post-glacial recolonization from southern refugia (Wallander, 2008). The inbreeding coefficients were positive for most of the loci/studies (Table 3.2), implying that this could be a general feature of common ash related to polygamy of the species (Wallander, 2008). The genetic structure of common ash in Latvia indicated low nuclear genetic diversity with a slight local specialization sugesting limited adaptability of the local population. However, the part of gene pool of the stands in Ķemeri showing relationships to another population might be considered as a source of additional genetic diversity.

Table 3.2

Comparison of allele number and inbreeding coefficients

Population Locus	Latvia[1]		Scotland[2]		France[3]		Italy[4]		Bulgaria[5]		Bosnia and Herzegovina[6]		Romania[7]	
-	Na	F	Na	F	Na	F	Na	F	Na	F	Na	F	Na	F
Femsatl4	25	0.46	nd	nd	37	0.10	32	0.27	50	0.08	20	0.39	37	0.01
Femsatl10	34	0.42	nd	nd	nd	nd	76	0.33	nd	nd	55	0.37	nd	nd
Femsatl11	17	0.09	nd	nd	40	0.03	42	0.31	32	0.08	24	0.33	32	0.01
Femsatl16	10	0.27	6	0.03	nd	nd	9	0.19	10	0.12	12	0.16	10	0.17
Femsatl19	21	0.08	19	0.33	36	0.13	55	0.08	33	0.13	26	0.17	27	–0.07
M2-30	37	0.14	30	0.19	56	0.16	nd	nd	59	0.11	nd	nd	42	0.04

Na – number of alleles; F – inbreeding coefficient; nd – no data. 1 – this study; 2 – Bacles et al., 2005; 3 – Morand et al., 2002; 4 – Ferrazzini et al., 2007; 5 – Heuertz et al., 2001; 6 – Ballian et al., 2008; 7 – Heuertz at al., 2003.

3.5. Succession in ADB affected stands

After various types of forest disturbances, including diseases, the regeneration of the understory and its dynamics can change dramatically, thus affecting the future composition of the stand and vitality of regenerating trees (Lygis et al., 2014; Thomas et al., 2018). Dolan & Kilgore (2018) indicated that after Emerald ash borer attack, the density of shade-tolerant shrubs and saplings, as well as density of native and non-native shrub species increased with decreasing abundance of ash.

According to the consolidated results from Paper VI and Paper VII, during 2005–2015, the mean density of AG and UG in ash stands was 6520 ±1401 and 11193 ±1751 individuals ha^{-1}, respectively. The understory in the middle-aged to overmature common ash stands generally consisted of broadleaved and pioneer species. The highest densities were observed for wych elm, common ash, Norway maple, and small-leaved lime *Tilia cordata* Mill., yet codominance of two to four AG species was often observed, suggesting formation of mixed stands. However, in the stands where Norway maple co-occurred in the canopy, AG of maple was facilitated by ADB, suggesting formation of pure maple stands in the future.

In most cases, AG consisted of the same species as the canopy. However, in the previously ash dominated stands, recolonization of UG by the shrub species (e.g., bird cherry *Padus avium* Mill. and common hazel *Corylus avellana* L.) occurred (Table 1 in Paper VI). Although common hazel is considered as a valuable species in ash forests (Loidi, 2004), together with bird cherry it comprised 53% of UG (Paper VI and VII), indicating suppression of AG regeneration (Runkle, 1990; Gillman et al., 2003; Royo & Carson, 2006). Furthermore, an increase in the abundance of adventitious (e.g. cherry plum *Prunus cerasifera* var. *divaricata* Bailey. false spiraea *Sorbaria sorbifolia* (L.) A. Braun and gooseberry *Ribes* spp.) UG species (Table 1 in Paper VI) that benefit from disturbances (Gonzales et al., 2002) occurred.

Since 2015, an increase (near double) in total density of understory was observed, but in contrast to other studies (de la Cretaz & Kelty, 2002; Coomes et al., 2003; Royo & Carson, 2006), it appeared independent of the local extent of ADB in most stands. This might be related to a delayed response of understory to ADB. Nevertheless, an increase in density of AG and UG remained similar compared to the 2005–2015 period (36% and 64%, respectively), with only slight fluctuations (up to 2%).

The observed ash AG densities suggest that pure ash stands would not regenerate naturally (low AG density 1987 ±397 ashes ha^{-1}), but that ash would likely remain in admixture. Although 81% of the studied ash trees were healthy, only 10% exceeded 0.5 m height, indicating high mortality due to self-thinning and likely ADB, as previously reported by Sakss (1958) and Giongo et al. (2017). Further decrease in health condition was observed as trees grew. For example, above 2 m height, 32% of trees were healthy, yet 17% were dead. It is expected that a small part of them (<5%) would reach the canopy, which is consistent with Laiviņš & Mangale (2004), who suggested that ash would remain as an admixture species.

The density of ash AG has been significantly affected by the dominant species in the canopy. Due to ADB, regeneration of ash under mother trees has drastically decreased, although, before ADB the number of ash seedlings was positively related to the number and proximity of parent trees (Harmer et al., 2005). Presence of black and grey alder in the canopy had a negative effect on ash regeneration density and health condition of saplings, while admixture of birch tended to have a positive effect. Nevertheless, a positive correlation between ash AG density and species richness in the understory was estimated ($\tau = 0.184$, $p = 0.03$), suggesting positive effects of stand diversity (Forrester & Bauhus, 2016). Although ash regeneration occurs in the affected stands, saplings are affected by the disease, which increases tree mortality and reduces competitiveness. Therefore, it is expected that other tree species, and especially shrub species, will compete with ash in the future and more likely will force ash into admixture, implying gradual transformation of the affected stands.

3.6. Natural regeneration of common ash in young stands

3.6.1. Species composition and ash regeneration

In Europe, including Latvia, the damaged ash stands are cut and reforested with other tree species, while a part of these stands are managed using natural regeneration, subjecting recruiting ash to competition with other species under open conditions (Lygis et al., 2014; McKinney et al., 2014). In the young stands of ash, the mean understory and AG density was 18410 ±1040 and 7150 ±558 individuals ha^{-1}, respectively (Paper VIII). In AG, ash (4185 ±401 ashes ha^{-1}), grey alder, birch and common aspen were the main species, but in UG two species – bird cherry and common hazel dominated (Fig. 3.5). The woody flora, however, appeared little affected by the dieback, as the same species have been observed before (Sakss,

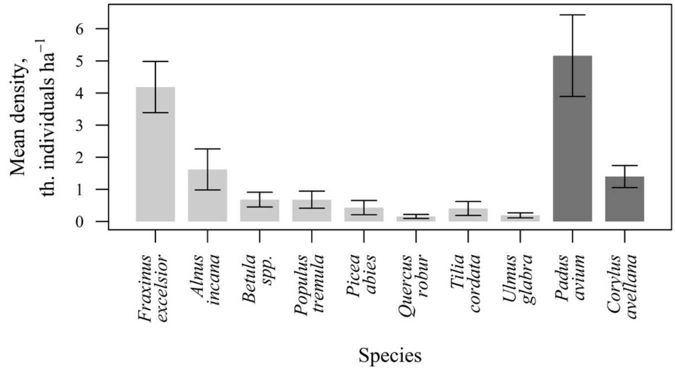

Fig. 3.5. **Mean density of the main understory (advanced- and undergrowth) species in young stands (≤40 years) of common ash**

1958) and after the ADB (Fig. 3.5; Lygis et al., 2014). Still, the composition of regenerating stands has been altered. Ash AG density was considerably higher than recently observed in neighbouring Lithuania (599 ashes ha^{-1}), Estonia (3500 ashes ha^{-1}) and Germany (653 ashes ha^{-1}) (Lygis et al., 2014; Drenkhan et al., 2017; Enderle et al., 2018), but lower than before the ADB in Europe (15–30 10^3 ashes ha^{-1}) (Sakss, 1958; Harmer et al., 1997; Tabari & Lust, 1999; Lygis et al., 2014). The ratio of AG and UG individuals was 48.4 vs. 51.6; the increased proportion of the UG species (Fig. 3.5) suggested ongoing changes in the affected ash stands.

ADB apparently facilitated rapid development of UG species, which were outcompeting the surviving ash, as negative correlations between density of ash and UG, as well as AG ($r = 0.24$ and 0.23, respectively) were found. Similar inter-species relationships in affected stands have been reported from other studies (Keer, 1998; Givnish, 2002; Royo &Carson, 2006; Skovsgaard et al., 2010).

3.6.2. Incidence of Hymenoscyphus fraxineus in ash advance growth

In Latvia, the proportion of dead ash saplings (from the accounted) was 10.0% (Paper VIII), which is comparable with the natural die-off of recruiting trees (Harmer et al., 2005). Unfortunately, based on available data, it was difficult to assess the compensatory and/or complimentary effects of ADB on mortality. For example, Sakss (1958) found that in Latvia ash AG in clear-cuts withered and died, similarly in England prior to ADB, after the removal of understory and thinning of canopy ash AG was reduced by 40–50% per year (Harmer et al., 2005). For comparison, the mortality of naturally regenerated young ash in Estonia, Lithuania, Germany and Italy was 7%, 17%, 16.2% and 17.6% respectively (Drenkhan et al., 2017; Enderle et al. 2017; Giongo et al., 2017), indicating regional differences in health condition of ash. Highly significant regional differences were also observed in Latvia, as health condition of ash was worse in the western than in the central and eastern part of Latvia.

A relationship was observed between health class and height ($r = 0.28$, $p < 0.001$), as well as age ($p < 0.001$, Fig. 3.6) of ash saplings. The proportion of healthy and dead ashes up to and above 3 m height was 81% vs. 4% and 54% vs. 33% respectively, which is similar to observations from the Italian Alps (70% vs. 14% and 38% vs. 20% in the height groups above and below 2 m; Giongo et al., 2017). This suggests that a certain time is needed for ash to be infected as well as to progressively increase disease pressure on older trees.

Increment analysis showed high site-specificity, indicating plasticity of the species. A significant (Paper VIII) linear relationship between the diameter as well as age and height of ash was observed. However, during the first 5–8 years, the height of ash increased irregularly and individually, possibly due to different growing conditions. Ash height and diameter were significantly affected by the disease suggesting cumulative effect of the pathogen as the trees grow. The lowest and thinnest trees were healthier, but health condition deteriorated significantly with age. In general, ash health condition in Latvia was better in young stands compared

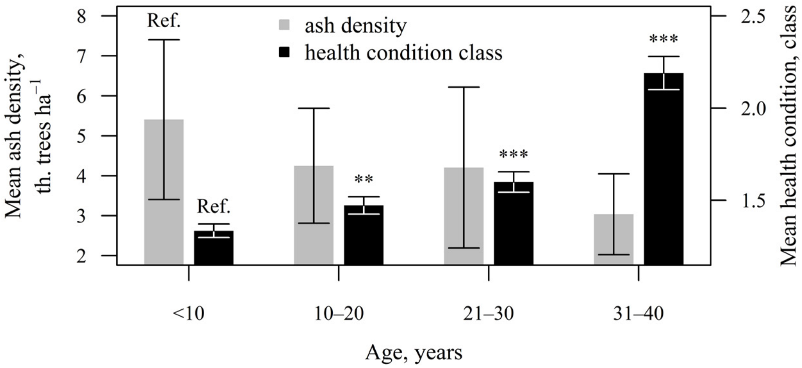

Fig. 3.6. **The mean density and health condition of common ash according to stand age**

The asterisks indicate the differences from the youngest age class used as the reference level (Ref.). Significance codes: * – $p \leq 0.05$, ** – $p < 0.01$, *** – $p \leq 0.001$.

to AG in a mature stand, which opposes the observations by Drenkhan et al. (2017) in Estonia, highlighting regional differences. Compared to Central Europe, ash in Latvia regenerated with higher density, and its mortality was lower. However, under intense interspecies competition in open conditions (after clearcut), height growth of infected ash was suppressed, thus allowing the formation of mixed stands with ash in admixture.

3.7. Factors affecting regeneration and health condition of common ash

The control of ADB in mature stands using silvicultural techniques is practically impossible (Gross et al., 2014). However, any management practice (e.g. stand establishment, thinning, etc.) and/or environmental aspect (moisture availability, fertility, etc.) showing positive effect on ash health condition should be exploited when establishing young ash stands for conservation, as well as for commercial purposes at the regional level (Havrdová et al., 2017; Skovsgaard et al., 2017).

The ash AG density was similar throughout the territory of Latvia (Paper VIII); it appeared independent on the stand age (Fig. 3.6), soil, and canopy species of the previous rotation. Although species richness can have positive effect on density of ash AG (Fraxigen, 2005; Dobrowolska et al., 2011; Ahlberg, 2014), composition of AG had no effect on density of ash saplings in Latvia. Nevertheless, density of ash AG tended to be higher when growing in mixture with grey alder (also before ADB; Sakss, 1958) and birch, and partially contrasting results were found in mature stands (Paper VII). Presence of spruce had contrasting effect on ash AG density, depending on composition. When a stand was formed by Norway spruce and ash, low density of spruce (<30%) had negative effect on ash density, while increased abundance of spruce (60–80%) showed the opposite effect. This relationship, however, might by coincidental.

Health condition of ash was affected by the structure of the regenerating stands. Mixed ordinal regression indicated that the combination of AG height and number of UG species were the best predictors of ash health condition, yet both their effects were interacted by region (Paper IX). The explicit regional differences in the effect of shrub (UG) species richness on ash health condition (Fig. 3.7) might be related to climatic conditions, as suggested by Papić et al. (2018). In the western part of Latvia, warmer climate apparently has facilitated development of *H. fraxineus* (Kowalski & Bartnik, 2010; Dvorak et al., 2016) in the longer term, suppressing growth of ash, as suggested by shorter tree height (Table 3.3, Fig. 3.7 B). Under such conditions, the admixture species apparently were outcompeting ash and decreasing its vigour, thus explaining positive linkage with the health class (Fig. 3.7 A). Under a cooler climate in the eastern part of Latvia, which is less favourable for *H. fraxineus* (Kowalski & Bartnik, 2010), the shrub (UG) species richness had a positive effect on health of ash (Fig. 3.7 A), likely acting as biological barriers (Kosawang et al., 2018) and hindering development of the pathogen (Jactel et al., 2005; Pautasso et al., 2005), thus highlighting the effects of species richness (Givnish, 2002). Ashes were taller, indicating better growing conditions under a more continental climate, as previously shown by Papić et al. (2018).

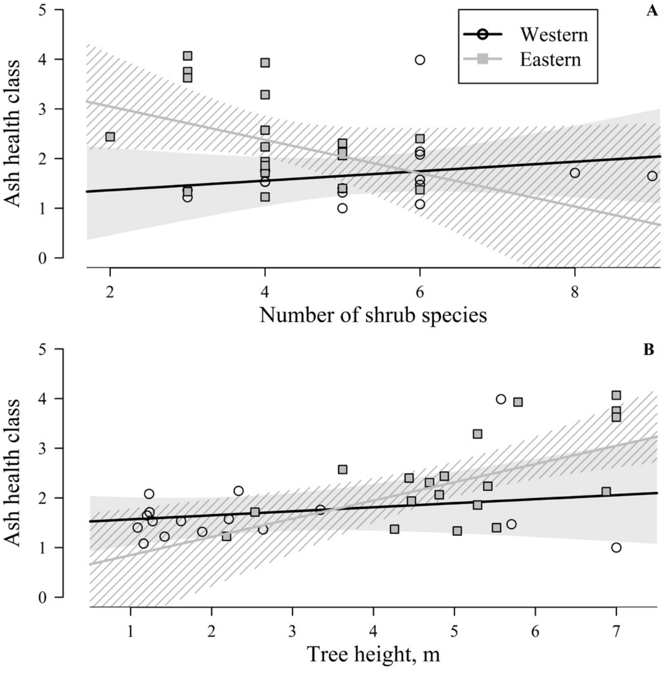

Fig. 3.7. **The relationship between health condition (health class) of common ash in young mixed stands and number of shrub species (A) and tree height (B) in the western and eastern parts (provenance regions) of Latvia**

The polygons (envelopes) denote confidence intervals.

Although there is no consensus on the effect of site type on the susceptibility to *H. fraxineus* damage (Bakys et al., 2013; Timmermann et al., 2017), in Latvia health condition of ash differed significantly (Paper VIII) in relation to soil type. Higher susceptibility of ash to the pathogen was observed in the overmoist sites, as previously shown by Gross et al. (2014); health condition was the best in stands growing in well-drained and dry mineral soils (>76% of trees had minimal or no symptoms), but worst condition on wet mineral soils, where 27% of trees were dead. The positive effect of a drainage system on ash health condition has been observed in other European countries (Dobrowolska et al., 2011; Schumacher, 2011; Ahlberg, 2014).

Table 3.3

Description of the best mixed ordinal regression model predicting health condition of common ash in young mixed stands based on stand and tree properties

Model (fixed part) analysis of deviance table, type II test

Term	Likelihood ratio, χ^2	Degree of freedom	*p*-value
Number of shrub species	0.14	1	0.71
Region	0.48	1	0.49
Tree height	245.04	1	<0.001
Number of shrub species × region	9.12	1	<0.01
Region × tree height	9.43	1	<0.01

Ash regeneration and health condition were better in mixed (82–95% of ash trees were healthy, 1% dead) compared to pure (66% vs 20%; Paper VIII) stands, as observed in Central and Western Europe as well (Dobrowolska et al., 2011; Schumacher, 2011; Stener, 2013). However, effect of species composition (dominant species) in AG was also significant. The greatest *H. fraxineus* damage was observed in young stands mainly formed of Norway spruce and Norway maple (in Norway spruce/ash stands – 40% of ash were dead, Norway spruce stands – 12% and Norway maple stands – 14%). This can be explained by crown and root system competition, as well as soil acidification (Lei et al., 2012), and weak barriers for the spread of the disease due to low understory diversity (Pautasso et al., 2005). As ash and Norway maple have similar growth strategies (Petritan et al., 2009), the competitiveness of ash due to ADB has weakened (Urbinati & Cillia, 1995), facilitating development of Norway maple. The best health condition of ash was observed in stands with birch and common aspen (in ash/birch stands 87% of ash were healthy, birch stands – 87% and common aspen 89%), as was previously observed by Givnish (2002) as well, suggesting positive effects of presence of pioneer species.

The health condition of ash can be improved by silvicultural activities like regular thinning (Niemelä et al., 1992; Guzman & Dirzo, 2001; Fraxigen, 2005) minimizing biotic (competition) and abiotic (increased moisture) stresses of common ash (Cech & Hoyer-Tomiczek, 2007; Skovsgaard et al., 2010; Bakys et al., 2013). Significantly better (Paper VIII) ash health condition (by four times) was observed in young stands, which were tended the most, and gradually decreased with decreasing number of tending events. Selection and retention of naturally regenerated ash young stands in accordance with environmental conditions and relevant management should contribute to preservation of ash in the future in Latvia.

CONCLUSIONS

1. Rapid large-scale dieback of common ash was observed in Latvia during 2005–2015 with mortality of mature trees reaching 6.9% year^{-1} and standing volume decreasing from 322 to 151 m^3 ha^{-1}. The survival of trees was similar throughout Latvia, however regional and local differences in health condition and susceptibility to pathogen suggested unequal future potential of ash stands.

2. The development of lesions displays a seasonal pattern; rapid development of lesions occurs during the warmer summer months, irrespectively of health condition of trees and number of existing lesions. The dynamics of lesion development indicate ability of ash to localize disease in the initially infected parts of trees. Chronic symptoms are observed in 78% of analysed trees; competitiveness is significantly reduced by dieback of tops, which has been observed in ca. one fifth of infected trees.

3. Restricted genetic diversity of common ash stands, as indicated by a prevailingly common origin and low nuclear genetic diversity (high inbreeding coefficient), suggested local specialization and limited potential for improvement of the resistance based on the local reproductive material.

4. Observed correlation between growth and meteorological factors indicated likelihood of increasing stress as a result of climate change, and, consequently, increased susceptibility to the pathogen. Trees showed generally similar sensitivity to meteorological factors, irrespective of their health status.

5. Ash regenerates in young stands (4185 ±401 ashes ha^{-1}), as well as in the affected mature stands (1987 ±397 ashes ha^{-1}), though in youg stands only ⅓ of the recruiting trees appear healthy and their proportion tend to decrease with ageing. In mature stands, 90% of recruiting ash dies already at the seedling stage. The competitiveness of ash is reduced by the admixture of broadleaved species. Still, mortality rates were lower compared to Central Europe indicating regional differences in the susceptibility.

6. Management and site properties have regional effects on ash health condition, but not on ash regeneration density (trees per hectare). Intensive tending improves the phytosanitary condition of ash. Trees are more vital in dry and drained forests. Stand composition has explicit regional effect on ash health condition; higher species diversity has positive effect in the eastern part of Latvia, although effects of admixture species may vary.

RECOMMENDATIONS

1. Considering contrasting condition of regenerating ash and positive effects of management, health condition of ash stands should be monitored. Survey during the growth season, when the identification of disease symptoms is the easiest, is advisable.
2. Efforts to conserve ash stands as a valuable genetic resource should primarily be focussed in areas in the eastern part of Latvia where the health condition of ash appears better. Nevertheless, the stands with best vitality should be conserved throughout Latvia, promoting local genetic diversity. Identification of the most genetically diverged populations for future breeding programmes is advised.
3. Specific silvicultural strategies should be used for young and mature ash stands: mixed stands with a small proportion of ash in admixture should be facilitated for ash conservation. Obviously, resistant ash trees should be identified and their long-term survival promoted using intense tending. In stands of other age groups, vital dominant trees should be left as a seed source. Stands with low vitality should not be preserved.

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SPREAD OF *HYMENOSCYPHUS FRAXINEUS* IN LATVIA: ANALYSIS BASED ON DYNAMICS OF YOUNG ASH STANDS

Māris Laiviņš[1,2,#], Agnese Priede[3], and Ilze Pušpure[1]

[1] Latvian State Forest Research Institute „Silava”, Rīgas iela 111, Salaspils, LV-2169, LATVIA

[2] Forest Sector Competence Centre, Dzērbenes iela 27, Rīga, LV-1006, LATVIA

[3] Nature Conservation Agency, Baznīcas iela 7, Sigulda, LV-2150, LATVIA,

[#] Corresponding author; maris.laivins@silava.lv

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In Latvia, during the last 15 years (2000–2015), the area of common ash Fraxinus excelsior *forest stands has decreased by 40.6%. The dieback was predominantly caused by the fungal pathogen* Hymenoscyphus fraxineus*. Mostly young stands (up to 40 years old) were affected, accounting for 77.3% of the area of dieback. In this paper, we analysed the dynamics of young ash stand area within nature regions in Latvia to attempt to determine patterns of spread and the major migration routes of* H. fraxineus*. As suggested by the available data, the invasion of the fungal pathogen began in the southern part of Latvia, and then gradually dispersed across the country. The largest decline of young ash stands, during the period 2000–2010, occurred in lowlands. According to our estimate, the average rate of dispersal in Latvia was 40 km per year. At the scale of Latvia, the dispersal routes of fungal pathogen* H. fraxineus *largely coincide with the major migration corridors of biota and are related to macro-relief forms and their configuration.*

Key words: *dieback,* Fraxinus excelsior*, young stands, migration routes.*

INTRODUCTION

Common ash *Fraxinus excelsior* is the most widespread deciduous broadleaved woody species in Latvia. According to the data of the State Forest Service (Anonymous, 2015), in 2015 the total cover of ash stands was 13 011.1 ha (0.4% of the total forest cover or 53.1% of the broadleaved deciduous forest area in Latvia).

During the last century, the absolute and relative area of ash stands in forests of Latvia varied considerably. In the first half of the 20th century, the cover of ash stands did not exceed 1000 ha (Anonymous, 1926; 1937; Kundziņš, 1937; Eihe, 1940; Kronītis, 1966) or 0.1% of the total forest area. However, in the 1960s to 1970s, targeted silvicultural measures resulted in a rapid increase of the total ash stand area (Saks, 1957; Sakss, 1958; Grauziņš, 1969, 1971; Grauzinsh, 1971) with a peak at the end of the 20th century (in 1998, the total area was 21 905.3 ha or 0.8% of the total forest area). On the basis of the literature sources mentioned above and forest statistics, the dynamics (change in area) of ash stands was estimated (Fig. 1).

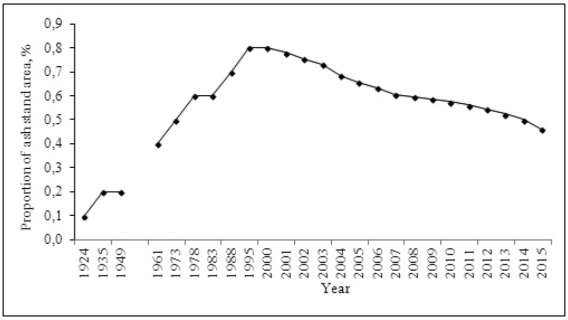

Fig. 1. Dynamics of common ash stands in the forests of Latvia within the last 90 years. Data summarized from the following sources: Anonymous, 1926; 1937; Kundziņš, 1937; Eihe, 1940; Saks, 1957; Kronītis, 1966; Sakss, 1958; Grauziņš, 1969; 1971; Grauzinsh, 1971; Anonymous, 2015.

Regeneration of ash and increase of the ash stand area were perhaps promoted also by environmental changes, particularly eutrophication of soil and synanthropization of vegetation, as suggested also by numerous studies in Europe (e. g. Kuhn *et al*., 1983; Fangmeier *et al*., 1994; Diekmann *et al*., 1999) including Latvia (Laiviņš, 1997, 1998).

The first cases of common ash dieback in Poland were recorded in the beginning of the 1990s (Przybyl, 2002). In Lithuania, the first cases of ash dieback were noted at the end of the 1990s (Juodvalkis and Vasilauskas, 2002; Kowalski and Lukomska, 2005). Soon the fungal pathogen *Hymenoscyphus fraxineus* was discovered to be the cause of the extensive ash dieback (Bakys *et al*., 2013; Pautasso *et al*., 2013; Gross *et al*., 2014). In the first decade of the new millenia, the fungi species was present in ash stands most of the European countries (Pautasso *et al*., 2013).

In Latvia, numerous cases of ash dieback were recorded in the end of the 20th century and in the beginning of the 21st century. However, the actual cause of dieback, *H. fraxineus*, was first proven in the western part of the country near Liepāja in 2006 and in the central part near Tukums in 2007 (Kēnigsvalde *et al*., 2010), several years after the massive dieback throughout the country. Thus, no precise data on the first establishment and dispersal rate of this fungi species in Latvia are available.

During the last 15 years (2000–2015), ash stands in Latvia are being damaged and die mostly due to infection caused by *H. fraxineus*. As a result, the total cover of ash stands has decreased by 8878.0 ha (40.6% of the area in 2000). The pathogen has affected mostly young ash stands (younger than 40 years) causing a loss of area of this age class by 77.3%, and of middle-aged stands by 51.1%, compared to the area in 2000. Similar patterns were observed also in Lithuania, where young and middle-aged stands were the most affected by pathogen infection (Juodvalkis and Vasilauskas, 2002). In mature and old-growth stands, the destruction and dieback is considerably slower.

In literature, the scientific name of the fungal pathogen species has changed several times. Currently, *Hymenoscyphus fraxineus* has been adopted as the taxonomically correct name (previously, *Chalara fraxinea* and *Hymenoscyphus pseudoalbidus*, currently used as synonyms) (Baral *et al*., 2014). In this paper, we used the latest accepted scientific name, *Hymenoscyphus fraxineus*.

In our study, we focused on the dynamics of young ash stands, which are known as the most susceptible against pathogen infections (Kowalski, 2006) and consequently the most dynamic age group among ash stands. Thus far, the cause of massive ash dieback in Europe has not cast doubt since the main reason was discovered. Therefore, in this paper we assume that this was the cause of young ash dieback also in Latvia, though the cause in each stand was not determined. The aim of this study was to describe regional differences of ash dieback caused by *H. fraxineus* and to determine the migration routes and the dispersal rate. We hypothesized that its dispersal routes in Latvia coincided with the major migration routes of biota largely defined by the pattern and configuration of macro-relief forms.

MATERIAL AND METHODS

Forest inventories and age groups of ash stands. In order to determine the dynamics of young ash stand area in different regions of Latvia within the time period from 2000 to 2015, data from the State Forest Service (Anonymous, 2015) were used. For each of the smallest administrative units (counties), data on the area and volume of ash stands (within time intervals 1–10, 11–20, ... > 201 years) were available. The ash stands are classified into five age groups: young stands (< 41 years), middle-aged stands (41–60 years), pre-mature stands (61–80 years), mature stands (81–120 years), and overmature stands (>121 years). Only data on the area per county of young stands (< 41) years) were selected and used in the analysis.

Nature regions and groups of regions. The selected data on young ash stands in 506 counties were used in a spatial analysis at the regional level. Using ArcView 9.1 software the counties were grouped within the borders of physiogeographical districts, or nature regions (Fig. 2) as defined by K. Ramans (Ramans, 1994), others (Sleinis, 1937; Zelčs and Šteins, 1989). If a county was crossed by the border of nature region, the county was joined to the region with the largest proportional area of the county.

The nature regions (Fig. 2) were merged into larger units: Southern, Central, and Northern Latvia. The Eastern Zemgale Plain (VII) and Augšzeme Upland (VIII) as well as Western Zemgale Plain (VI), Eastern Kursa Upland (IV), and Western Kursa Upland II were grouped into a unit

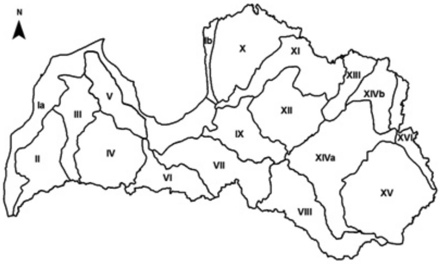

Fig. 2. Nature regions of Latvia.

I – Coastal Lowland, Ia – Kurzeme and Rīgava Coastal Plain, Ib – Vidzeme Coastal Plain; II – Western Kursa Upland; III – Venta River-land; IV – Eastern Kursa Upland; V – Northern Kursa Upland; VI – Western Zemgale Plain; VII – Eastern Zemgale Plain; VIII – Augšzeme Upland; IX – Southern Vidzeme Tilted Plain; X – Northern Vidzeme Plain; XI – Gauja River-land; XII – Vidzeme Upland; XIII – Eastern Vidzeme Upland; XIV – Aiviekste River-land, XIVa – Lubāna and Jersika Plain, XIVb – Adzele Rise; XV – Latgale Upland; XVI – Eastern Latgale (Mudava) Lowland. In the text the region numbers are given in parentheses.

called Southern Latvia, south of the Daugava River. The Southern Vidzeme Tilted Plain (IX), Lubāna and Jersika Plain (XIVa), and Vidzeme Upland (XII) were merged into a group called Central Latvia. The Northern Kursa Upland (V), Vidzeme Coastal Plain (Ib), Northern Vidzeme Plain (X), and Adzele Rise (XIVb) were combine in a Northern Latvia group of regions. The Northern Latvia and Southern Latvia groups were analysed in detail.

Data from nature regions in which the area of young ash stands in 2000 was smaller than 200 ha (Kurzeme and Rigava Coastal Plain (Ia, Ib), Venta River-land (III), Gauja River-land (XI), Latgale Upland (XV), Eastern Vidzeme Upland (XIII), and Eastern Latgale Lowland (XVI)) were excluded from the analysis. In these regions, the distribution of ash is limited by climatic or edaphic factors (von Sievers 1903; Sakss 1958; Laiviņš and Mangale 2004).

Data analysis. For each region, the annual increase (+) or decrease (-) of young ash stand area was calculated in hectares (transformed into percent) as compared to the previous year. This analysis was performed for each region over the time period from 2000 to 2010. The distribution maps were prepared using Arc View 9.1 software.

RESULTS

Dynamics of young ash stand areas in Latvia. In comparison to 2000, in 2015 the total area of young ash stands in Latvia had decreased by 4.4 times (Fig. 3). The rate of decline during the last 15 years was uneven: in 2000–2006, the decline was rapid (average 805 ha/year), while in 2007–2015 the rate had declined (average 279 ha/year).

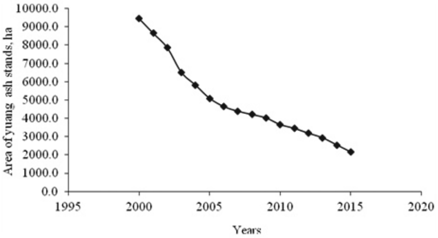

Fig. 3. Total area of young ash stands in Latvia (2000–2015).

During the last 16 years, the damage caused by *Hymenoscyphus fraxineus* has dramatically changed the age structure of ash stands at the national scale. In 2000, young ash stands comprised nearly one half (43%) of all ash stands in Latvia. In 2015, the area of young ash stands was only 17% of the total ash stand area in the country.

Regional differences in young ash stand dynamics. Intensive dieback of young ash stands began in Southern Latvia. In 2000, the highest rate of dieback was observed in the Eastern Zemgale Plain (VI) where a loss of 2333 ha had occurred (decrease of the total young ash stand area by 4.3 times), and in the Eastern Zemgale Plain (loss of 1083 ha; decrease by 3.1 times) (Fig. 4).

A year later, in 2001, the total area of young ash stands had decreased by one half (373 ha or 34.4% of the area in 2000). In Augšzeme (VIII), during the period from 2000 to 2002, the total area of young ash stands decreased almost

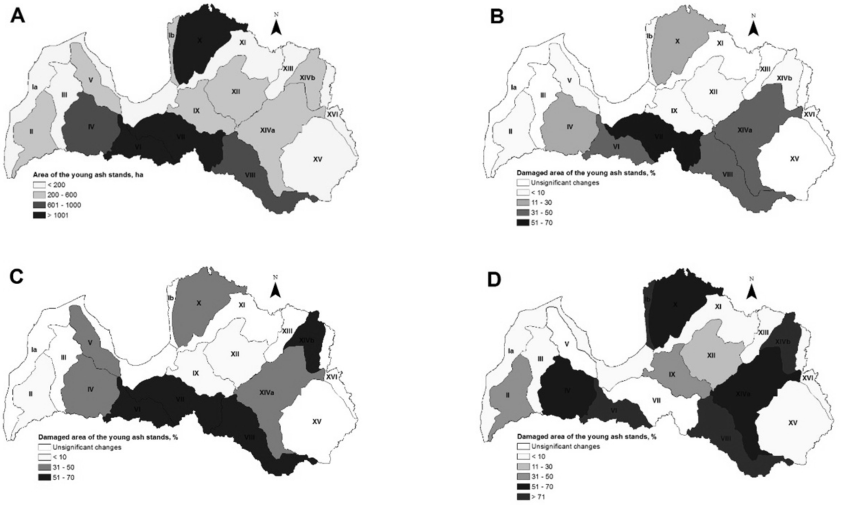

Fig. 4. The dynamics of ash young stand dieback across different nature regions in Latvia (A – 2000, B – 2003, C – 2006, D – 2009).

Table 1

THE DYNAMICS OF THE YOUNG ASH STAND AREAS IN NATURE REGIONS OF LATVIA*

Year	Nature regions in Southern Latvia					Nature regions in Central Latvia			Nature regions in Northern Latvia			
	II**	IV	VI	VII	VIII	IX	XIVa	XII	Ib	V	X	XIVb
2001	+5.8	+2.7	–17.4	–34.4	–1.6	+5.6	–21.7	–0.1	+2.3	+2.7	+11.2	+0.2
2002	+0.6	+0.7	–1.2	–7.1	–47.3	–0.6	–15.7	+1.7	–6.6	–3.0	–12.2	–8.1
2003	–5.5	–25.0	–31.8	–32.2	–0.2	–17.4	–2.7	–4.1	–2.1	–2.2	–13.6	–1.1
2004	–4.7	–9.5	–7.0	0.0	–1.8	–0.6	–2.8	+3.2	–66.2	–1.7	–28.7	–1.3
2005	–1.1	–3.0	–17.1	–1.7	–12.3	–2.9	–4.6	+1.5	–1.1	–36.1	–3.7	–65.2
2006	–0.4	–4.7	–29.3	–3.3	–3.1	–3.9	–10.0	0.0	–1.5	–5.2	–4.6	–1.6
2007	–8.1	–0.2	–8.7	–9.8	–2.0	–7.0	–0.1	–4.5	–5.1	–9.3	–4.5	–3.3
2008	–5.7	–2.7	–4.6	+0.4	–3.6	–9.4	–9.8	+0.8	–6.5	+0.2	–5.0	–0.9
2009	–1.9	–7.6	–3.2	–1.5	–7.4	–0.3	12.4	–0.3	+3.4	–19.3	+3.6	–3.1
2010	–2.6	5.0	–9.4	–7.4	–15.5	–9.6	–7.8	–6.4	–15.1	+3.7	–17.5	–9.2

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* ±, % as compared to the previous year; ** The number code of regions are presented in Fig. 2. Annual decreases exceeding –10% are underlined.

twice — loss of 354 ha (48.8% of the area in 2000). In the Western Kursa (II) Upland and Eastern Kursa (IV) Upland, where the total area in 2001 was 236 ha and 967 ha, respectively, the total area slightly increased (Table 1).

In the nature regions of Central Latvia, the total area of young ash stands in 2001 decreased only in the Lubāna and Jersika Plain (XIVa) — by 106 ha (21.6% of the area in 2000). In the Vidzeme Upland (XII), the area of young ash stands did not change, while in the Southern Vidzeme Tilted Plain (IX) it slightly increased.

In all nature regions of Northern Vidzeme, the total area of young ash stands in 2001 increased in the next year, but only a year later, in 2002, in all these regions a slight decrease of the area was observed, with the largest decline in the Northern Vidzeme Plain (X) — loss of 160 ha (12.2%) from the area in 2000 (Table 1).

Spread of *H. fraxineus* can be modelled by the dynamics of young ash stand area across the nature regions of Latvia (Fig. 4). In 2003, in the Eastern Zemgale Plain more than 50% of the young ash stands recorded in statistics of 2000 were dead (Fig. 4A). In Western Zemgale, where the largest area of young ash stands was recorded in 2000 (2333 ha) as well as in Augšzeme Upland and Lubāna and Jersika Plain (total 1083 ha), two regions bordering with Eastern Zemgale, the total area of young ash stands decreased by more than 30%. Similarly, in the Northern Vidzeme Plain with relatively large areas of young ash stands in 2000 (totally 1177 ha), about 10% of them died in the period up to 2003 (Fig. 4B). This means that within three years after the outbreak in Southern Latvia, the disease had reached also the northern part of the country.

In the period up to 2006, the decline of young ash stands reached nearly 70% in the Western Zemgale Plain, Augšzeme Upland, Adzele Rise, and Vidzeme Coastal Plain, which means that massive dieback had occurred throughout the country. Loss of about one-third of young ash stands had occurred also in the Eastern Kursa Upland, Northern Kursa Upland, and Northern Vidzeme Plain (Fig. 4C). In the period from 2007 to 2010, in most of regions the dieback of young ash stands had slowed (Fig. 4D, Table 1).

DISCUSSION

In Latvia, ash dieback caused by *Hymenoscyphus fraxineus* began in the first years of the 21st century along with dispersal of spores of the fungi northward from Lithuania. In Lithuania, the first signs of dieback caused by *H. fraxineus* were recorded in 1996–1997, and massive dieback was recorded a few years later in 2001 (Juodvalkis and Vasilauskas, 2002; Vasaitis, 2012). According to Lithuanian forest monitoring data, moderately poor (crown defoliation > 25 %) condition of common ash was first recorded in 1994 and 1998 (crown defoliation 26.9 and 27.9%, respectively), and in all years since 2000 (Ozolinčius *et al.*, 2005; Stakenas *et al.*, 2013). In Poland, spreading dieback of common ash was first recorded in the second half of the 1990s, although the first signs were observed earlier with recording of the fungal pathogen *H. fraxineus* (Kowalski, 2001; Kowalski and Lukomska, 2005). Also in countries north of Latvia (Estonia and South Finland including the Åland Islands), invasion of the fungal pathogen was recorded during the first decade of the 21st century (Rytkönen *et al.*, 2011).

According to data of the State Forest Service, increased dieback of common ash began in Southern Latvia in two nature regions located south of the Daugava River. In 2001, a dramatic decline of young ash stands was recorded in Western Zemgale Plain (VI), where the young ash stand area had decreased by 405 ha (17.4% of the area in 2000), and Eastern Zemgale (VII), by 373 ha (34.4% of the area in 2000).

Both regions are located on the border of districts in Lithuania (Biržai, Panevežys, and Rokiškis forest massifs), where the majority of the damaged ash stands occurred at that time (Juodvalkis and Jankauskas, 2002). In 2001, the area of young ash stands had decreased by 106 ha (21.7% of the area in 2000) also in the Lubāna and Jersika Plain (XIVa). Thus we can assume that the diaspores of the fungal pathogen had spread northward across Daugava River and reached the Eastern Latvia Lowland. Between 2000 and 2001, the area of young ash stands did not decrease in other nature regions in Latvia, and in some cases had even slightly increased. A few years after invasion of *H. fraxineus* in Southern Latvia, dieback of common ash was recorded also in Northern Vidzeme (Fig. 5), reaching a peak in 2004 (Table 2).

The intensity of young ash stand decline in 2000–2015 was uneven: in 2000–2006, the decline was rapid (average 805 ha/year), and relatively slower in 2007–2015 (average 279 ha/year). One of the reasons for the observed young ash stand decline (loss of area) might be that the young stands had survived and were included in the category of pre-mature stands, but this would include only a small proportion of stands. The forest statistics data did not allow to estimating the area of young ash stands that transform into pre-mature stands annually. However, according to the forest statistics, the proportion of total ash stand area that transformed into the next age category did not exceed 14% during the period 2000–2015. The forest statistics do not show a notable increase of pre-mature stand area, and thus it is very probable that the area of young stands has declined, and not transformed into pre-mature stands. Although we did not analyse this in more detail, also the area of pre-mature ash stands continuously declined over the study period, allowing us to assume that the major cause of the decline of area was dieback.

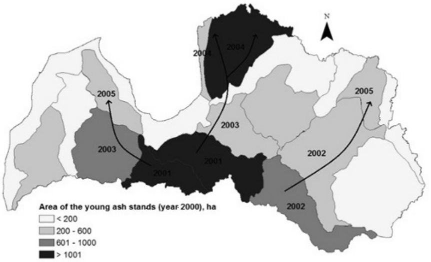

Fig. 5. Hypothesized migration routes of *Hymenoscyphus fraxinea* in Latvia.

Table 2

ANNUAL CHANGES OF THE YOUNG ASH STAND AREA IN THE SOUTHERN AND NORTHERN NATURE REGIONS OF LATVIA*

Year	Southern regions of Latvia	Northern regions of Latvia
2001	–15.0	+6.5
2002	–10.8	–9.4
2003	–24.8	–7.9
2004	–5.5	–26.9
2005	–10.2	–24.8
2006	–13.9	–4.1
2007	–6.1	–5.2
2008	–3.2	–3.9
2009	–2.0	+4.6
2010	–6.4	–13.5

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* ±, % as compared to the previous year. Annual decreases exceeding –10 % are underlined.

Perhaps the high rate of decline can be explained by the large proportion of young ash stand areas in the beginning of the studied period. Being more widespread, the ash stands became easily invaded by the fungal pathogen, consequently serving as stepping stones and promoting further spread into other stands.

On the basis of map analysis, we can assume that the diaspores of the fungal pathogen spread from the south to the north, at least during the first years (2001–2003) after invading the territory of Latvia. The dispersal occurred in the south-north oriented lowlands using the landforms as migration corridors (Fig. 5). The Eastern Latvia migration corridor coincides with the Eastern Latvia Lowland. It is likely that the diaspores spread from the southern slope of Aknīste Rise (bordering with Biržai and Rokiškis forest massifs in Lithuania) toward the Jersika and Lubāna Lowland reaching and massively invading young ash stands in the Adzele Rise in 2005.

The Central Latvian migration corridor, connects Western Zemgale Lowland with Northern Vidzeme and the Coastal Lowland in Vidzeme along the coast of the Gulf of Riga and the Southern Vidzeme Tilted Plain. In 2003, the fungal pathogen caused significant damage to ash stands in Southern Vidzeme and Northern Vidzeme. Most probably, *H. fraxineus* spread into Northern Kurzeme from the Western Zemgale region (Fig. 5).

The dispersal of diaspores was less intensive and less damaging to young ash stands on uplands. In Vidzeme Upland and Western Kursa Upland, dieback of young ash stands in the period up to 2010 was recorded only in small areas. This is perhaps due to the low cover of young ash stands in the particular regions (in 2000, there were 366 ha in Vidzeme Upland, and 236 ha in Western Kursa Upland). Additionally, due to terrain conditions in uplands, the distribution pattern of ash stands has a mosaic character and they are naturally more isolated from each other. Augšzeme Upland is an exception, where intensive dieback was observed already in 2002, most probably because the region is adjacent to the most invaded districts in Lithuania at the time. Augšzeme Upland has also a relatively warm climate in comparison to many other nature regions in Latvia and early spring (Zirnītis 1956), which could be beneficial for the spread of *H. fraxineus*.

The dispersal and direction of *H. fraxineus* migration in Latvia could be well portrayed by the dynamics of young ash stands, particularly when comparing the southern and northern regions of the country (Table 2). In the northern regions (150_180 km to the north from the southern regions), the most intensive dieback started four years later than in the southern regions. Thus, we can assume that the dispersal rate was about 40 km per year. Dispersal rate estimations in other European countries differ, e. g. 50–60 km per year in North East Italy (Luchi *et al*., 2012) and 30 km in Norway (Solheim *et al*., 2012) suggesting that at the European scale the rates may increase southward.

The dispersal corridors of *H. fraxineus* in Latvia coincide with the most important migration corridors of biota. For example the migration routes of birds (e. g. northern lapwing *Vanellus vanellus*, Eurasian skylark *Alauda arvensis*, common cuckoo *Cuculus canorus*, and many other) are across northward oriented lowlands (Strautzels, 1939; Kalniņš, 1943). Also numerous non-native plant species spread along the lowland rivers, as suggested by several studies, e. g. Laiviņš and Gavrilova (2003); Laiviņš *et al*. (2006); Laiviņš and Čekstere (2014).

At the scale of Latvia, the dispersal routes of the fungal pathogen *H. fraxineus* largely coincide with the major migration corridors of biota and are associated with macro-relief forms and their configuration.

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PATOGĒNAS SĒNES *HYMENOSCYPHUS FRAXINEUS* IZPLATĪŠANĀS LATVIJĀ: PARASTĀ OŠA JAUNAUDŽU DINAMIKAS ANALĪZE

Latvijā pēdējos 15 gados (2000–2015) oša audžu platība ir samazinājusies par 40,6%, galvenokārt patogēnās sēnes *Hymenoscyphus fraxineus* izraisīto slimību dēļ. Šajā periodā visstiprāk (par 77,3%) ir samazinājusies līdz 40 gadiem vecu oša audžu jeb jaunaudžu platība. Šajā rakstā, pamatojoties uz jaunaudžu platību izmaiņām dabas reģionos, analizēta sēnes izplatīšanās dinamika un galvenie izplatīšanās ceļi. Noskaidrots, ka oša jaunaudžu atmiršana vispirms sākās valsts dienvidu reģionos, bet pēc tam pakāpeniski aptvēra visu valsti; intensīvāk oša jaunaudžu platība 2000.–2010. gadā samazinājusies zemienēs. Parastā oša infekcijas masveida izplatīšanās Latvijā ir notikusi aptuveni ar ātrumu 40 km gadā. Kopumā slimības izplatīšanās ceļi sakrīt ar galvenajiem biotas migrācijas koridoriem Latvijā — reljefa lielformām un to konfigurāciju.

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Ilze Matisone, Roberts Matisons, Māris Laiviņš and Tālis Gaitnieks

Statistics of ash dieback in Latvia

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Highlights

- Dynamics of ash dieback during 2005–2015 were summarized.
- The area of ash dominated stands decreased twofold.
- The number of mature ash trees and their standing volume decreased by 53.1 and 69.9%, compared to 2005, respectively.
- The mortality of trees was higher during the first part of the survey, the decrease of standing volume culminated later.

Abstract

Dieback of the common ash (*Fraxinus excelsior* L.) has been spreading throughout Europe since the 1990s, causing severe ecological and economical consequences; however, detailed statistics on its dynamics have been published rarely. This paper presents the dynamics of mature ash-dominated stands in Latvia for the period 2005–2015. Data from the national forest inventory and a permanent sampling plot network were summarised. According to the official statistics, the dieback has caused a twofold decrease in area of the ash stands (from 21 891 to 13 011 ha, which respectively comprised ca. 0.8 to ca. 0.4% of the total forest area). The official statistics on standing volume appeared biased, as they did not account for increased mortality. According to the permanent sampling plots, standing volume and stand density have been affected even more, having decreased by 53.1 and 69.9%, respectively, compared to 2005 (the stand density and standing volume of ash in 2015 was 77 individuals ha^{-1} and 151 m^3 ha^{-1}, respectively). The mortality of the trees has not been stable. Stand density decreased faster during 2005–2009 compared to 2010–2015, with mortality rates of 9.6 and 8.2% $year^{-1}$, respectively. In contrast, the decrease in standing volume in 2005–2009 was slower than in 2010–2015 (mortality rates were 4.7 and 7.7% $year^{-1}$, respectively) because trees with smaller dimensions were more susceptible to the dieback. Nevertheless, the observed mortality rates clearly indicate negative prospects for ash stands in Latvia.

Keywords *Fraxinus excelsior*; mortality rate; national forest inventory; permanent sampling plots
Address Latvian State Forest Research Institute (LSFRI) Silava, Rigas str. 111, Salaspils, Latvia, LV2169
E-mail ilze.matisone@silava.lv
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1 Introduction

In Europe, the common ash (*Fraxinus excelsior* L.) is a widespread, economically and ecologically important tree species (Dobrowolska et al. 2011). In the late 1990s, ash dieback broke out in Poland (Kowalski 2006) and Lithuania (Juodvalkis and Vasiliauskas 2002), rapidly spreading across Europe, and, at present, more than 20 countries have been affected (Vasaitis and Enderle 2017). After the initial symptoms, e.g., wilting of leaves and shoots (Schumacher et al. 2010), the infected trees can die within a few months or years (Bakys et al. 2011; Bengtsson et al. 2014); however, some trees may show chronic symptoms, or even some signs of recovery (Schumacher et al. 2010; Bengtsson et al. 2014). The ascomycete *Hymenoscyphus fraxineus* (T. Kowalski) is the primary agent of the dieback (Kowalski 2006), while the secondary infection, often by *Armillaria* spp. that colonise root collars, causes tree death and uprooting (Skovsgaard et al. 2010; Bakys et al. 2011).

Ash dieback is usually large-scale, reducing densities of ash stands down to a few individuals per hectare (Bengtsson et al. 2014; Pliūra et al. 2015). Consequently, it has a pronounced economic impact (Thomsen and Skovsgaard 2012; Worell 2013). Still, publication of national or regional statistics concerning the dynamics of this are mostly lacking, except for some scarce reports, derived from data from national forestry information systems (cf. Worell 2013; Vasaitis and Enderle 2017), which, however, might not be quite accurate. The aim of this study was to summarise the statistics on the dynamics of ash dieback in Latvia, combining data from forest inventory (FI) and a permanent sampling plot (PSP) network for the period 2005–2015. We hypothesised that ash mortality was higher at the beginning of the dieback, decreasing afterwards.

2 Material and methods

2.1 Datasets

The FI data on standing volume (living) and area of ash-dominated stands in Latvia, (Fig. 1), were acquired from the Latvia State Forest Service database. The data for 2005–2015, and additionally for 1998, when the maximum coverage of ash forests was observed, were used.

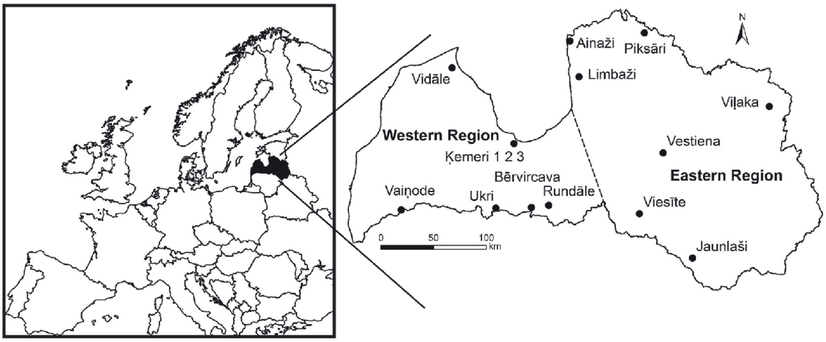

Fig. 1. Location of the studied permanent sampling plots. Broken line indicates arbitrarily division of Latvia in two regions with differing growth patterns of broadleaved trees.

Empirical data were acquired from 15 PSPs, scattered over the territory of Latvia (Fig. 1). The PSPs were established in 51- to 138-year-old, uneven-aged stands, which have been dominated by ash, yet have been subjected to different degrees of dieback. The sampled stands grow on flat terrain on fertile mineral soil. In some stands, spring waterlogging occurred. The climatic conditions were temperate, with mean annual temperature (± standard error) in the western and eastern parts of Latvia of 7.0 ± 1.4 and 6.1 ± 1.6 °C, respectively; January was the coldest (–1.8 ± 0.7 and –4.5 ± 1.0 °C, respectively) and July was the warmest (17.4 ± 0.3 and 17.9 ± 0.3 °C, respectively) month (Harris et al. 2014). The mean annual precipitation was 706 ± 67 mm; the highest monthly precipitation occurred in the summer months (June–September; 72 ± 29 mm).

Each PSP was a circle with a radius of 15 m (ca. 706 m^2). Within each PSP, all trees, including deadwood, with stem diameter at breast height (DBH) of ≥ 6 cm were measured. For each tree and log/snag, DBH and height/length (H) were recorded, with an accuracy of 1 cm and 50 cm, respectively. The measurements were repeated in 2005, 2010, and 2015.

2.2 Data analysis

The standing volume of the PSPs was calculated, based on stemwood volume (V, m^3 ha^{-1}) of individual trees, according to Liepa (1996):

$$V = \psi \times H^{\alpha} DBH^{\beta \lg(H)+\varphi}, \tag{1}$$

where $\psi = 0.8530 \cdot 10^{-4}$, $\alpha = 0.73077$, $\beta = 0.0682$, and $\varphi = 1.91124$ are the coefficients, H was height of tree (length of stem) and DBH was stem diameter at breast height. To assess the mortality of ash during each of the periods analysed, the mortality rate, r%, was calculated:

$$r\% = \frac{N_1 - N_t}{tN_1}, \tag{2}$$

where N_1 was the amount (number, or standing volume, of trees) of ash at the beginning of the observation period, N_t was the amount of ash at the end of the observation period, t was the length of the observation period, in years. Considering the regional diversity in growth patterns of broadleaved trees in Latvia (Matisons et al. 2012), the differences in ash mortality between the western and eastern parts of Latvia were assessed, for each period, by generalised linear mixed model, applying the binomial distribution of residuals and a "logit" link function. Sampling plot was used as a random effect. Regional differences in standing volume were assessed by t-test. Data were analysed using the program R v. 3.3.3 (R Core Team 2016), applying the "lme4" package (Bates et al. 2015).

3 Results

According to the FI data, the dieback decreased the area of ash-dominated stands in Latvia nearly twofold, from 21 891 ha (ca. 0.8% of the forest area) in 1998, when the maximum was observed, to 13 011 ha (ca. 0.4% of the forest area) in 2015 (Table 1). The decrease was slightly faster (ca. 6%) in the western part of Latvia, where the total area of ash stands was higher (not shown). Surprisingly, the official (FI) statistics indicated a gradual increase in the standing volume of the remaining ash-dominated stands in Latvia during 2005–2015 (Table 1), contradicting the dieback.

Table 1. Statistics of ash stands in Latvia according to data from Latvia State Forest Service and permanent sampling plots for the period 2005–2015. The 95% confidence intervals are shown for the permanent sampling plot data.

	Year 2005	 2010	 2015
Latvia State Forest Service data			
Total stand area, ha	18 315.7	16 263.5	13 673.71
Total standing stock m^3	373 7303	369 8663	341 0624
Mean standing volume, m^3 ha^{-1}	204.05	227.42	249.43
Permanent sampling plot data			
Living			
Stand density, individuals ha^{-1}	256±55	136±45	77±32
Standing volume, m^3 ha^{-1}	322±89	246±77	151±59
Deadwood			
Stand density, individuals ha^{-1}	74±50	178±82	233±79
Standing volume, m^3 ha^{-1}	18±14	119±54	212±66

In the PSPs at the beginning of the survey in 2005, 340 ashes of different dimensions were accounted, of which 69 were already dead. In the second and third surveys, only 52.0%, and 30.6%, respectively, of the initially accounted trees had survived (Table 1). The survival of trees was similar between the regions (p-value$>$0.10). Accordingly, in 2005–2009 and 2010–2015, r% was 9.6 and 8.2% year^{-1}. The r% calculated for the entire period (2005–2015) was ca. 6.9% year^{-1}. During the survey, only six new ashes with DBH$\geq$6 cm were accounted in the PSPs.

In 2005, the standing volume of living ash in the PSPs was ca. 320 m^3 ha^{-1} (Table 1), and stand density was ca. 260 trees ha^{-1}. The volume of ash deadwood was low, ca. 18 m^3 ha^{-1}. In the following surveys (in 2010 and 2015), the standing volume of living trees decreased to 76.4 and 46.9% of the initially accounted, respectively. The volume of deadwood increased accordingly (up to 212 m^3 ha^{-1} in 2015). The changes in the standing volume of ash were similar for the regions (p-value$\geq$0.06). The r%, calculated based on standing volume, was lower in the first than the second observation period (4.7 and 7.7% year^{-1}, respectively), and the r% for 2005–2015 was 5.3% year^{-1}.

4 Discussion

In the Baltics, ash dieback has been observed for more than 15 years (Juodvalkis and Vasiliauskas 2002). Despite this, the decrease in stand area (by ca. 40% in 17 years; Table 1) in Latvia was slightly lower, compared to the neighbouring Lithuania and Central Europe, where ash forests were more common (cf. Vasaitis and Enderle 2017). A slower decrease in the area of stands (Table 1), particularly in the eastern part of Latvia, might be partly related to lower connectivity between them (Liepiņš et al. 2016), which might have limited the spread of the disease. The official statistics (FI data) on the standing volume of ash, which showed a stable increase (Table 1), were obviously biased. The bias can be explained by the growth models employed in the calculation system to extrapolate stand growth between inventories, which were based solely on site type and age, yet did not account for increased mortality due to the disease. Nevertheless, the statistics concerning the area of the stands are credible, as they are based on verified observations (inventories). On the other hand, observation data from a limited number of PSPs might not be completely representative of diverse stands across the country.

The overall mortality of affected ash in Latvia (Table 1) was lower than in Lithuania and Germany (6.9 vs. 8.7 and 10.1% year^{-1}, respectively; cf. Lenz et al. 2016; Vasaitis and Enderle 2017), suggesting a higher resistance of the Latvian ash stands against the pathogens. Still, it was considerably higher than observed for "veteran" (i.e., long lived; ca. 1% year^{-1}) trees in Sweden (Bengtsson 2014), which, apparently, are the most resistant. The decrease in standing volume (r% = 5.3% year^{-1}; Table 1) was slower compared to stand density (number of ash), which might be explained by the presence of "veteran" trees in the studied population.

As hypothesised, the mortality of the affected ash, as indicated by changes in the number of trees, was higher at the beginning of dieback (Table 1), indicating that most of the population was highly sensitive to the pathogen (McKinney et al. 2014), and hence died promptly. The remaining trees, apparently, had higher resistance (Bengtsson 2014), explaining the decreased mortality (Table 1). The mortality rate calculated for the standing volume was higher in the second period of observation (Table 1), indicating that the larger, thus more vigorous, trees were able to survive longer (Skovsgaard et al. 2010; McKinney et al. 2011). Potentially, the larger trees were able to resist *H. fraxineus*, but the secondary agents (e.g., *Armillaria* spp.) likely caused their death (Bakys et al. 2011; Thomsen and Skovsgaard 2012).

Based on the decrease in stand area and the mortality rate of ash, it could be concluded that the prospects for the species in Latvia is clearly negative. Some regeneration of the affected stands was observed, as a few new trees were registered during the surveys, yet the rate of recruitment still appeared insufficient to counteract the dieback. Still, the decrease in mortality rate suggests that the most vigorous trees might survive, providing a gene source for a new generation of more resistant trees to form in the longer term (McKinney et al. 2014).

Acknowledgements

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Seasonal development of lesions caused by *Hymenoscyphus fraxineus* on young *Fraxinus excelsior* trees in Latvia

**Ilze Matisone,
Roberts Matisons,
Kristine Kenigsvalde,
Talis Gaitnieks,
Natalija Burneviča**

The spread of the ascomycete *Hymenoscyphus fraxineus*, causing dieback of common ash (*Fraxinus excelsior*) in Europe, is rapid and the damage is pronounced, as young ashes can perish over the course of only a few months following infection. The objective of this study was to investigate the rate and extent of lesion formation on young (5-8-year-old) ashes during a vegetation season in the hemiboreal zone in Latvia. Continuous surveys (with monthly intervals) of the health condition of 30 young ash and measurements of lesion area in three stands were performed during the vegetation season of 2015. From June to September of that year, the number of observed lesions gradually rose from 58 to 87. New lesions emerged on branches (55%, 0.5 per tree), top shoots (28%, 0.3 per tree), and stems (17%, 0.2 per tree), mostly appearing at the beginning of the observation period (45%, 52%, and 3% in June, July, and August, respectively). During the vegetation season, 20% of the existing and 28% of the newly-emerged lesions on branches, as well as 20% and 25% of top shoot lesions, respectively, reached the main stem. Some (< 20% of cases) transitions of lesions from the tops and branches to the stems were observed. The extension of lesions was significant until August, and ceased afterwards in a similar fashion in all stands. The mean extension of area significantly differed between the previously-existing and newly-emerged lesions. During the vegetation season, the new lesions expanded by 25.1 ± 4.8 cm^2, whereas the existing ones grew by only 7.3 ± 1.1 cm^2. The extension of the new lesions varied according to their location on a tree. The spread of emerging lesions on stems was considerably slower than on branches or top shoots (1.9 ± 0.7, 7.3 ± 1.5, and 14.5 ± 4.1 cm^2 per lesion per month, respectively). During the studied vegetation season (summer), the overall health score of trees decreased twice, yet the relationship between heath status and development of lesions lacked significance.

Keywords: Common Ash, Ash Dieback, Lesion Length, Sapling Wilting

Introduction

The ascomycete *Hymenoscyphus fraxineus* is a disease agent that has caused dieback of common ash (*Fraxinus excelsior* L.) in Europe in recent decades (Pautasso et al. 2013). Since the 1990s, ash dieback has been observed in Lithuania (Vasiliauskas et al. 2006) and Latvia (Laivinš et al. 2016), although in Latvia, it was only confirmed in 2007 (Kenigsvalde et al. 2010). The number of countries affected by ash dieback continues to increase and already exceeds 20 (Gross et al. 2014). This dieback affects stands of different ages and compositions (Pliura & Heuertz 2003, Schumacher et al. 2010), yet young stands are the most susceptible to infection, and hence are critical for development of the pathogen (Skovsgaard et al. 2010, Bengtsson et al. 2014).

The spread of *H. fraxineus* within its host is rapid, irrespective of tissue type, and proceeds in three dimensions (Schumacher et al. 2010). Some of the affected trees can be destroyed promptly, particularly as the fungus girdles the main stem, whereas others can have chronic symptoms (Thomsen & Skovsgaard 2012, Pautasso et al. 2013). Primary symptoms of the disease are macroscopic cankers on leaves and leafstalks (Skovsgaard et al. 2010), brown spots on buds (Bengtsson et al. 2014), and wilting of leaves and/or top shoots (Schumacher et al. 2010). These symptoms are followed by the formation of necrotic lesions spreading along rachises onto shoots, branches, and stems, resulting in dieback of the affected parts of a tree (Bakys et al. 2009, Skovsgaard et al. 2010, Bengtsson et al. 2014). Skovsgaard et al. (2010) noted, however, that cankers may also appear before wilting and dieback of shoots occurs. The appearance of lesions on undamaged stems suggests that the fungus could have entered through the lenticels (Husson et al. 2012). In addition, several strains of the fungus can attack a host simultaneously (Bengtsson et al. 2014). After dieback of the primary shoots, the affected trees can recover growth by formation of epicormic shoots, resulting in a bushy appearance of tree crowns (Gross et al. 2014). Nevertheless, for most ash trees, lifespan is considerably reduced (Skovsgaard et al. 2010).

Due to the threatened existence of ash

Latvian State Forest Research Institute Silava, Rigas str. 111, Salaspils, LV-2169 (Latvia)

@ Ilze Matisone (ilze.matisone@silava.lv)

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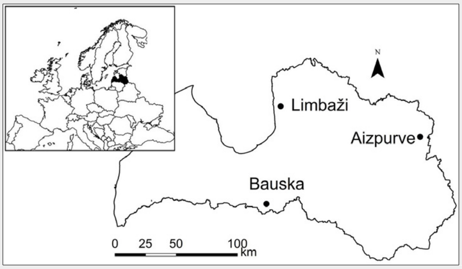

Fig. 1 - The location of the study stands in Latvia.

(Pautasso et al. 2013), natural resistance against the pathogen and methods for improving this resistance have been among the most commonly investigated issues regarding the dieback (Kjaer et al. 2012, McKinney et al. 2014). Seasonal dynamics of lesion development, specifically balance between emergence and entering the latent phase, has been shown to be a proxy of the dieback process (Bengtsson et al. 2014). Pliura et al. (2015) concluded that none of the tested provenances or progenies of ash had complete resistance to the infection or development of the disease, yet their susceptibility notably differed. In contrast, McKinney et al. (2014) were more sceptical, arguing that much more time is required for ash to form a resistance to *H. fraxineus* by means of natural selection. Alternatively, varying susceptibility to the dieback might be related to phenological differences in the seasonal cycles of trees and the fungus (McKinney et al. 2011, Bengtsson et al. 2014). Among abiotic factors, seasonal temperature has been shown to significantly affect the development of lesions (Bengtsson et al. 2014); however, McKinney et al. (2011) observed development and spread of the fungus during the dormant period, suggesting only a partial role of this factor. Hence, more comprehensive information concerning the factors affecting the formation of lesions is required.

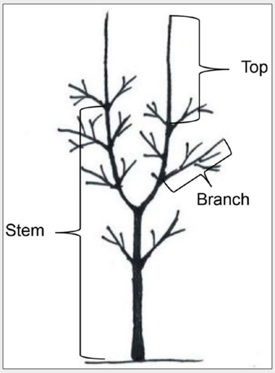

Fig. 2 - The categories used for distribution of lesions according to their location on trees.

The aim of this study was to assess the pattern of lesion development caused by *H. fraxineus* in young ash trees during a growing season. We hypothesised that the development of lesions varied during the vegetation season, and that it was more intense at the beginning of summer when the newly-formed tissues of ash had not yet matured. We also hypothesised that the development of emerging lesions occurred more quickly than that of those already existing.

Materials and methods

Studied sites

This study focused on 30 young ash trees (5-8 years of age, according to inventory data) growing in three naturally regenerated post-clear-cut stands in Latvia (Fig. 1) that were dominated by ash in the preceding rotation. These trees were monitored in 2015 from June to September, when the greatest fungal activity is expected (Timmermann et al. 2011, Bengtsson et al. 2014). The stands were fertile and corresponded to the *Aegopodiosa* site type, according to the national classification by Bušs (1976). Trees grew on flat terrain with a well-drained fertile loamy soil, although water excess occurred during the moist springs. The climate could be classified as moist continental, with the mean annual temperature (± standard error) during the most recent three decades ranging from 6.2 ± 1.6 to 7.1 ± 1.5 °C and the mean annual precipitation ranging from 665 ± 13.9 to 618 ± 12.4 mm in the eastern and central parts of Latvia, respectively. The highest monthly precipitation occurred in July and August. The mean temperature in June, July, and August 2015 was 14.9 ± 0.3, 16.9 ± 0.4, and 18.7 ± 0.6 °C in the central part, and 14.7 ± 0.3, 16.2 ± 0.4, and 17.6 ± 0.6 °C in the eastern part of Latvia, respectively, which were ca. 0.3 °C cooler than the 30-year mean values of the respective regions. The precipitation in these months was similar to the long-term mean (ca. 76.9 ± 6.5, 70.8 ± 6.2, and 62.6 ± 5.1 mm, respectively).

The densities of the Bauska and Aizpurve stands were the greatest (ca. 8000 and 3500 trees ha^{-1}, respectively), and their compositions were mixed. The Bauska stand was dominated by ash (ca. 5000 trees ha^{-1}), with an admixture of common aspen (*Populus tremula* L.) and goat willow (*Salix caprea* L.; ca. 1500 trees ha^{-1} each). In the Aizpurve stand, ash had a considerably lower density (ca. 1500 trees ha^{-1}), and was mixed with Norway maple (*Acer platanoides* L.) and common aspen (ca. 1500 and 500 trees ha^{-1}, respectively). In the Limbaži stand, ash was the dominant species (density ca. 1900 trees ha^{-1}), with a small admixture of silver birch (*Betula pendula* Roth; ca. 200 trees ha^{-1}). The dieback process was apparent in all studied stands, as indicated by ash saplings with obvious damage (lesions on top shoots, branches, and stems), confirming the presence of the pathogen. No obvious signs of other diseases or damages were observed.

Sampling and measurements

In each stand, 10 dominant unsheltered ash saplings with heights of 2.5-3.0 m were selected. At the beginning of the observation, all chosen saplings had one to three lesions that were necroses (discolorations) on stems (40%), tree tops (32%), or branches (28% – Fig. 2). Only trees with a small initial lesion area (the maximum area of the lesions on stems, branches, and tree tops were 37 cm^2, 5 cm^2, and 18 cm^2, respectively) were selected. At the beginning of the survey in June, height and diameter at breast height of the sample trees were measured with accuracies of 5 cm and 0.5 mm, respectively. For four months (from

Tab. 1 - Grades of ash sapling health condition. (AGB): aboveground biomass.

Grade	AGB Damage (%)	Damage visual characteristics
I	0 - 10	Tree looks healthy or slightly damaged individual leaves
II	11 - 25	Damaged several leaves, some necroses of the bark
III	26 - 60	Fully damaged/dead separate branches; damaged part of the foliage; necroses of the bark on large areas
IV	61 - 99	Completely broken up dead part of the crown; partially damaged the entire crown; live separate branches in secondary crown
V	100	Tree is completely dead

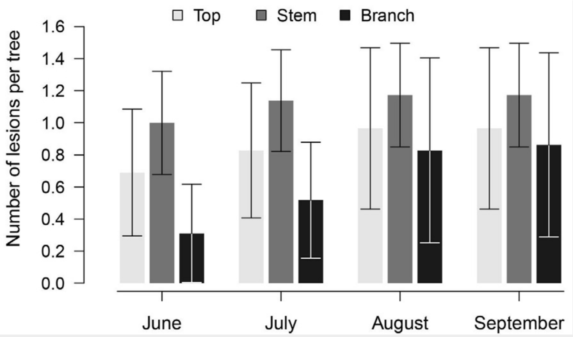

Fig. 3 - The number of the accounted lesions per tree during the observation period according to their location on trees. Whiskers indicate standard errors.

the 10th to the 15th of each month), the health condition of each sapling (quality of crown, and proportion of damaged leaves and shoots) was graded (Tab. 1) according to the methodology reported in Pušpure et al. (2015). In June, all visible lesions on branches and stems were marked on a transparent film. Development of lesions – the area of the extension since the last measurement, as well as the emergence of new lesions – was marked on the films at monthly intervals. All measurements were taken by the same person. The location of each lesion, such as necrosis on stems (elongated axis from the apical shoot to the root collar), or necrosis and wilting of top shoots (top shoot down to the uppermost axil) and branches (from stem to top of shoots), was recorded (Fig. 2). For bifurcated trees, all tree tops were considered individually (Fig. 2). After the final survey in September, the damaged parts of the saplings were sampled, bark was removed, and the area of the discoloured wood was marked on the film. To confirm the presence of *H. fraxineus* in the studied trees, samples from the symptomatic material, *i.e.*, from the inner bark or wood from the largest lesions, where the quantity of *H. fraxineus* mycelium presumably was the highest (Schumacher et al. 2010) were collected. In the Bauska, Limbaži, and Aizpurve stands, seven, six, and four samples were collected, respectively.

In a laboratory, the areas of the lesions from each tree and month were measured on the films with the accuracy of 0.01 cm^2 using a TAMAYA digital planimeter PLANIX 10S "Marble". To isolate *H. fraxineus*, samples of the infected material were surface sterilised by submersion in 35% hydrogen peroxide for 30 seconds and washed twice in distilled water for one minute. After draining, the samples were placed on a Petri dish containing 1% malt agar and incubated in darkness at 20°C for four weeks. As the study was focused on *H. fraxineus*, any other emerging fungi were mechanically removed from the samples every three days to facilitate development of the target species. The systematic affiliation of the isolate was confirmed microscopically according to Kowalski (2006).

Data analysis

Based on the rate of extension during the observation season, lesions were divided into three groups: active, inactive, and latent. A lesion was considered active if it had expanded since the last measurement, and considered latent if no expansion occurred during the entire observation period. Newly-emerged lesions (NL), which appeared during the observation period, exhibited patterns of development that differed from those of existing lesions (EL), and were therefore analysed separately. The differences in size and rate of extension of the lesions (per tree) according to their location on a tree (Fig. 2), their age (NL or EL), overall health condition of the sapling at the beginning of the observation period, and site were evaluated using analysis of variance (ANOVA). A paired-sample *t*-test was applied to assess differences in the extension of the lesions between the consecutive months, and a Bonferroni transformation was used to adjust the *p*-values of the differences. A generalised linear model (GLM) utilising the binomial distribution of residuals was applied to assess differences in the activity of the lesions (active or inactive, as well as latent or non-latent) according to their location, date of observation, health condition of the sapling, and site. Differences in the number of active/inactive/latent lesions per tree, as well as NL and EL, were assessed according to the same factors *via* GLM, using a Poisson distribution of the residuals. In all cases, Tukey's honest significant difference *post-hoc* test was applied to compare the levels of significant factors. Relationships between the lesion area above and below the bark at the end of the survey were quantified through bootstrapped (Johnson 2001) Pearson correlation analysis, whereas relationships between health condition class at the end of the study and sapling height and diameter were quantified via bootstrapped (Johnson 2001) Kendall correlation analysis. The sapling was considered as the statistical unit. All data analyses were conducted in R ver. 3.3.3 (R Core Team 2016) using the "multcomp" package (Hothorn et al. 2008), with a significance level of $\alpha = 0.05$.

Results

Activity of lesions

In total, 319 observations of 87 lesions were made. The observed lesions (Fig. 3) were necroses on stems (39.8%, the mean ± standard error number of lesions per tree was 1.2 ± 0.8), tree tops (31.8%, 0.9 ± 0.5 per tree), and branches (28.4%, 0.9 ± 0.3 per tree). Most of the lesions (67%) were EL, of which 26% were latent; NL comprised 33% of all observed. Among the EL, 50%, 34%, and 16% were located on stems, tree tops, and branches, respectively, whereas 17%, 28%, and 55% of the NL appeared on those same parts, respectively. The number of NL significantly (p-value < 0.001) differed among months and sites. Overall, NL appeared at the beginning of the observation period (45%, 52%, and 3% in June, July, and August, respectively). The number of NL was significantly (p-value < 0.001) lower in the Limbaži and Aizpurve stands (12% and 20% of the accounted lesions; 0.3 and 0.1 lesions per tree that emerged in June and July, respectively) than in the Bauska stand (62% of the accounted lesions), where 0.7, 1.3, and 0.1 lesions per tree emerged in June, July, and August, respectively. *H.*

Tab. 2 - The proportion of active (expanding since the last observation) lesions during the observation period according to their location on tree. (N_sept): number of accounted lesions in September.

Period	Existing lesions			Newly emerged lesions		
	Top	Stem	Branch	Top	Stem	Branch
June (%)	58	83	43	33	75	17
July (%)	67	83	86	100	100	92
August (%)	50	39	29	67	50	42
Total (% mean)	58	68	52	67	75	50
N_sept	12	23	7	6	4	12

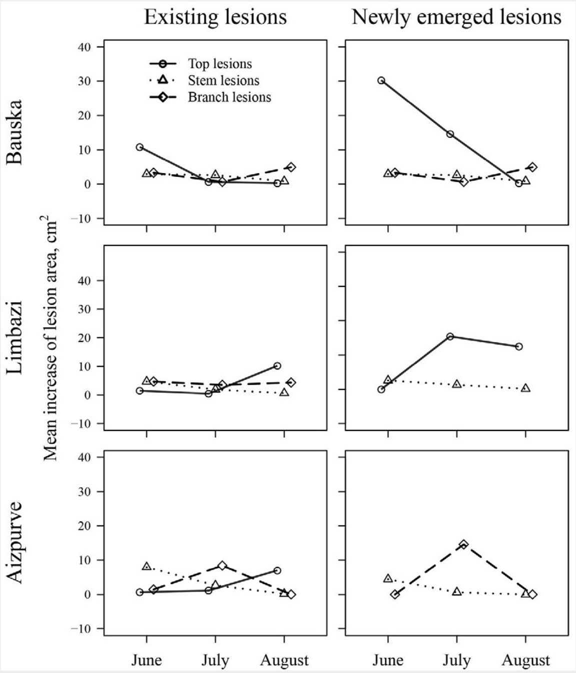

Fig. 4 - The mean monthly increase in area of the lesions existing before the observation period and newly emerging lesions in the studied stands according to the location on trees. The symbols are shifted for clarity.

fraxineus was isolated in six of the 17 samples collected (35% – four in Bauska and two in Aizpurve).

The mean proportion of latent lesions was similar (p-value = 0.23) among the locations on trees (25 ± 10 %), yet it was significantly (p-value = 0.007) higher in Limbaži than in Bauska (39 ± 9% and 12 ± 6%, respectively); in the Aizpurve stand, it was intermediate (30 ± 11%). The proportion of active lesions (Tab. 2) differed between EL and NL (p-value < 0.01), and among locations on trees (p-value = 0.02), and varied during the season, with significant differences observed among months (p-value < 0.001). Nonetheless, the activity of lesions was similar among the stands (p-value = 0.13). During the entire observation period, the highest activity was observed for lesions on stems (68 and 75% for EL and NL were active, respectively) and tree tops (58 and 67%, respectively). The activity of lesions on branches was the lowest, as 52% of EL and 50% of NL expanded during the observation period. For both EL and NL, activity was the highest in July (83% and 100%, respectively), and then sharply decreased in August (39% and 50%, respectively).

Extension of lesions

During the season, the area of lesions ± standard error increased from 52.5 ± 11.3 cm² per tree in June to 92.1 ± 14.7 cm² per tree (91.9 ± 60.1, 42.3 ± 11.5, and 26.6 ± 15.9 cm² per tree for top, branch, and stem lesions, respectively) at the end of the observation period (Fig. 4). The extension of the lesions was highly significant (p-value < 0.001) until August, but ceased thereafter, similarly across all stands. The mean extension of lesion area significantly differed between EL and NL (p-value < 0.01). During the season, NL extended by 25.1 ± 4.8 cm², whereas EL only expanded by 7.3 ± 1.1 cm². The rate of extension of EL was similar among stands (p-value = 0.84) and locations on trees (p-value = 0.47; 2.48 ± 0.70 cm² per lesion per month). The extension of NL was also similar among the stands (p-value = 0.50); however, it differed among locations on trees (p-value = 0.02). The extension of lesions on the stems was considerably slower than on the branches and tree tops (1.9 ± 0.7, 7.3 ± 1.5, and 14.5 ± 4.1 cm² per lesion per month, respectively). The development of lesions on the stems and branches culminated in June and July, respectively, similarly for both EL and NL. The patterns of development of EL and NL on tree tops differed; NL had the fastest enlargement after appearance (mostly in June), whereas EL exhibited a maximum extension later in the season (in August; Fig. 4). A strong correlation (r = 0.99) between the area of the lesions above and below the bark in late September suggested a clear linear dependence of these variables (Fig. 5). Nevertheless, the difference between the lesion areas below and above the bark was significantly (p-value < 0.001, by ca. 6%) higher for EL than it was for NL (ca. 2%), yet it was similar among stands and locations on trees.

As the season advanced, 28% of EL and 20% of NL girdled branches, causing their complete dieback. Some transitions of lesions from tree tops and branches to stems

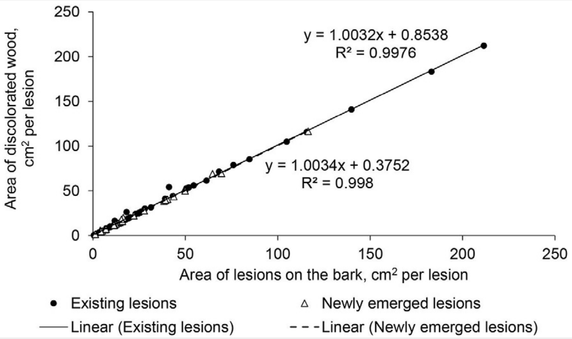

Fig. 5 - The relationships between the visible area and area below the bark of the lesions existing before the observation period and newly emerging lesions.

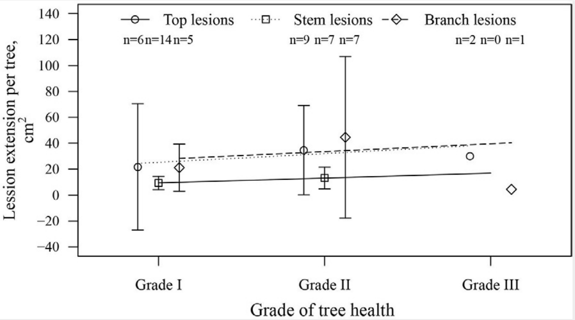

Fig. 6 - The mean increase in the lesion area (extension) per tree during the observation period according to the health grade of saplings in June 2015 (beginning of the observation period). Whiskers indicate standard error (where applicable). (n): number of trees.

were also observed. At the first observation, five necroses on stems coincided with dead branches, whereas in the following months, three lesions from girdled branches expanded onto the stems. The dieback of the infected tops was slightly weaker, as 20% (5% in June, 15% in July) and 25% (in August) of the EL and NL, respectively, girdled tree tops, causing their dieback. Five of the observed 28 tree top lesions expanded onto the stem.

Health condition of trees

The growing number of lesions and the area they covered decreased the overall health condition of saplings; their health grade increased from 1.6, 1.6, and 1.7 in June to 2.7, 3.2, and 2.9 at the end of the observation period (September) for the Limbaži, Bauska, and Aizpurve stands, respectively. Nevertheless, mortality of the studied saplings was low, as only one sapling in the Aizpurve stand died in August. That sapling was strongly mechanically damaged in July and had an extensive lesion area (215 cm^2).

There were no significant relationships between the health grade at the beginning of the observation period and the expansion rate of EL or NL, nor between health grade and the number of lesions, although slight positive tendencies were visible (Fig. 6). There was no significant relationship between the number of EL and NL, suggesting a similar probability of a lesion emerging, irrespective of preceding infection. The morphometric parameters showed no effects on the development of lesions, as the correlations between the total lesion area per sapling in September and height, as well as between total lesion area and diameter of the saplings, were not significant (r = -0.09 and 0.11, p-value = 0.55 and 0.47, respectively). Health condition class in June was negatively affected by tree diameter (τ = -0.21, p-value = 0.01), but not by tree height (τ = -0.02, p-value = 0.79).

Discussion

Agent and occurrence of lesions

The isolation of *H. fraxineus* confirmed its involvement in the formation of lesions on ash saplings. The proportion of positive samples was intermediate (35%) in comparison to that found in Sweden (Bengtsson et al. 2014) and lower than that in nurseries in Germany (Schumacher et al. 2010), suggesting the involvement of other agents (Husson et al. 2012). As this study focused on *H. fraxineus*, other agents were not quantified. Alternatively, the low occurrence of the pathogen in samples might be explained by seasonal (McKinney et al. 2011, Bengtsson et al. 2014) and tree-vice (Schumacher et al. 2010) variation in the number of propagules, or by intense surface sterilisation of samples prior to incubation.

Most of the observed lesions were located on stems (Fig. 3), which were the largest parts of the trees, and hence featured most EL, including those previously occurring on tops. Considering that propagules of *H. fraxineus* (Schumacher et al. 2010) infect their host through leaves and shoots (Cleary et al. 2013), most NL were found on branches (twigs) and tops (Fig. 3). The occurrence of NL on the lower parts of stems suggests that infection may have occurred through an alternative pathway (*e.g.*, *via* lenticels or fine roots – Husson et al. 2012, Fones et al. 2016), but genotyping would be necessary to validate this hypothesis. Nonetheless, there was no visible discoloration of wood below EL on stems leading towards shoots, as observed by Bengtsson et al. (2014), although young hyphae may not be visible (Schumacher et al. 2010).

Lesion activity

Although sporulation of many pathogenic fungi is synchronised with the biological cycles of their host, *H. fraxineus* has an extended period of spore release (Kirisits & Cech 2009), indicating a high possibility of infection throughout the season. Most NL emerged in June and July (Fig. 3), however, implying a seasonal pattern, which might be explained by the maturation of leaves and shoots (Schumacher et al. 2010, Timmermann et al. 2011). The majority of NL appeared on branches (Tab. 2), where most leaves, which are the primary infected organs (Kirisits & Cech 2009, Kirisits et al. 2009), occurred.

The development of lesions explicitly differed among the stands, as indicated by the number and activity of lesions (Fig. 4). These differences might be related to the structure of the stands, as well as to the connectivity between them (Liepinš et al. 2016), and hence also to the abundance of propagules. The moister microclimate of a denser stand is considered to facilitate development of the pathogen (Timmermann et al. 2011), hence the occurrence of lesions. Although Bengtsson et al. (2014) and McKinney et al. (2011) suggested a weak effect of temperature on the development of lesions, differences among the stands (Fig. 4) still might be related to local climate. The highest and lowest numbers of active lesions was observed in the Bauska and Limbaži stands, where summers were the warmest and coolest, respectively. The effect of temperature was also supported by the seasonal pattern of lesion development, as the highest activity, particularly for NL, occurred in July (Tab. 2), when the temperature was the warmest (ca. 15-18 °C). Previously, the highest activity of *H. fraxineus* has been observed when the mean temperature is close to 20 °C (Kowalski & Bartnik 2010, Timmermann et al. 2011). The sharp decrease in lesion development after August (Fig. 3, Tab. 2) might be linked to shortening of the photoperiod and initiation of cold hardening, which ceases the development of lesions (McKinney et al. 2011). It remains difficult, however, to overtly distinguish the influence of temperature on fungal and tree life-cycles (Bengtsson et al. 2014).

Lesion extension

The extension of lesions, which could be considered the main process affecting the overall condition of saplings (McKinney et al. 2011), differed between EL and NL, as well as amongst the affected parts of trees, and were modulated by local factors (Fig. 4). The extension of lesions mostly decreased as the season advanced (Fig. 4), similarly to the emergence of NL. Nonetheless, higher rates of extension of NL indicated that after emergence, lesions rapidly grew to a certain minimum size within the first month. Apparently, a month was necessary for ash to partially compartmentalise the development of the pathogen

(Pallardy 2008), slowing extension of the lesion. Alternatively, this might be connected to the higher activity of younger strains of fungus (Lygis et al. 2016). The opposite was observed for EL on tree tops in two of the three stands, which exhibited maximum extension in August (Fig. 4). This could likely be related to slower compartmentalisation of the pathogen at the cessation of the growing period, when formation of callus is slower (Pallardy 2008). In the Bauska stand, however, EL on tree tops were the most active in June, presumably due to the moister microclimate of the denser stand.

At the end of the observation period, the largest lesions were observed on tree tops, which were the largest shoots. The fastest spread of the pathogen within its host occurs in the stem pith, which is closest to the surface of shoots (Schumacher et al. 2010). In addition, the bark on shoots is thinner, facilitating the extension of lesions (Husson et al. 2012). Larger lesions on top shoots might also be related to microclimatic conditions, as apical shoots receive more atmospheric moisture during the night (dew), particularly in dense stands, as suggested by NL in the Bauska stand (Fig. 4). In the older parts of a tree, hyphae must apparently penetrate wood ray parenchyma (Schumacher et al. 2010), which limits their development, explaining the slower extension of lesions on stems (Fig. 4). The opposite was observed by Bengtsson et al. (2014) in Sweden, likely due to differences in climate and/or tree genetics (Pliura et al. 2011, 2015). Nevertheless, the actual size of lesions (under bark) was larger for EL (Fig. 5), implying some latent extension, as observed by Schumacher et al. (2010) and McKinney et al. (2011). The actual and visible sizes of NL were more similar (Fig. 5), likely because of the rapid extension (Fig. 4).

Health condition of trees

The intermediate proportion of NL (33% of the accounted) and low mortality of the affected parts of trees (ca. 22%) indicated chronic formation of lesions, suggesting a certain resistance to the dieback (Pliura et al. 2015). The presence of latent EL suggested that trees were able to sufficiently compartmentalise the pathogen (Pallardy 2008). In addition, biological limitations (*e.g.*, inactive physical defence) of development (Bengtsson et al. 2014) were observed, as most of the lesions stopped expansion when reaching the shoot base or the main stem. This might be related to the anatomical properties of wood, such as differences in vessel size and lateral connectivity, or pith diameter between the transitions of height increments of consecutive years (Schweingruber 2007). Regarding shoots, this might also be linked to differences in the thickness of primary and secondary bark compared to stem diameter (Husson et al. 2012). Nevertheless, previous studies have shown that the mortality of affected trees increases drastically in subsequent years (Kirisits & Freinschlag 2011, Pliura et al. 2011, 2015, Bengtsson et al. 2014).

Although the overall health condition of the saplings decreased differently during the observation period, the non-significant relationships between initial health condition and the number and extension of lesions (Fig. 6) suggested comparable infection pressure for all saplings, regardless of previous infection. This was supported by the non-significant relationship between the number of EL and NL in September, indicating the influence of stochastic processes or microclimate. The opposite was observed by McKinney et al. (2011) in Denmark, where susceptibility to the disease appeared to be prevailingly controlled by deterministic factors, such as genetics. Such differences could be related to the milder and more humid climate in Denmark that facilitates development of *H. fraxineus* and extension of lesions (Kowalski & Bartnik 2010, Timmermann et al. 2011), enhancing the tree-vice differences in susceptibility (Schumacher et al. 2010). Nevertheless, relationships between morphometrics (diameter) and health grade were observed, suggesting that the largest trees were also the healthiest.

Conclusion

As hypothesised, the development of lesions followed a seasonal pattern that could be linked to meteorological and phenological conditions. The emergence, activity, and extension of lesions was greatest during June and July, when the plant tissue had not yet matured and ambient temperature was close to the optimum for *H. fraxineus* development. Accordingly, the most rapid extension of the lesions was observed on top shoots, the youngest parts of trees, suggesting that height growth would be notably affected. Differences in the development of lesions amongst the stands indicated effects of climate, as well as local factors (*e.g.*, microclimate); hence, alterations in lesion development might be expected with the changing climate. The extension of newly-emerging lesions was faster than that of existing ones, particularly during the first month of observation, suggesting that approximately one month was necessary for trees to compartmentalise the infection. The development of lesions was not related to the health condition of trees, and a relationship between the number of existing and emerging lesions did not exist, indicating that infection was stochastic. Biological limitations of lesion development were observed, as most lesions remained in the initially-infected parts of trees. The proportion of emerging lesions exceeded the proportion of latent lesions, indicating a progressive decrease in the overall health condition of trees; however, both this proportion and tree mortality appeared to be lower than in other studies. Nonetheless, a longer observation season involving a wider spectrum of sites is required to evaluate the annual cycles in development of the infection, as well as to allow the assessment of secondary infections.

Acknowledgements

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Tree-ring Width of European Ash Differing by Crown Condition and its Relationship with Climatic Factors in Latvia

ILZE PUŠPURE, LINDA GERRA-INOHOSA, ROBERTS MATISONS* AND MĀRIS LAIVIŅŠ

LSFRI "Silava", Rigas str. 111, Salaspils, Latvia, LV2169

**Corresponding author: robism@inbox.lv, tel. +371 29789581*

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Abstract

The spreading dieback of European ash (*Fraxinus excelsior* L.) that is a serious threat to the existence of the species in Europe, has been related to climatic changes. Still, not all trees in stands are damaged equally, suggesting that sensitivity to weather conditions might have affected the susceptibility to the disease. Climate-growth sensitivity of ash with visually healthy and damaged crowns growing in four stands in the central and eastern part of Latvia was assessed by dendrochronological techniques. The patterns of tree-ring width variation showed high diversity amongst trees, stands and regions; differences were observed between the damaged and healthy trees. Tree-ring patterns showed higher diversity amongst the healthy trees in the central part of Latvia, but, in the eastern part of Latvia, amongst the damaged ones. Mainly, the damaged trees were ca. 10–15 years older than the healthy ones suggesting age related differences in susceptibility, which might be related to vigour. The damaged and healthy trees differed also by growth trends, suggesting affiliation to different crown class, particularly at younger age. The sets of the significant climatic factors differed between the central and eastern part of Latvia. In the central part of Latvia, ash was mainly affected by the precipitation and daily temperature difference in the summer preceding formation of the tree-ring. Although the damaged trees were more sensitive to daily temperature difference and precipitation in the preceding August, the healthy trees were also additionally affected by maximum temperature in the preceding August. In the eastern part of Latvia, the sets of the significant factors were site specific, however, trees were mainly affected by temperature in the preceding autumn and current spring. In one site, the damaged ashes were more sensitive to temperature in July and September, while in other site the damaged trees were more affected by precipitation in July; the healthy trees were additionally affected by precipitation in September and temperature in April. Hence, the susceptibility to the disease appears partially related to the climatic sensitivity of trees.

Keywords: *Fraxinus excelsior*; ash dieback; dendroclimatology; climate-growth relationships; tree-ring width; radial growth patterns.

Introduction

The dieback of ash, which has been spreading across the Europe (Kowalski et al. 2010, Timmermann et al. 2011), is considered as a serious threat to the existence of the species (Kowalski 2006, Bakys et al. 2009a). The dieback is a rapid process, as the infected tree might die within a few years after the first symptoms, such as reduction of crown, appear or, in some cases, symptoms might not be even visible (Bakys et al. 2009b, Timmermann et al. 2011, Enderle et al. 2013). Although the mechanisms of ash dieback are not completely understood, it is considered to be caused by a complex of factors including climate and pathogens (Pautasso et al. 2010, Skovsgaard et al. 2010). The fungus *Hymenoscyphus fraxineus*, has been considered as a initiator of the dieback process (Kowalski 2006), which promotes further infestation and damage by the secondary agents (Kowalski et al. 2010, Skovsgaard et al. 2010, Bakys et al. 2011). The fungus, which attacks root system of ash (Bakys et al. 2008) affecting physical stability (susceptibility to uprooting) and water relations (increasing risk of water deficit) (Tulik et al. 2010), is considered to infest stressed trees, e.g. by unfavourable weather conditions (Thomsen and Skovsgaard 2006, Pautasso et al. 2010). Still, not all trees within a stand are damaged equally (Kirisits and Freinschlag 2011, Pliūra et al. 2011), suggesting different resistance to the disease (McKinney et al. 2011, Stener 2012). Hence, the sensitivity to climate might be one of the factors affecting the susceptibility of ash to the dieback. Similar has been observed for the declining pedunculate oak (*Quercus robur*) in Southern Finland, which showed different climate-growth sensitivity also before the decline (Helama et al. 2009).

Climate is one of the main factors affecting vigour and growth of trees, which are archived in the variation of wood increment (Fritts 2001). Hence, detailed information about the sensitivity of tree growth to climatic factors can be obtained from a retrospective analysis of the variation of tree-ring width (TRW) (Speer 2010). As tree growth has an explicit biological i.e., age trends (Fritts 2001), the effect of climatic factors is commonly assessed from the high-frequency variation of TRW (Cook et al. 1992). Considering that a tree-ring forms during a certain period of the vegetation period, combined effects of several factors might be recorded in TRW (Cook 1992, Schweingruber 1996).

The aim of this study was to assess the variation of TRW of ash with different crown condition and its relationship to climatic factors at the inter-annual scale. We hypothesized that the damaged trees had different growth patterns and were more sensitive to climatic factors than the healthy ones, and that the sets of the significant factors differed.

Material and methods

Studied sites, sampling and measurements

Four mature stands dominated or co-dominated by ash with different crown condition located in the central and eastern part of Latvia near Ukri (UKR), Rundāle (RND), Gulbene (GBN) and Barkava (BAR) (Figure 1) were studied. Sites in these regions were selected, as differences in growth have been observed for other species (Matisons et al. 2012, Baumanis et al. 2001). All of the stands were situated on a flat terrain in a normal moisture conditions on loamy soil. According to the national classification by Bušs (1976), site type in all stands was *Aegopodiosa*. The elevation of stands was ca. 35 and 110 m above the mean sea level in sites in the central (UKR and RND) and eastern (GNB and BAR) part of Latvia, respectively. The maximum age of ash in the BAR, GBN, RND and UKR sites, as determined from the obtained wood samples, was ca. 70, 190, 110 and 100 years, respectively. Advanced regeneration occurred in all stands.

The climate in the studied sites is determined by the dominant western winds, which bring cool and moist air masses from the Baltic Sea and the Atlantic. The weather conditions are harsher in the eastern part of Latvia. The mean annual temperature is ca. +6.4 and +5.5 °C; the mean monthly temperature ranges from -4.3 to +17.5 °C and from -6.2 to +17.4 °C in January and July in the central and eastern part of Latvia, respectively. The vegetation period, when the mean diurnal temperature is above +5°C, extends from mid-April to mid-October; it is usually 10–15 days longer in the central part of Latvia. The mean annual precipitation is about 610 mm in all sites. The highest monthly precipitation sums occur in the summer months, usually resulting in a positive water balance (Klavins and Rodinov 2010). Climatic changes are reflected as an increase of temperature in the autumn-spring period, which is extending the vegetation period (Lizuma et al. 2007). In the same time, summer precipitation regime is becoming more variable (Avotniece et al. 2010).

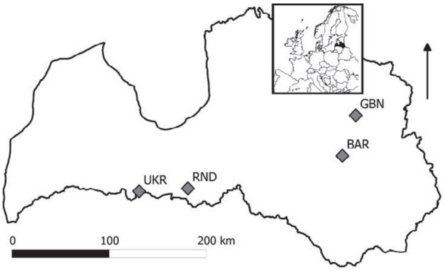

Figure 1. Location of studied sites near Barkava (BAR), Gulbene (GBN), Rundāle (RND) and Ukri (UKR)

In each stand, 10 dominant ashes with visually healthy crowns (crown reduction, i.e. dry branches, ≤ 15%) and 10 ashes with damaged crowns (crown reduction 30–60%) were selected. From each tree, two increment cores from the opposite sides of stem were collected with a Pressler increment corer at ca. 1.4 m height, avoiding reaction wood if trees were tilt. The sampling was done at the end of May 2015, when leaves had fully flushed. In the laboratory, increment cores were air dried and mounted on fixation planks for grinding. Sandpaper of four roughness grits (120, 140, 320 and 400 grains per inch) was applied, using hand sanding machine. The polished surface of samples was rubber with chalk to increase the contrast between early and latewood and to aid the recognition of tree-rings. The TRW was measured by a Lintab 5 measurement system (RinnTECH, Heidelberg, Germany) with the precision of 0.01 mm.

Data analysis

All of the measured chronologically ordered series of TRW were crossdated (i.e. their dating and synchronicity compared against each other) and their quality was checked by graphical inspection and statistically, by the program COFECHA (Grissino-Mayer 2001). Series showing low agreement with the rest of the dataset (r < 0.35) were omitted from further analysis. The TRW series of the healthy trees were used as a reference for crossdating of the damaged trees. The crossdated series were then averaged for trees and their quality was verified. For description of the datasets, expressed population signal, signal to noise ratio (Wigley et al. 1984), Gleichläufigkeit, interseries correlation (based on detrended series) and the first order autocorrelation coefficients were calculated.

For assessment of high-frequency variation of TRW, residual chronologies based on the crossdated datasets were produced by the program ARSTAN (Cook and Holmes 1986) for each site and group of trees (damaged and healthy). Double detrending, firstly by a negative exponential curve and secondly by the cubic spline with rigidity of 64 years and 50% frequency cut-off level, was applied. The relationships between climatic factors and high-frequency variation of TRW was assessed by a bootstrapped Pearson correlation analysis (Johnson 2001) conducted for the common period from 1934 (1948 for the healthy trees in the RND site) to 2010. The significance of correlations was determined at $\alpha = 0.05$, performing 10000 iterations. The climatic factors showing significant correlations with TRW were tested for collinearity. The tested climatic factors were the minimum, maximum and mean temperature, potential evapotranspiration (PET), precipitation sums and mean daily temperature difference for months. The climatic window from January in the year preceding formation of tree-ring to September in the year of tree-ring formation (21 months) was used. Climatic data were obtained from the high-resolution gridded datasets provided by the Climatic Research Unit of UEA for the closest to the sites grid entries (Harris et al. 2014). The statistical analysis were conducted in the program R (R Core Team 2014) using the library “dplR” (Bunn 2008).

Results

After the crossdating and quality checking, from 75 to 100% of the series per group/site were maintained for further analysis. The crossdated datasets covered the periods beginning from 1824 to 1948 in GBN and RND sites, respectively (Table 1). Generally, TRW series showed better agreement in the central part of Latvia, as shown by higher values of interseries correlation and expressed population signal. The agreement of TRW of the healthy trees was better in sites in the eastern part of Latvia, where values of interseries correlation (0.35 vs. 0.15), Gleichläufigkeit (0.62 vs. 0.58) and expressed population signal (0.75 vs. 0.55) were higher. The opposite was observed in sites in the central part of Latvia, where the environmental signal was stronger, as shown by a higher signal to noise ratio (4.8 and 2.3, respectively). The agreement of TRW was considerably weaker, hence the noise was considerably stronger for the damaged trees in the BAR site, compared to the rest of the datasets. The value of expressed population signal exceeded 0.85 only for the damaged trees in the UKR site. Nevertheless, common tendencies, such as the decreased TRW in 1940, 1963, 1984, 1990 and 2006 were observed in all chronologies (Figure 2).

Table 1. Statistics of the crossdated datasets of tree-ring width (TRW) of ask with the damaged and healthy crowns in sites near Barkava, Gulbene, Rundāle and Ukri. A – stand age, D– mean diameter of trees, S – mean sensitivity, N – number of crossdated trees, IC – mean interseries correlation, AC – autocorrelation, GLK – Gleichläufigkeit, EPS – expressed population signal (Wigley et al. 1984), SNR – signal to noise ratio

	N	Period	A, years	D, cm	Min. TRW, mm	Max. TRW, mm	Mean TRW, mm	St. dev. TRW, mm	S	IC	AC	GLK	EPS	SNR
Barkava (BAR)														
Healthy	8	1933–2014	91	32.9	0.68	5.69	2.53	0.90	0.20	0.39	0.70	0.63	0.79	3.67
Damaged	10	1928–2014	91	28.9	0.10	10.00	1.99	0.99	0.22	0.09	0.80	0.58	0.42	0.71
Gulbene (GBN)														
Healthy	10	1824–2014	195	48.6	0.17	5.39	1.48	0.61	0.18	0.31	0.77	0.60	0.72	2.53
Damaged	10	1831–2014	195	43.9	0.12	4.77	1.38	0.56	0.19	0.23	0.79	0.58	0.69	2.20
Rundāle (RND)*														
Healthy	9	1948–2014	68	27.2	0.46	6.24	2.05	0.81	0.21	0.36	0.73	0.60	0.82	4.49
Damaged	10	1916–2014	112	34.2	0.16	6.35	1.79	0.88	0.19	0.36	0.83	0.60	0.83	4.90
Ukri (UKR)														
Healthy	7	1933–2014	106	33.2	0.53	5.28	1.99	0.99	0.19	0.40	0.86	0.60	0.81	4.32
Damaged	8	1925–2014	106	29.5	0.20	5.80	1.65	0.89	0.17	0.44	0.87	0.64	0.86	5.73

* – uneven aged stand.

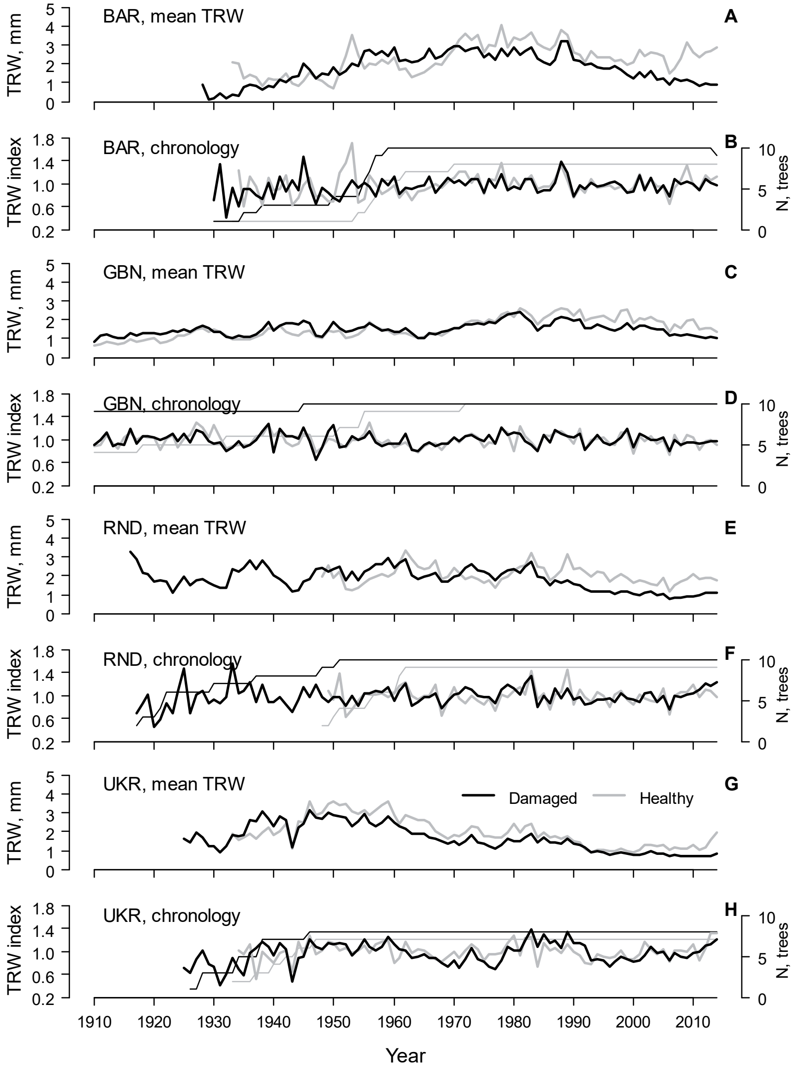

Figure 2. Mean series (A, C, E, G), residual chronologies (thick lines) and sample depth (thin line) of tree-ring width (TRW) (B, D, F, H) of ash with damaged (black lines) and healthy (grey lines) crowns for sites near Barkava (BAR), Gulbene (GBN), Rundāle (RND) and Ukri (UKR), respectively. Curves are based on crossdated datasets. For GBN site, only data for period from 1910 to 2014 are shown

In most of the cases, the healthy trees were younger, had higher mean TRW and contained less autocorrelation (0.77 vs. 0.82) than the damaged ones (Table 1). The damaged trees showed growth suppression during a few recent decades, although in some sites, they have been growing faster than the healthy trees at younger age (Figure 2).

The indices of chronologies generally ranged from ca. 0.60 to ca. 1.40, but the range tended to become narrower during a few recent decades (Figure 2). The agreement among the chronologies was weak, as the mean values of correlation calculated between them was 0.26, although Gleichläufigkeit was 0.63. The correlation between chronologies of the healthy and damaged trees ranged from 0.36 to 0.70 in the BAR and GBN sites, respectively. Nevertheless, the agreement between the chronologies of trees with different crown condition increased with age, particularly during the recent decades, as shown by the mean values of correlation coefficients of 0.50 and 0.65 at the beginning and at the end of the 20th century, respectively (not shown).

From the tested 132 climatic factors, 18 significantly correlation with TRW of ash (Figure 3). The number of significant correlations was higher in sites in the central than in the eastern part of Latvia (23 and 13, respectively). The values of correlation coefficients were generally low and did not exceed 0.32, except for precipitation in March (*r* = 0.48). The climate-growth relationships were quite individual, particularly in the eastern part of Latvia, as the sets of the significant correlations differed amongst the sites. In the central part of Latvia, TRW was affected by climatic factors (precipitation and daily temperature difference) related to the previous vegetation season (May–August), as well as some correlations with precipitation in February and March of the current year were observed. In the eastern part of Latvia, TRW of ash appeared mainly sensitive to weather conditions (maximum and mean monthly temperature) in the previous autumn (September and October) and in the current vegetation season (April–September). Generally, temperature and its mean daily difference had a negative effect on TRW, as shown by the prevailing negative correlations, while precipitation had a positive effect.

Only a few pronounced and systematic differences in the sets of the significant climatic factors were observed between the healthy and damaged trees (Figure 3). The damaged trees in the central part of Latvia displayed stronger correlation to the mean daily temperature difference and precipitation in August of the preceding year. The healthy trees showed additional sensitivity to the maximum temperature in previous August. In the RND site, TRW of the healthy trees showed the strongest of the observed correlations with precipitation in March. In the UKR site, the healthy trees showed stronger correlation with PET in May of the preceding year. In the eastern part of Latvia in the BAR site, the damaged trees were more affected by temperature in July and September of the current year, while the healthy trees were additionally affected by temperature in September and October of the preceding year. In the GNB site, the healthy trees were affected by precipitation in previous September and temperature in April of the current year, but they were less sensitive to precipitation in previous July.

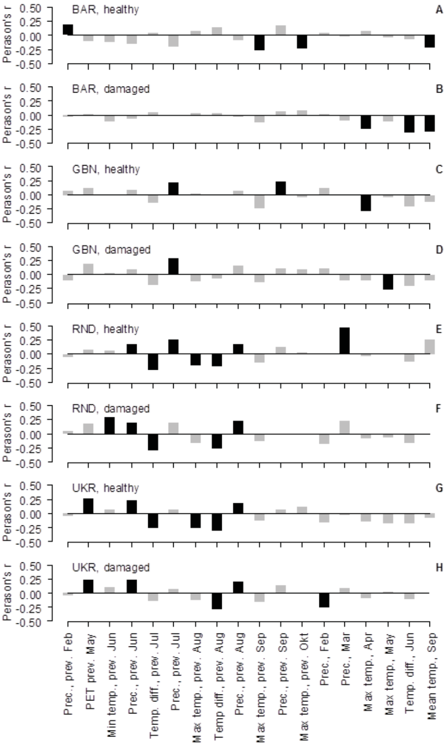

Figure 3. Bootstrapped Pearson's correlation coefficients calculated between climatic factors and residual chronologies of tree-ring width of ash with healthy (A, C, E, G) and damaged crowns (B, D, F, H) for sites near Barkava (BAR), Gulbene (GBN), Rundāle (RND) and Ukri (UKR), respectively. The period from 1934 (1948 for healthy trees in RND site) to 2010 was analysed. The significant correlations (at α = 0.05) are shown in black. Only the significant factors are plotted. PET – potential evapotranspiration

Discussion and conclusions

Most of the TRW series were sufficiently crossdated, as they had common signature years (narrow tree-rings in certain years). Yet the individuality of growth was rather pronounced (Table 1), as the values of expressed population signal were mainly below 0.85 (Wigley et al. 1984). Such individual growth patterns apparently caused the noise in the datasets, hence the values of signal to noise ratio were below 5.8 (Table 1). Pronounced site-specifics in the growth patterns were also evidenced by weak correlation amongst the established chronologies (Figure 2), despite the fact that, in Latvia, ash occurs near its northern distribution limit (EUFORGEN 2009), where distinct effect of a common climatic factor(s) is expected (Fritts 2001). The observed regional differences in agreement of TRW series (Table 1) matched with the current knowledge on the diversity of tree growth patterns between the central (western) and eastern part of Latvia (Baumanis et al. 2001, Matisons et al. 2012). Nevertheless, weaker agreement of the TRW series was observed in the eastern part of Latvia that might be explained by stressed growth under harsher climate, when the effect of competition diversifies growth patterns (Speer 2010). In contrary, individuality of growth has been observed also for trees growing in optimum conditions, where a strict limiting factor is lacking (Fritts 2001). In the central part of Latvia, where climate is milder, ash, apparently, was less suppressed, hence the common signatures in TRW were clearer (Table 1). This is supported by the differences in agreement between the healthy and damaged trees. In the central part of Latvia, trees, which had stronger crown damage, showed better agreement of TRW likely due to higher sensitivity to environmental variability, suggesting the role of climatic factors in the dieback process (Thomsen and Skovsgaard 2006, La Porta et al. 2008). In the eastern part, crown damage, apparently, additionally stressed the trees, diversifying their growth patterns (Speer 2010).

The susceptibility of ash to fungal infection (Enderle et al. 2013) appeared to be age-related, as the healthy trees were mainly younger than the damaged ones (Table 1). With age, maintenance costs increases (Ryan 1990) and less resources might be allocated for production of defence substances (Pallardy 2008). This is supported by higher autocorrelation in TRW of the damaged trees (Table 1), suggesting stronger dependence on nutrient reserves (Fritts 2001). Nevertheless, the differences in growth rates between the tree groups (Figure 2) suggested that the susceptibility to disease might be also related to the social status (i.e., crown classes) of trees (Martin-Bento et al. 2008). The damaged trees, apparently, had a higher social status at the young age, as shown by faster growth (wider tree-rings) during a few earliest decades of their life. In contrast, trees, which grew slower, were less susceptible to crown damage at the older age. In all sites, the damaged trees showed reduction of TRW for ca. three recent decades compared to the healthy ones (Figure 2) suggesting that previously suppressed trees have been infected (Timmermann et al. 2011). Similarly, stronger symptoms of decline has been observed for the suppressed oaks (Helama et al. 2009). Simultaneous reduction of TRW in all sites during the recent decades (Figure 2) might be explained by the effect of weather conditions, such as the extreme drop of temperature in the winter of 1978/1979, striking the insufficient hardened trees and causing shifts in their growth trends (Matisons et al. 2013). The reduction of TRW decreased its annual variation particularly for the damaged trees (Figure 2) due to the suppression of growth (Speer 2010), but the patterns became more similar for both tree groups (Figure 2) suggesting clearer effect of common limiting factor(s).

High-frequency variation of TRW was significantly affected by the tested climatic factors (Figure 3). Still, the rather low values of correlation coefficients might be explained by the individuality of TRW variation within a site due to stresses, when the common response is reduced (Speer 2010). Similarly, the diversity of the significant factors amongst the sites and tree groups, particularly in the eastern part of Latvia (Figure 3), might be explained by the individuality of growth rhythms (Table 1). This apparently explains the number of observed significant correlations. In the sites in the central part of Latvia, the sets of significant climatic factors were similar (Figure 3), suggesting that trees have been able to show clearer reaction to weather conditions. The TRW mainly correlated with climatic conditions in the preceding summer, suggesting effect of nutrient reserves on wood increment (Barbaroux and Breda 2002). In the ring porous species, including ash (Carlquist 2001), nutrient reserves are mainly deployed for early growth (Barbaroux and Breda 2002) that affects water relations of tree in the following vegetation period (Tyree and Zimmermann 2002) and hence the increment. The amount of precipitation in summer showed positive correlation with TRW for all groups (Figure 3), suggesting that in the central part of Latvia ash has suffered water deficit. Shifting temperature conditions can burden assimilation and physiological processes (Pallardy 2008), as certain time is needed for the adjustment of photosynthetic apparatus to current conditions (Berry and Downton 1982), explaining the observed negative correlations between mean difference in daily temperature and TRW (Figure 3). The effect of precipitation and temperature in the preceding August was significant in all sites (Figure 3), as it is the time when the formation of nutrient reserves initiates (Barbaroux and Breda 2002).

In the sites in the eastern part of Latvia, TRW of ash was mainly negatively affected by temperature (Figure 3), but the mechanisms of influence shifted during the season. In autumn, raised temperature can increase respiration, causing losses of stored nutrients (Ögren et al. 1997). In September, increased temperature, apparently, might also increase evapotranspiration (Traykovic 2005) causing water deficient, as positive correlation with precipitation was observed (Figure 3). This applies to current and previous September temperature. The negative effect of temperature in current spring might be explained by earlier onset of the active period or earlier leaf flush, subjecting trees to damage from late frosts (Gu et al. 2008), which are quite common in the eastern part of Latvia (Avotniece et al. 2010).

In the context of regional and local diversity, the sets of the significant factors between the healthy and damaged trees differed slightly (Figure 3), suggesting that climatic sensitivity had non-drastic effect on susceptibility to the disease and crown reduction (Figure 2), although the effect of weather extremes might not be visible in the residual chronology (Schweingruber 1992). Still, some regional or stand differences were observed (Figure 3). In the central part of Latvia, the damaged trees were more affected by water deficit and temperature regime in the preceding August, as shown by higher values of correlation (Figure 3). Stronger relationships with climatic factors suggested that under unfavourable conditions, i.e. warm and dry summers, which are becoming more frequent (Avotniece et al. 2010), trees are more stressed hence predisposed to the infection and damage. Nevertheless, negative effect of the maximum temperature in the preceding August was observed for the healthy trees (Figure 3), which apparently have been able to react to additional factor, probably due lower stress (Speer 2010). On the other hand, the negative effect of maximum temperature in August, which is the second warmest month (Lizuma et al. 2007), might be related to slower growth of the healthy trees before 1970s (Figure 2). In the RND site, only the healthy trees showed positive relationship with precipitation in March, which had the highest value of correlation coefficients (Figure 3). Precipitation in March is usually in the form of snow, and its effect might be explained by the insulating properties of snow layer, which influences soil temperature, decreases soil freeze (Hardy et al. 2001) and hence winter mortality of fine roots (Tierney et al. 2001), affecting water relations of a tree later in spring (Tyree and Zimmermann 2002). The absence of such relationship might suggest that the damaged trees have had less sensitive root system before the infection. The effect of potential evapotranspiration in the previous May in the UKR site (Figure 3) might be related to excess of soil water at the beginning of vegetation season, which influence root respiration (Pallardy 2008). Still, the differences in reaction to this factor between the healthy and damaged trees is difficult to explain. Probably, it might be related to slower growth of the health trees at younger age, when they have been more influenced by this factor. In the eastern part of Latvia in the GBN site, where trees were the oldest, TRW was influenced by precipitation (Figure 3) that might be explained by the age-related sensitivity to water deficit (Carrer and Urbinati 2004). The damaged trees showed stronger correlation to precipitation (Figure 3), suggesting increased susceptibility to water deficit, hence they were more stressed in the dry years that, presumably, led to the infection by fungus and reduction of crown (Kowalski et al. 2010). In the BAR site, the differences in sets of the significant factors are difficult to explain and might be caused by the district individuality of growth patterns (Table 1).

Although located near its northern distribution limit, in Latvia, ash showed site-specific growth patterns and climatic sensitivity, probably due to the stresses caused by the pathogen(s). This also approves the complexity of ash dieback process. In all sites, ash of different crown conditions was affected by the climatic factors related to water deficit in summer, suggesting that under the changing climate, growth of ash might become even more stressed. The sensitivity of growth was higher under more continental conditions. Trees with different crown condition, apparently differed by social status, as the damaged trees grew faster at young age, but the TRW of the healthy trees was the highest at medium age. Still, the damaged trees showed growth reduction during recent three decades. The susceptibility to disease also appeared age-related, as the damaged trees were older than the healthy ones, likely due to the increasing maintenance costs. Still, some differences in climatic sensitivity were observed. In general, trees with the damaged crowns were more affected by the climatic factors, suggesting that climatic stresses have been at least partially involved in the development of the disease. Nevertheless, the healthy trees also showed effect of some climatic factors not observed for the damaged ones, suggesting influence of e.g. microclimate and microtopography on the susceptibility to the disease. For better understanding of the role of tree water relations in ash dieback, analysis of wood vessels might be useful.

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ANALYSIS OF THE GENETIC DIVERSITY AND POPULATION STRUCTURE OF LATVIAN ASH (*FRAXINUS EXCELSIOR* L.) STANDS USING NUCLEAR AND CHLOROPLAST SSR MARKERS

Dainis Ruņģis#, Anna Korica, Agnese Gailīte, Ilze Pušpure, and Ilze Veinberga

Latvian State Forest Research Institute “Silava”, Rīgas 111, Salapils, LV-2169, LATVIA

Corresponding author

Contributed by Dainis Ruņģis

*Common ash (*Fraxinus excelsior *L.) has a widespread distribution throughout Europe, and Latvia is almost at the north eastern edge of the distribution range. In Europe, ash is threatened by ash dieback, a disease caused by the introduced ascomycete* Hymenoscyphus fraxineus*. Chloroplast and nuclear DNA markers have been used to study the genetic diversity and population structure of ash both in a broader pan-European context as well as in more restricted regions. Some of the markers analysed in these previously published reports were also utilised in this study, enabling comparisons of the genetic parameters calculated from the nuclear SSR marker data and of the haplotypes identified with the chloroplast markers. Analysis of chloroplast markers revealed one dominant haplotype in Latvian stands, which corresponds to the haplotype previously found in Eastern Europe and Scandinavia. A second haplotype, corresponding to a previously reported central European haplotype was found in all individuals from the Ķemeri stand, indicating that this stand was naturally established from introduced germplasm, which was planted in a neighbouring park. The nuclear SSR markers revealed low levels of differentiation of Latvian* F. excelsior *stands, probably due efficient pollen flow between stands. The analysis of both chloroplast and nuclear DNA markers has revealed different aspects of the structure and provenance of Latvian* F. excelsior *populations.*

Key words: *pollen flow, population differentiation, introduced germplasm, post-glacial migration.*

INTRODUCTION

Common ash (*Fraxinus excelsior* L.) has a widespread distribution throughout Europe, with the exception of the central and southern parts of the Iberian Peninsula, south-east Turkey and northern Scandinavia (Dobrowolska *et al.*, 2011, and references therein). Latvia is almost at the north eastern edge of the distribution range (Anonymous, 2009), and *F. excelsior* comprises approximately 0.5% (14 582 ha) of the total forested area (Anonymous, 2014). It is mainly found in mixed stands, together with other deciduous species: elm (*Ulmus glabra* Huds.), oak (*Quercus robur* L.), aspen (*Populus tremula* L.), birch (*Betula pendula* Roth.). In moist sites, it mostly occurs with black alder (*Alnus glutinosa* (L.) Gaertn.), as observed in other European countries (Anonymous, 2005; Dobrowolska *et al.*, 2011). Ash grows on a wide range of site types but dominates mainly on fertile, biologically active, moist and seasonally excessively moist soil conditions (Laiviņš and Mangele, 2004; Anonymous, 2005). In Europe, ash is threatened by ash dieback, a disease caused by the introduced ascomycete *Hymenoscyphus fraxineus*. This fungus originates from East Asia, and was confirmed as the causal agent of European ash dieback in Poland in 2006 (Kowalski, 2006). Since the probable introduction of this fungus into Europe in the 1990s, it has spread throughout Europe, and is currently threatening common ash throughout most of its distributional range (Kowalski and Holdenrieder, 2009).

A limited number of nuclear microsatellite or simple sequence repeat (SSR) markers have been developed for *F. excelsior* (Brachet *et al.*, 1999; Lefort *et al.*, 1999), which have been used to study the genetic diversity and population structure of common ash both in a broader pan-European context as well as in more restricted regions. In addition, a set of conserved chloroplast SSR markers have been developed that have been applied to a wide range of angiosperm species (Weising *et al.*, 1999). European *F. excelsior* populations have been investigated using nuclear SSR markers (Heuertz *et al.*, 2004a) as well as chloroplast markers (Heuertz *et al.*, 2004b). Nuclear SSRs have also been used to study ash populations in Bulgaria (Heuertz *et al.*, 2001), France (Morand *et al.*, 2002), Romania (Heuertz, 2003), Scotland (Bacles *et al.*, 2005), Germany (Hebel *et al.*, 2006), Italy (Ferrazzini *et al.*, 2007) and Bosnia and Herzegovina (Ballian *et al.*, 2008). In many of these studies, the DNA markers utilised are at least partially overlapping, enabling direct comparisons of the genetic parameters calculated from the nuclear SSR marker data and the haplotypes identified with the chloroplast markers.

Analysis of the European populations of *F. excelsior* using chloroplast DNA markers suggest post-glacial recolonization of Europe from refugia located in Iberia, Italy, the Alps and the Balkan Peninsula, as suggested by the allelic richness in these southern populations. In addition, strong signatures of the post-glacial migration routes remained in the current northern European populations, which showed high levels of differentiation of chloroplast haplotypes, indicating only a small overlap of recolonising maternal lineages (Heuertz *et al*., 2004b). In contrast, analysis of European populations of *F. excelsior* with nuclear SSR markers revealed very little differentiation of populations ranging from the British Isles over central Europe to Lithuania and Latvia (Heuertz *et al*., 2004a). The discrepancy between the high levels of population differentiation identified by the chloroplast analyses, and the low levels revealed by nuclear SSR marker analyses has been suggested to be due to efficient postglacial pollen flow (Heuertz *et al*., 2004b).

Smaller scale analyses of *F. excelsior* populations using nuclear SSR markers have revealed patterns of genetic diversity and population differentiation which are in agreement with the pan-European studies. In general, southern populations have high genetic diversity (e.g Heuertz *et al*., 2001; Ferrazzini *et al*., 2007; Ballian *et al*., 2008), while the northern populations have lower levels of genetic diversity (Bacles *et al*., 2005). The previous studies also reported low levels of population differentiation, even between fragmented populations at the northern boundary of the distribution range of *F. excelsior* (Bacles *et al*., 2005). In addition, many of the previous studies reported high inbreeding coefficients (Fis) detected with the nuclear SSR markers (e.g. Bacles *et al*., 2005; Ferrazzini *et al*., 2007; Ballian *et al*., 2008). These increased Fis values could be due to biological factors and population structure (i.e. assortative mating or a Wahlund effect) or to the presence of null alleles. Null alleles are caused by mutations leading to the non-amplification of specific alleles by PCR, leading to an apparent excess of homozygosity. This can have an influence on the estimation of population genetic parameters (Chapuis and Estoup, 2007). However, five nuclear SSR markers (M2-30, Femsatl4, Femsatl5, Femsatl11, Femsatl19) were used to genotype the progeny of controlled (test) crosses, which allows the Mendelian segregation of alleles to be observed, thus enabling detection of null alleles. Potential null alleles were only detected at one locus (Femsatl5), with the other loci conforming to the expected segregation ratios (Morand *et al*., 2002). In addition, the Fis values were not significantly positive in all studies utilising these nuclear SSR markers (e.g. Heuertz *et al*., 2003), and therefore the high inbreeding coefficients are probably due to biological factors and/or structure within the previously analysed *F. excelsior* populations.

The aim of this study was to initiate a survey of the genetic diversity and population structure of ash (*Fraxinus excelsior* L.) in Latvia. Knowledge of the distribution of genetic diversity within and between populations is required for optimal management of forest genetic resources. In addition, the analysis of haplotypes using chloroplast markers enables the post glacial recolonisation pathways to be determined and also the identification of possible introductions of germplasm. The results obtained from this study will form a basis for further studies of *F. excelsior* in Latvia, and allow comparison of the obtained results with previously published studies of *F. excelsior* populations in other European regions.

MATERIALS AND METHODS

Six nuclear and six chloroplast SSR markers were utilised to analyse 16 naturally regenerated ash stands located throughout the territory of Latvia. The markers utilised in this study were also utilised in previous studies of European *F. excelsior* populations, thus enabling comparisons of genetic diversity with these previously obtained results. Leaves were collected from approximately 24 young ash individuals (1–2 metres in height) from 16 naturally regenerated *Fraxinus excelsior* stands (Fig. 1, Table 1). The 'Skrīveri' and 'Bērvircava' stands have been designated as *F. excelsior* genetic resource stands. A total of 372 *Fraxinus excelsior* individuals were analysed with the nuclear and chloroplast SSR markers.

DNA from leaves was isolated using a CTAB-based method (Porebski *et al*., 1997). Genotyping was done using six nuclear SSR markers — Femsatl4, Femsatl10, Femsatl11, Femsatl16, Femsatl19 (Lefort *et al*. 1999) and M2-30 (Brachet *et al*. 1999) and six angiosperm chloroplast markers ccmp2, ccmp 4, ccmp6, ccmp10, ccmp7 and ccmp3 (Weising *et al*., 1999). Each forward primer was labelled with a different fluorophore (6-FAM, HEX or TMR) to facilitate visualisation using capillary electrophoresis. The PCR reactions for the nuclear SSR markers were carried out in a 20 µl solution containing, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 1.5 µl DNA solution, 1x Taq buffer and 1U of recombinant Taq DNA polymerase (Thermo Scientific). PCR cycling conditions consisted of an initial denaturation step of 95 °C for 4 min; 35 cycles of 94 °C for 30 s, 52 °C for 45 s (56 °C for M2-30), and 72 °C for 60 s; followed by a final extension step of 72 °C for 10 min. The PCR reactions for

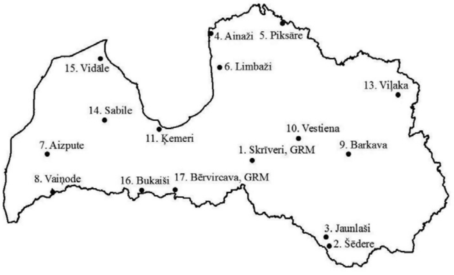

Fig. 1. Location of the sampled Latvian *Fraxinus excelsior* stands.

Table 1

COORDINATES AND ELEVATION OF THE SAMPLED LATVIAN *FRAXINUS EXCELSIOR* STANDS

Region	Stand	Coordinates		Elevation (m above sea level)
		X	Y	
Coastal lowland	4. Ainaži	523032	6409092	9
	15. Vidāle	411202	6383595	24
	11. Ķemeri	470181	6312350	8
Western Kurzeme	7. Aizpute	357289	6287409	75
	14. Sabile	415074	6321517	80
	8. Vaiņode	362909	6248835	110
Western Zemgale	17. Bērvircava	486569	6251149	34
	16. Bukaiši	452675	6250481	66
Augšzeme	3. Jaunlaši	639266	6203264	130
	2. Šēdere	642444	6194070	171
Northern Vidzeme	6. Limbaži	531633	6375057	52
	5. Piksāre	595114	6419336	96
Southern Vidzeme	1. Skrīveri	564760	6280660	90
Vidzeme highlands	10. Vestiena	611348	6302999	202
Aiviekste	9. Barkava	661933	6287151	94
	13. Viļaka	712240	6346960	122

the chloroplast SSR were similar, but with the addition of a final concentration of 1% BSA to the reaction. The PCR profile consisted of an initial denaturation at 96 °C for 5 min followed by 25 cycles of denaturation at 94 °C for 60 s, annealing at 55 °C for 60 s, and extension at 72 °C for 60 s. Final extension of amplified DNA occurred at 72 °C for 10 min. All PCR reactions were carried out in an Eppendorf Mastercycler ep *gradient* thermal cycler. Amplification fragments were separated on an ABI Prism 3130*xl* Genetic Analyzer (Applied Biosystems) and visualised with GeneMapper 3.5.

The chloroplast marker data was combined into haplotypes. Analysis of nuclear SSR data was done using Fstat version 2.9.3.2 (Goudet, 2001) and GenAlEx 6.5 (Peakall and Smouse, 2012). Dendrograms were constructed using the software MEGA 5.2 (Tamura *et al.*, 2011). A Bayesian clustering approach, implemented using the software STRUCTURE version 2.1 (Pritchard *et al.*, 2000), was used to estimate the most likely number of clusters (K) into which the nSSR genotypes were assigned with certain likelihood. The population priors were not used, and a burn-in period of 50 000 iterations followed by 50 000 iterations was used. K was set from 1 to 16, and each run was replicated 20 times. The most likely number of clusters was identified by the delta K criterion (Evanno *et al.*, 2005) with the STRUCTURE HARVESTER Web version 0.6.93 software (Earl and vonHoldt, 2012).

RESULTS

Of the six analysed chloroplast SSR markers, only three were polymorphic (ccmp6, ccmp7, ccmp10). The genotypes of these three loci were combined into two haplotypes, of which one was found in all individuals from all stands except for the 'Ķemeri' stand, where all individuals had the second identified haplotype. While the sizes of all the chloroplast alleles amplified in this study were not identical to the values reported previously, it was possible to unambiguously match the identified haplotypes to previously reported European *F. excelsior* haplotypes (Heuertz *et al.*, 2004b). The most common haplotype in this study, which was found in all stands except 'Ķemeri' was the haplotype H01, which is widespread in Eastern Europe and Scandinavia. The allele sizes for this haplotype were as follows (with the previously reported sizes in brackets): ccmp6 – 97 (97); ccmp7 – 115 (118); ccmp10 – 104 (103). The haplotype found exclusively in the 'Ķemeri' stand corresponds to H02, which is the most common haplotype found in central Europe. The allele sizes for this haplotype were as follows (with the previously reported sizes in brackets): ccmp6 – 99 (99); ccmp7 – 114 (117); ccmp10 – 105 (104). The H01 and H02 alleles were the most common alleles in European *F. excelsior* populations, together representing 68% of the analysed individuals (Heuertz *et al.*, 2004b). In addition, the other previously reported haplotypes were able to be excluded from comparisons with the results from this study, given the size differences of the polymorphic alleles. The differences in allele sizing are probably due to the use of differing genotyping platforms, protocols and reagents (Pasqualotto *et al.*, 2007).

The total number of alleles identified by each nuclear SSR markers ranged from 10 (Femsatl16) to 37 (M2-30) (mean 23.17 alleles). The number of effective alleles was considerably lower, ranging from 1.96 (Femsatl16) to 20.88 (M2-30) (mean 9.55 alleles), indicating the high proportion of low frequency alleles. The expected heterozygosity ranged from 0.49 (Femsatl16) to 0.95 (M2-30) (mean 0.82), while the observed heterozygosity was lower, ranging from 0.37 (Femsatl16) to 0.85 (Femsatl19) (mean 0.64). As a result, the fixation index (F) was positive for all loci, ranging from 0.06 (Femsatl19) to 0.43 (Femsatl4) (mean 0.23). These were all significantly higher than zero, with the exception of the locus Femsatl19 ($p < 0.001$) (Table 2).

The mean number of alleles found in each population over all the analysed nuclear SSR loci was similar, ranging from

Table 2

GENETIC DIVERSITY STATISTICS OF THE ANALYSED NUCLEAR SSR MARKERS

Locus	N	Na	Ne	Ho	He	F
Femsatl4	364	23	5.23	0.46	0.81	0.43
Femsatl10	348	33	11.68	0.54	0.91	0.41
Femsatl11	370	16	6.79	0.78	0.85	0.09
Femsatl16	369	10	1.96	0.37	0.49	0.25
Femsatl19	368	20	10.77	0.85	0.91	0.06
M2-30	363	37	20.88	0.83	0.95	0.12

N – number of individuals, Na – number of alleles, Ne – number of effective alleles, Ho – observed heterozygosity, He – expected heterozygosity, F – inbreeding coefficient.

Table 3

GENETIC DIVERSITY STATISTICS OF THE ANALYSED LATVIAN *F. EXCELSIOR* STANDS

Population	Skrīveri	Šēdere	Jaunlaši	Ainaži	Piksāre	Limbaži	Aizpute	Vaiņode	Barkava	Vestiena	Ķemeri	Viļaka	Abava	Vidāle	Bukaiši	Bērvircava	Mean
Na	12.33	10.67	11.00	10.17	10.50	11.17	12.00	9.00	11.83	9.83	10.50	8.17	11.00	11.83	11.00	11.33	10.77
Na (freq. ≥ 0.05)	5.33	5.67	4.67	6.33	5.33	5.50	6.67	4.50	4.83	5.00	5.00	4.50	4.50	6.17	5.33	5.67	5.31
Ne	7.32	5.71	5.91	6.85	5.57	6.57	8.28	4.72	6.38	5.08	6.04	4.00	5.84	7.07	6.95	7.11	6.21
No. of private alleles	0.33	0.67	0.33	0.00	0.17	0.33	0.17	0.17	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.15
He	0.80	0.76	0.75	0.77	0.77	0.76	0.82	0.74	0.77	0.73	0.78	0.67	0.76	0.78	0.80	0.83	0.77

Na – number of alleles, Ne – number of effective alleles, He – expected heterozygosity.

8.17 (Viļaka) to 12.33 (Skrīveri) (mean 10.77). The number of alleles with a frequency of over 0.05 was also similar, ranging from 4.50 (Vaiņode) to 6.67 (Aizpute) (mean 5.31). A small number of private or unique alleles (found in only one stand) were only found in eight stands (Skrīveri, Šēdere, Jaunlaši, Piksāre, Limbaži, Aizpute, Vaiņode, Vidāle) (ranging from a mean over all loci of 0.17-0.67 alleles) (Table 3). A total of 14 private alleles were identified with five nuclear SSR markers (Femsatl4, Femsatl10, Femsatl16, Femsatl19, M2-30), of which only three had a frequency above 0.05 in their respective stand (Table 4).

The level of population differentiation detected by the analysed nuclear SSR markers was low, but significant ($Fst = 0.045$, $p < 0.001$), as calculated by AMOVA (999 permutations). The maximum pairwise population Fst value was 0.151 between Vaiņode and Viļaka, and these two stands were the most differentiated from each other and also from the other analysed stands. The pairwise Fst are shown in Table 5. The most genetically differentiated stands are Viļaka, Šēdere, Vestiena and Vaiņode, with pairwise Fst values above 0.05. These stands were also differentiated from each other, with all pairwise Fst values between these four stands being higher than 0.075.

The pairwise Nei genetic distances between stands ranged from 0.10 (Jaunlaši-Ainaži) to 0.75 (Vaiņode-Viļaka) (mean 0.28). The pairwise Nei genetic distances were used to construct a neighbour-joining dendrogram (Fig. 2). As can be seen from the branch lengths, the most differentiated stands were Viļaka, Vaiņode, Vestiena and Šēdere, as previously shown by the pairwise Fst results. The clustering of the populations did not show any geographical or other structure, which was expected due to the low level of population differentiation as previously reported by the AMOVA.

The Bayesian clustering approach, implemented using the software STRUCTURE version 2.1, identified that the most likely number of clusters was three (Fig. 3). However, there were additional peaks in the delta K plot corresponding to a larger number of clusters. These clusters did not correspond to any geographic grouping, and differentiated individual stands with a higher proportion of membership of a particular cluster. In the case of three clusters, three stands (Šēdere, Limbaži, Vestiena) had a relatively high proportion (over

Table 4

FREQUENCY OF PRIVATE ALLELES ACROSS LOCI

Population	Locus	Allele size (bp)	Frequency
Skrīveri	Femsatl4	208	0.042
Skrīveri	M2-30	210	0.021
Šēdere	Femsatl4	210	0.042
Šēdere	Femsatl10	261	0.021
Šēdere	Femsatl16	173	0.063
Šēdere	M2-30	188	0.021
Jaunlaši	Femsatl19	165	0.021
Jaunlaši	M2-30	267	0.021
Piksāre	Femsatl10	161	0.043
Limbaži	Femsatl10	267	0.042
Limbaži	Femsatl16	185	0.063
Aizpute	Femsatl16	194	0.100
Vaiņode	M2-30	225	0.022
Vidāle	Femsatl16	204	0.021

bp – base pairs.

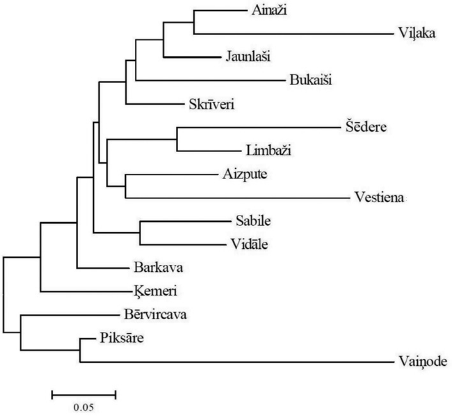

Fig. 2. Neighbour-joining dendrogram of pairwise Nei genetic distances between the analysed Latvian *F. excelsior* stands.

Table 5

PAIRWISE FST VALUES BETWEEN ANALYSED *F. EXCELSIOR* STANDS

Skrīveri	Šēdere	Jaunlaši	Ainaži	Piksāre	Limbaži	Aizpute	Vaiņode	Barkava	Vestie-na	Ķemeri	Viļaka	Abava	Vidāle	Bukaiši	Bērvir-cava	
	0.001	0.026	0.290	0.002	0.001	0.001	0.001	0.062	0.001	0.005	0.001	0.002	0.006	0.009	0.004	Skrīveri
0.030		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Šēdere
0.012	**0.056**		0.454	0.002	0.001	0.001	0.001	0.004	0.001	0.014	0.001	0.001	0.001	0.001	0.001	Jaunlaši
0.002	0.050	0.000		0.002	0.002	0.001	0.001	0.039	0.001	0.034	0.001	0.001	0.001	0.002	0.003	Ainaži
0.026	0.047	0.028	0.031		0.001	0.001	0.001	0.004	0.001	0.004	0.001	0.001	0.001	0.001	0.005	Piksāre
0.021	0.028	0.042	0.024	0.042		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Limbaži
0.024	**0.057**	0.048	0.034	0.047	0.049		0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	Aizpute
0.072	**0.109**	**0.084**	**0.075**	0.045	**0.106**	**0.086**		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Vaiņode
0.009	0.035	0.020	0.012	0.019	0.022	0.023	**0.067**		0.001	0.014	0.001	0.003	0.001	0.001	0.005	Barkava
0.047	**0.077**	**0.058**	0.046	**0.070**	**0.052**	0.048	**0.121**	0.043		0.001	0.001	0.001	0.001	0.001	0.001	Vestie-na
0.017	**0.052**	0.015	0.013	0.020	0.030	0.038	**0.061**	0.015	0.049		0.001	0.001	0.001	0.001	0.001	Ķemeri
0.045	**0.091**	**0.054**	0.035	**0.091**	**0.063**	**0.063**	**0.151**	**0.058**	**0.107**	**0.076**		0.001	0.001	0.001	0.001	Viļaka
0.020	**0.057**	0.028	0.033	0.039	0.041	0.038	**0.090**	0.019	**0.066**	0.037	**0.072**		0.025	0.001	0.006	Abava
0.014	**0.050**	0.036	0.031	0.034	0.027	0.027	**0.084**	0.019	**0.054**	0.032	**0.067**	0.011		0.001	0.001	Vidāle
0.013	0.049	0.024	0.021	0.031	0.048	0.039	**0.082**	0.032	**0.059**	0.026	**0.069**	0.029	0.040		0.001	Bukaiši
0.017	**0.059**	0.031	0.031	0.020	0.049	0.024	0.045	0.016	**0.061**	0.022	**0.074**	0.020	0.025	0.028		Bērvir-cava

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Fst values below diagonal, probability P (rand >= data) based on 999 permutations above diagonal. Pairwise Fst values ≥ 0.05 are highlighted.

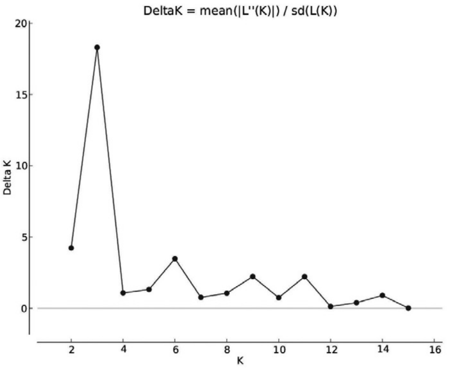

Fig. 3. Plot of delta K (the second order rate of change of the likelihood function with respect to K) vs. K (predefined number of clusters). The mode of delta K at K = 3 indicates the most probable number of clusters (3) in the analysed Latvian *F. excelsior* individuals.

0.6) of individuals assigned to one cluster; while the Vaiņode and Viļaka stands had a high proportion (over 0.7) of individuals assigned to each of the other two clusters. The remaining stands had an approximately equal proportion of individuals assigned to each of the three clusters (data not shown).

DISCUSSION

The chloroplast DNA markers identified one stand (Ķemeri), where all analysed individuals had the haplotype H02, in contrast to the common haplotype H01 found in all other stands and individuals. The H02 haplotype is common in central Europe, and the closest previously identified natural *F. excelsior* stands with this haplotype were in Poland (Heuertz *et al.*, 2004b). The individuals that were sampled were young, naturally regenerated individuals, and the Ķemeri stand from which they were collected did not show any signs of being artificially established. Given the uniform presence of this haplotype within the Ķemeri stand, and the complete absence of it in the other analysed populations, it seems likely that this stand has been established by ash individuals that have escaped from nearby parks or other artificial plantings, where genotypes from central Europe may have been introduced. The Ķemeri stand is adjacent to the Ķemeri sanatorium, which has been in operation since 1796 (Kupcis, Lībietis, 1933/34). In the period between 1839 and 1846, the nursery owner C. H. Wagner established a park adjoining the sanatorium (Dambis *et al.*, 2007), planting material (including *F. excelsior*) obtained from commercial nurseries, the majority of which was sourced from Western Europe (Zigra, 1805; Wagner, 1822). This stand and surrounding stands should be examined in more detail, including sampling mature individuals, to determine the extent of this haplotype in this area. In addition,

Table 6

COMPARISON OF ALLELE NUMBER AND INBREEDING COEFFICIENTS

	Latvia[1]		Scotland[2]		France[3]		Italy[4]		Bulgaria[5]		Bosnia and Herzegovina[6]		Romania[7]	
Locus	Na	F	Na	F	Na	F	Na	F	Na	F	Na	F	Na	F
Femsatl4	25	0.455	nd	nd	37	0.102	32	0.274	50	0.081	20	0.393	37	0.007
Femsatl10	34	0.421	nd	nd	nd	nd	76	0.332	nd	nd	55	0.371	nd	nd
Femsatl11	17	0.088	nd	nd	40	0.033	42	0.312	32	0.080	24	0.328	32	0.003
Femsatl16	10	0.274	6	0.032	nd	nd	9	0.191	10	0.124	12	0.156	10	0.165
Femsatl19	21	0.075	19	0.334	36	0.130	55	0.078	33	0.133	26	0.167	27	-0.07
M2-30	37	0.136	30	0.191	56	0.161	nd	nd	59	0.114	nd	nd	42	0.043

Na – number of alleles, F- inbreeding coefficient, nd – no data. 1 – this study, 2 – Bacles *et al.*, 2005, 3 – Morand *et al.*, 2002, 4 – Ferrazzini *et al.*, 2007, 5 – Heuertz *et al.*, 2001, 6 – Ballian *et al.*, 2008, 7 – Heuertz *et al.*, 2003.

a survey of the known artificial plantings within the area would enable comparison to the naturally regenerated stands in order to determine any genetic relationships between them. Interestingly, analysis of the nuclear SSR marker results did not differentiate the Ķemeri stand from the other analysed stands. This is probably due to the efficiency of pollen flow over larger distances, which would reduce the differentiation of the naturally regenerated individual that were descended from the artificially introduced genotypes, while maintaining the maternally inherited chloroplast haplotype. Analysis of older individuals may enable a higher level of differentiation from other Latvian *F. excelsior* stands to be identified with the nuclear SSR markers, however, given that these artificial introductions have been occurring since the early 19th century, the original founder genotypes may not be present in this area, and only the chloroplast haplotype persists. In addition, populations from central Europe could be analysed with the chloroplast and nuclear markers in conjunction with the Latvian samples. This would enable a direct comparison of the obtained haplotypes, and to determine the level of differentiation identified with the nuclear markers between Latvian and central European ash populations.

The genetic diversity identified by the nuclear SSR markers utilised in this study was lower than identified in more southern populations in previous studies (Table 6). At almost all loci that were also analysed in previous studies, the number of alleles was higher in previous studies, with the exception of Scotland, where the number of alleles was consistently lower. This is in agreement with the post-glacial expansion of *F. excelsior* from southern refugia, with populations at the northernmost edge having a lower level of genetic diversity. However, the inbreeding coefficients were significantly positive for most of the loci/studies, with the exception of Romania, indicating that this could be a feature of *F. excelsior* stands, regardless of their location within the distribution areal. This could be related to the mating system of *F. excelsior*, which is polygamous, with a continuum between pure male and pure female individuals with hermaphroditic intermediates (Wallander, 2008), or to other population structure processes.

The overall level of genetic differentiation identified with the nuclear SSR markers between the analysed Latvian stands was low, which is in agreement with previous pan-European studies, which found little differentiation of *F. excelsior* populations stretching from the British Isles over central Europe to Lithuania and Latvia (Heuertz *et al.*, 2004a). Four of the Latvian *F. excelsior* stands were slightly more differentiated (Viļaka, Šēdere, Vestiena, Vaiņode). These were not geographically clustered; however, it is interesting to note that all of these stands had an elevation above sea level of over 100 m. The remaining stands were all located at an elevation of less than 100 m above sea level, with the exception of the Jaunlaši stand, which had an elevation of 130 m above sea level. The Jaunlaši stand was geographically close to the Šēdere stand (Fig. 1); however they were not clustered together in the neighbour-joining dendrogram and the difference in elevation between the two stands was 41 m. One possibility is that the flowering time of the stands with a higher elevation is altered in comparison to the other stands, thus reducing the amount of gene flow between these stands and those at a lower elevation. Each of the elevated stands is geographically separated from each other as well, also decreasing the level of gene flow between the elevated stands, as demonstrated by the pairwise differentiation between the elevated stands as well as between the stands with a higher elevation and the stands with a lower elevation. The phenology of these stands should be further investigated to determine the extent of the role of flowering time in the differentiation of these stands, or if there are other possible factors influencing the population structure of Latvian *F. excelsior* stands.

The health status with regard to *H. fraxineus*, the causal pathogen of ash dieback, was assessed for a subset of the analysed *F. excelsior* populations (I. Pušpure, unpublished results). This revealed that the lowland coastal stands had the least favourable health status in comparison to the Eastern stands, which could be a result of more favourable environmental conditions for the pathogen. There was no discernible correlation of genetic population structure identified in this study with health status. Pliūra *et al.* (2011) found significant population differences with regard to resistance to *H. fraxineus* in Lithuania. However, these results were obtained from progeny trials in three locations. Therefore, the resistance status of Latvian *F. excelsior* populations should be assessed in a common environment, and the results of this study could be used as guide for the selection of the most genetically diverged populations for inclusion into future *F. excelsior* progeny trials, as well as the designation of additional genetic resource stands.

CONCLUSIONS

Analysis of chloroplast markers revealed only one dominant haplotype in Latvian stands, which corresponds to the haplotype previously found in Eastern Europe and Scandinavia. In addition, the central European haplotype was found in all individuals from the Ķemeri stand, indicating that this stand has originated from introduced germplasm. The lack of differentiation by the nuclear SSR markers of the Ķemeri stand from the other analysed stands indicates efficient pollen flow and the natural regeneration of the stand over several generations since the initial introduction. The nuclear SSR markers revealed low levels of differentiation of Latvian *F. excelsior* stands, again, probably due the efficient pollen flow. The slightly higher differentiation of stand found at a higher elevation above sea level could be due to phenological differences, however further investigation is required to elucidate this. The analysis of both chloroplast and nuclear DNA markers revealed different aspects of the structure and provenence of Latvian *F. excelsior* populations.

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LATVIJAS OŠU (*FRAXINUS EXCELSIOR* L.) AUDŽU ĢENĒTISKĀS DAUDZVEIDĪBAS UN POPULĀCIJAS STRUKTŪRAS ANALĪZE, IZMANTOJOT KODOLA UN HLOROPLASTU SSR MARĶIERUS

Parastais osis (*Fraxinus excelsior* L.) ir plaši izplatīts Eiropā. Latvija ir ošu izplatības areāla ziemeļos. Eiropā novērojama ošu audžu masveida atmiršana, ko izraisa patogēnā sēne askomicēte *Hymenoscyphus fraxineus*. Ošu ģenētiskās daudzveidības un populāciju struktūru izpētei Eiropas līmenī, kā arī tās atsevišķos reģionos, ir izmantoti hloroplastu un kodola DNS marķieri. Darbā izmantotie marķieri ar tiem vismaz daļēji sakrīt, dodot iespēju salīdzināt Latvijas un citus Eiropas reģionu ošu audžu ģenētiskos parametrus. Ar hloroplastu marķieriem veiktās analīzes Latvijas audzēs uzrādīja tikai vienu dominējošo haplotipu, kas sakrita ar Austrumeiropas un Skandināvijas haplotipu. Savukārt visos Ķemeru audzes indivīdos atrasts Centrāleiropas haplotips, kas norāda, ka šī audze dabīgi izveidojusies no blakus esošajā parkā introducētajiem ošiem. Kodola SSR marķieri uzrādīja zemu Latvijas ošu audžu diferenciāciju, kas, iespējams, ir saistīts ar efektīvu putekšņu plūsmu starp audzēm. Gan ar hloroplastu, gan kodola DNS marķieriem veiktās analīzes atklāj dažādus Latvijas ošu populāciju struktūras un izcelsmes aspektus.

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UNDERSTORY CHANGES IN *FRAXINUS EXCELSIOR* STANDS IN RESPONSE TO DIEBACK IN LATVIA

Ilze Pušpure[1 #], Māris Laiviņš[1, 2], Roberts Matisons[1], and Tālis Gaitnieks[1]

[1] Latvian State Forest Research Institute Silava, Rīgas iela 111, Salaspils, LV-2169, LATVIA

[2] Forest Sector Competence Centre, Dzērbenes iela 23, Rīga, LV-1006, LATVIA

[#] Corresponding author; ilze.puspure@silava.lv

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Intense dieback of Fraxinus excelsior *L. has been causing rapid changes in advance growth of trees and understory shrub growth of the affected stands. In this study, changes in composition and density of understory were studied in 15 permanent plots (each 235.6 m^2), repeatedly sampled in 2005, 2010, and 2015. Within each plot, the number and average height of understory individuals were determined. The successional changes in understory were assessed by Detrended Correspondence Analysis. In total, 11 advance growth and 20 undergrowth species were recorded. A significant increase in the density of understory was observed only in 2015, mainly due to understorey growth of* Corylus avellana *L.,* Padus avium *Mill., and* Lonicera xylosteum *L. Regarding advanced growth, the highest density was observed for* Ulmus glabra *Huds.,* F. excelsior *and* Acer platanoides *L.; the density of* A. platanoides *and* F. excelsior *increased particularly in the period from 2010–2015. The observed successional changes suggested individuality of development of the affected stands according to the composition of the remaining and neighbouring canopy trees.*

Key words: Fraxinus excelsior*, succession, advance growth, undergrowth,* Hymenoscyphus fraxineus*, recovery.*

INTRODUCTION

Studies of understory dynamics, i.e. advance growth of trees (AG) and understory growth of shrubs (UG), are mostly related to the changes fallowing disturbance of canopy (Klinka *et al*., 1996) caused by weather, harvesting (Mallik, 2003), pests (Ehrenfeld, 1980) or disease (Mackey and Sivec, 1973; McCormick and Platt, 1980; Lygis *et al*., 2014). Since the mid-1990s, in Europe, intense dieback caused by the pathogenic fungus *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz, Hosoya, comb. nov. has severely decreased the abundance of *Fraxinus excelsior* L. (Vasiliauskas *et al*., 2006). Studies conducted prior to the dieback showed successful self-regeneration of *F. excelsior* (Laiviņš and Mangale, 2004; Anonymous, 2005; Dobrowolska *et al*., 2011), but since the dieback, the regeneration has decreased sharply (Bakys, 2013). Simultaneously, changes in the understory species composition and density have been observed in the affected stands (Lygis *et al*., 2014). A similar succession was observed also in stands after Dutch elm disease (*Ophiostoma novo-ulmi* Brasier), as significant changes in UG were observed in stands with severe dieback, while minor changes were observed in healthy stands (Dunn, 1986). Huenneke (1983) showed that after Dutch elm disease, species composition and its change were influenced by the progression of individual tree dieback, the number of dead trees, gap size and the presence of adjacent gaps. McCormick and Platt (1980) suggested that changes in species composition after chestnut-blight mainly depended on the time since the disturbance and on local conditions. A dense UG layer is also known to strongly compete with AG regeneration (Beckage *et al*., 2000; Royo and Carson, 2006), thus altering the rate and direction of the succession (Givnish, 2002).

Considering that *F. excelsior* planting has been stopped (Kirisits *et al*., 2011, Bakys, 2013), knowledge about the natural succession of affected stands is crucial for assessment of their potential and for planning of further management. The future development of stands might be already guessed from the present composition of understory. Bakys (2013) and Lygis *et al.* (2014) showed that damaged *F. excelsior* stands tend to transform to stands dominated by early successional tree species like *Betula pendula* Roth, *Alnus incana* L. and *Populus tremula* L. The absence of pre-disturbance data is a major problem for many studies on forest response to sudden changes; therefore, long-term vegetation surveys are useful for understanding of successional processes (Sulser, 1971; Henry and Swan, 1974; Brewer, 1980; Sheil, 2001). The aim of the study was to evaluate changes in AG and UG species composition in affected *F. excelsior* stands during the recent decade and to assess potential transformations in the future. We hypothesised that the incidence of *F. excelsior* regeneration in previously *F. excelsior* dominated stands has decreased, and hence the species might be replaced by others (early successional tree species).

MATERIALS AND METHODS

Studied sites, sampling, and measurements. In this study, 15 permanent plots were established in 2005 in uneven-aged stands initially dominated by *F. excelsior*, but later subjected to a different degree of dieback. The age of the stands ranged from 51 to 138 years. The plots were scattered across the territory of Latvia (Fig. 1) to characterize the climatic differences and the prevailing site types of *F. excelsior* stands. Most of the plots were situated on soils with a normal moisture regime and a few plots (e.g. Ķemeri, Ainaži) were located on over-moist soils. Site types of the plots mostly were *Aegopodiosa, Dryopteriosa, Oxalidosa turf. mel,* and *Filipendulosa* (Bušs, 1976). The climate can be classified as moist continental. The mean annual temperature is +7.2 and +6.1 °C, with January being the coldest (–1.8 and –4.5 °C) and July being the warmest (+17.4 and +17.9 °C) months in the western and eastern region of Latvia, respectively. The mean annual precipitation in western Latvia is 748, and in eastern Latvia — 665 mm.

The data were collected in three observation periods during the recent decades, i.e. in 2005, 2010, and 2015. For the description of the overstorey, in each stand, one circular plot with a radius of 15 m was established, where all trees with diameter at breast height (DBH) exceeding 6 cm were measured and their health condition (living or dead) recorded. Within each plot, three smaller circular plots with the total area of 235.6 m^2 were placed in three directions (0°, 120°, 240°) at seven meter distance from the centre for the description of understory. In these plots, height of all UG and AG individuals with $DBH \le 6$ cm was measured with the precision of 0.5 m.

Data analysis. The composition of the understory and separately of AG and UG species among the sites and observation periods was compared using a chi-square test. The *t*-test was used to assess the significance of differences in mean height between the three periods for the understory as well as for individual species. ANOVA was used to assess the differences in total and individual species density between the observation periods. The relationships between understory as well as AG and UG density separately and the density of dead *F. excelsior* were determined by Pearson correlation analysis. Detrended Correspondence Analysis (DCA), based on the AG density, was used to assess the successional changes in species composition during the observation period following canopy *F. excelsior* dieback. A randomization test with 10^5 iterations was performed to determine the significance of DCA components. All analyses were calculated in the software R v. 3.1.2 (Anonymous, 2014) at the significance level $\alpha = 0.05$.

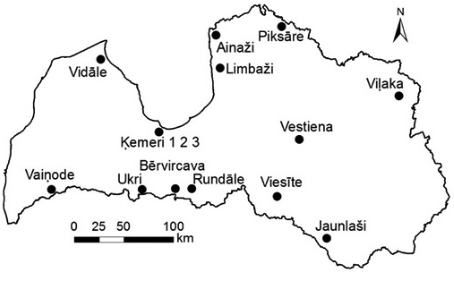

Fig. 1. The location of the studied sites (plots).

RESULTS

In total, in all observation periods, 13 canopy species were recorded (Table 1), but their distribution differed among the sites; e.g. *P. tremula* and *Tilia cordata* Mill., *Quercus robur* L., and *Alnus glutinosa* (L.) Gaertn. occurred only in three, four and five sites, respectively; *F. excelsior*, *Ulmus glabra* Huds., *Acer platanoides* L., and *Picea abies* (L.) H. Karst. occurred in more than eight sites. The highest density (> 70 trees ha^{-1}) was observed for *F. excelsior*, *P. abies*, *U. glabra,* and *A. glutinosa* (Table 1), although, after the second observation period, the density of *F. excelsior* decreased sharply (Fig. 2a). A slight decrease in the density of canopy *U. glabra* was observed after the second observation period, as in the Ainaži, Piksāre, and Viesīte sites, many *U. glabra* entered the canopy from AG to canopy, while in the third observation period, a dieback (mortality of 35%) occurred in the Ķemeri2 and Ķemeri3 sites. In the Vidāle site, a decrease (65%) of *P. abies* density occurred due to a windfall in the second period. An increase in number of *T. cordata* (from 142.86 trees ha^{-1} in 2005 to 285.71 trees ha^{-1} in 2015), *P. tremula* (from 1.00 trees ha^{-1} in 2005 to 242.68 trees ha^{-1} in 2015) and *A. platanoides* (from 28.57 trees ha^{-1} in 2005 to 471.42 trees ha^{-1} in 2015) was observed in the Viļaka, Jaunlaši, and Limbaži sites, respectively.

In total, 11 AG and 20 UG species were recoded (Table 1). Significant differences (*p*-value 0.001) in the composition of understory species were observed between all observation periods. *A. incana* was observed in AG only in 2005; *Cerasus avium* (L.) Moench and *Crataegus curvisepala* Gand. were observed in UG only in 2010. The highest number of species was in 2015, when seven species, which were absent before, were found (Table 1). Species with the highest occurrence in all three periods remained unchanged; in the AG layer — *F. excelsior*, *A. platanoides* and *U. glabra*, but in the UG — *Padus avium* Mill., *Corylus avellana* L., and *Sorbus aucuparia* L. (Table 1). The occurrence of *Q. robur*, *A. glutinosa*, *T. cordata,* and *Viburnum opulus* L. increased gradually. Nevertheless, the proportion of AG and

Table 1

UNDERSTORY AND CANOPY SPECIES DENSITY (D, INDIVIDUALS HA^{-1}) AND OCCURRENCE (O, %) CALCULATED BASED ON ALL PLOTS IN THREE OBSERVATION PERIODS

	Understory						Canopy		
	2005		2010		2015		2005	2010	2015
	D	O	D	O	D	O	D	D	D
Acer platanoides	920.03	73	696.39	73	1729.65	73	24.52	42.44	60.36
Alnus glutinosa	22.65	13	16.99	20	373.67	20	71.68	71.68	72.62
Alnus incana	14.15	13	0.00	0	0.00	0	-	-	-
Betula pendula	11.32	13	22.65	13	16.99	13	25.46	21.69	17.92
Fraxinus excelsior	645.44	73	871.90	80	1930.64	100	256.53	131.10	94.31
Picea abies	87.76	33	79.26	33	62.28	40	106.57	99.97	101.86
Populus tremula	93.42	27	56.62	27	192.50	20	8.49	18.86	21.69
Quercus robur	31.14	27	33.97	33	53.79	53	12.26	11.32	13.20
Tilia cordata	62.28	13	121.73	20	124.56	20	18.86	26.41	29.24
Ulmus glabra	1554.14	47	1406.94	60	1208.78	73	96.20	105.63	83.94
Cerasus avium	0.00	0	2.83	7	0.00	0			
Cornus sanguinea	25.48	7	0.00	0	110.40	7			
Corylus avellana	2692.14	73	2697.81	80	3204.53	73			
Crataegus curvisepala	0.00	0	2.83	7	0.00	0			
Dafne mezereum	0.00	0	0.00	0	2.83	7			
Euonymus europaeus	90.59	33	59.45	33	110.40	33			
Frangula alnus	16.99	13	8.49	20	48.12	13			
Lonicera xylosteum	266.10	33	418.97	33	854.92	33			
Malus sylvestris	8.49	13	8.49	13	59.45	27	0.94	0.94	2.83
Padus avium	2635.53	100	2525.12	100	4996.46	100	11.32	9.43	18.87
Prunus divaricata	0.00	0	0.00	0	2.83	7			
Rhamnus cathartica	5.66	7	2.83	7	5.66	13			
Ribes nigrum	90.59	27	62.28	27	342.53	40			
Ribes pubescens	0.00	0	0.00	0	2.83	7			
Ribes rubrum	0.00	0	0.00	0	11.32	7			
Salix caprea	0.00	0	0.00	0	2.83	7	1.89	1.89	1.89
Sambucus nigra	0.00	0	0.00	0	2.83	7			
Sorbaria sorbifolia	0.00	0	0.00	0	14.15	7			
Sorbus aucuparia	67.94	67	124.56	67	229.30	67	7.55	6.60	4.72
Viburnum opulus	53.79	10	50.96	33	325.55	53			

UG individuals remained relatively stable (36 and 64%, respectively), with fluctuations of only up to 2%.

In most of the sites, density of understory individuals was quite similar in 2005 (688 ± 221 and 1190 ± 178, respectively) and 2010 (660 ± 145 and 1192 ± 183, respectively), but in 2015, it was higher (*p*-value 0.001) and nearly doubled (1138 ± 228 and 2065 ± 312, respectively) (Fig. 3), except for the Ķemeri3 site, where a decrease was observed (Fig. 2b). During all periods, *U. glabra* (1390 trees ha^{-1}), *F. excelsior* (1149 trees ha^{-1}) and *A. platanoides* (1115 trees ha^{-1}) had the highest densities in AG (Fig. 4), but in 2015, significantly higher density was observed for *A. platanoides*, *F. excelsior,* and *A. glutinosa* (Fig. 4, Table 1). In UG, density of *P. avium* (3386 individuals ha^{-1}), *C. avellana* (2865 individuals ha^{-1}), and *Lonicera xylosteum* L. (513 individuals ha^{--1}) remained stable during all observation periods. However, their density, as for *Ribes nigrum L., V. opulus*, *Frangula alnus* Mill., and *Euonymus europaeus* L. was significantly higher in 2015 (Fig. 4). Thus, the increase of UG or AG density was caused by a particular species (Table 1). With the rapid increase in number of recruits, the average height of AG and UG decreased, particularly in the third observation period (mean height of AG decreased from 2.21 to 0.94 cm in 2005 and 2015, respectively) (Fig. 3).

The DCA ordination (Fig. 5) showed that the sites had an expressed grouping according the composition of AG species, hence three groups were arbitrarily distinguished. The first group consisted of the Ainaži (Fig. 5, site 1), Piksāre (8), Ķemeri1 (4) and Ķemeri2 (5) sites, which were dominated by *U. glabra*. The second group was characterized by the Bērvircava (2), Vidāle (13), Ukri (10), Vestiena (12), Jaunlaši (3) sites, which were distinguished by the dominance of *F. excelsior* with *Q. robur,* and *B. pendula*. The third group consisted of the Viesīte (14), Vaiņode (11), Limbaži (7), Viļaka (15) and Rundāle (9) sites, in which, however, AG was dominated by many species. The successional changes in AG were the most expressed in the

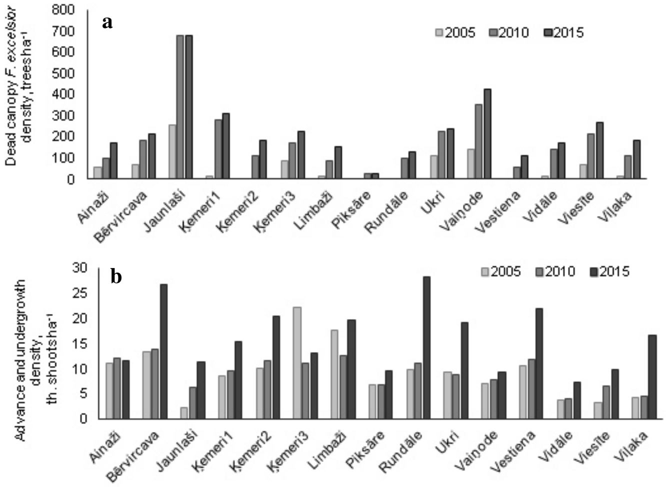

Fig. 2. Changes in the number of declining (dead) canopy *F. excelsior* trees (a) and total density of understory tree advance growth and shrubs (b) in the studied plots during the three observation periods.

first and second group, but the third group showed the weakest changes. Hence, the direction of changes were site specific. The sites from the first group showed the changes characterized by increasing abundance of *A. glutinosa* or *T. cordata*. The successional vectors of the sites from the second and third group mainly had opposite directions indicating changes among the broadleaved species, suggesting interchange of AG composition. Nevertheless, the abundance of *F. excelsior* increased in the Bērvircava (2), Ukri (10) and Jaunlaši (3) sites as shown by similarity of the vectors. Some sites (Ainaži (1), Ķemeri1 (4) and Vaiņode (11)) had only slight and reversing vectors, suggesting stability of the species composition in AG. The AG composition and its changes mainly coincided with the canopy species in the particular sites (Table 1). The correlation between understory density and the amount of dead canopy *F. excelsior* was not significant in any period (Fig. 6), although in a few sites, density of understory increased with the *F. excelsior* dieback (Jaunlaši, Ķemeri1, Ķemeri2) (Fig. 2a, b).

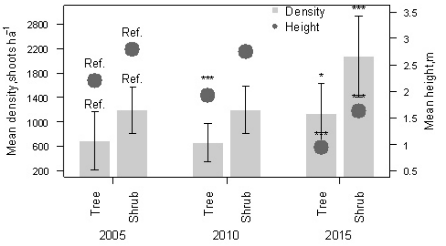

Fig. 3. Average density and height of understory tree advance growth and shrubs in the studied plots.

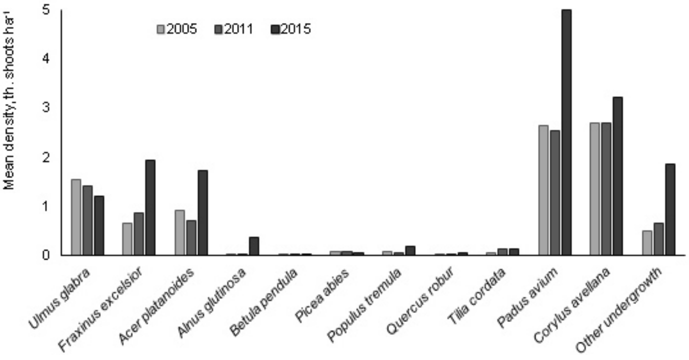

Fig. 4. Mean density of the main understory tree advance growth and shrub species.

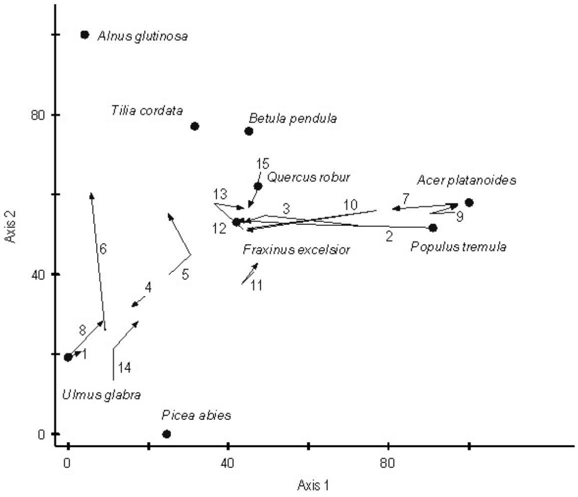

Fig. 5. DCA ordination of advance growth density in 2005, 2010, and 2015 in 15 studied plots (numbers) and species (dots). Site codes: 1 – Ainaži, 2 – Bērvircava, 3 – Jaunlaši, 4 – Ķemeri1, 5 – Ķemeri2, 6 – Ķemeri3, 7 – Limbaži, 8 – Piksāre, 9 – Rundāle, 10 – Ukri, 11 – Vaiņode, 12 – Vestiena, 13 – Vidāle, 14 – Viesīte, 15 – Viļaka. The successional changes in species composition among the observation periods are indicated by the vectors.

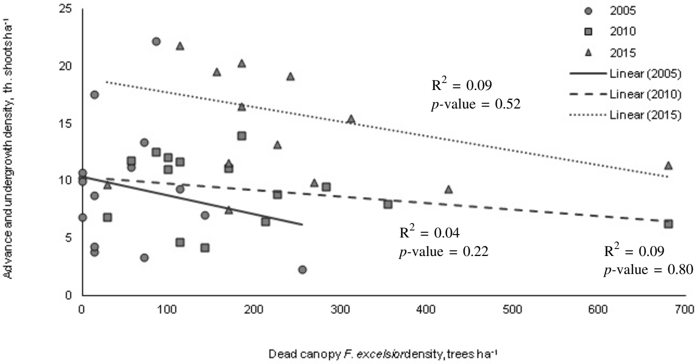

Fig. 6. Linear regression of understory tree advance growth and shrub density vs. dead canopy *F. excelsior* density in 15 studied plots in three observation periods.

DISCUSSION

Although understory is known to form a monodominant layer with abundant ground cover vegetation after the dieback of canopy trees (Royo and Carson, 2006), our results indicated the opposite, as an increase in number of UG species followed the dieback of canopy *F. excelsior*, while the number of AG species remained the same (Table 1). This could be explained by the fact that UG species, which are adventitious and/or temporary (e.g. *Prunus divaricate* Ehrh.*, Sorbaria sorbifolia* (L.) A. Braun and *Ribes sp.*), can adapt to altered environmental conditions more quickly than AG (Gonzales *et al*., 2002). Changes in species composition were minimal in the sites where the mortality of canopy *F. excelsior* was low, e.g., Piksāre and Viļaka (Fig. 2a), as observed after Dutch elm disease, when abundance of UG increased only in larger gaps (after death of two or more canopy trees) (Huenneke, 1983). Hence, changes in understory after disturbance can alter the course of succession, and diversification of stand composition might be expected in future (de la Cretaz and Kelty, 2002; Mallik, 2003).

The DCA, and particularly directions of the successional vectors, showed that the course of succession in the affected *F. excelsior* stands was diverse (Fig. 5), likely due to the differences in growing conditions and seed sources. The DCA also showed that in the first observation period *U. glabra* was a major species in AG in several sites (e.g. Ainaži, Piksāre, and Ķemeri3), but afterwards its abundance decreased due to poor health condition and dieback of the canopy trees, reducing the seed source (Fig. 5), probably due to *Ophiostoma novo-ulmi* (Huenneke, 1983). At present, *U. glabra* is being rapidly replaced by *A. glutinosa* (Fig. 5), likely due to moist conditions, for which alder is better adapted. In the third period, in sites where *A. platanoides* co-occurred in canopy, i.e. Limbaži and Rundāle, the growth of AG *A. platanoides* was released by the *F. excelsior* dieback (Figs. 4, 5), suggesting that it could become the dominant species there.

In Lithuania a rapid increase in the number of primary species (*P. tremula*, *B. pendula*) has been observed after canopy *F. excelsior* dieback, (Bakys, 2013; Lygis *et al*., 2014), but this was observed only in the Rundāle site (Figs. 4, 5), probably due to lack of these species in the canopy of the surrounding stands of other sites. Nevertheless, the increase in the number of AG *F. excelsior*, especially in the third observation period (Fig. 4), suggested recovery of *F. excelsior*, which might be explained by the natural selection of the most resistant genotypes (Pliura *et al*., 2015).

Many studies indicate that the increase of understory density has been caused by canopy thinning, hence reduction of competition and the improvement of light conditions (de la Cretaz and Kelty, 2002; Coomes *et al*., 2003; Royo and Carson, 2006). In contrast, in this study, the increase of understory density was not associated with the dieback of canopy *F. excelsior* (Fig. 6), suggesting that *F. excelsior* has had minimal effect on the UG species, except in the Jaunlaši, Ķemeri1 and Ķemeri2 sites, where such tendencies persisted. Alternatively, this could be related to delayed response of UG to such changes, as the strongest dieback was observed between first two observations (132.98 shoots ha^{-1}), hence the increase in understory density might still be expected. Some UG species are also known to persist within a territory for a long time after the disturbance, competing with AG in longer term (Latham, 2003; Mallik, 2003; Royo and Carson, 2006), hence making AG more susceptible to other disturbances (Pallardy, 2008).

Increase of UG due to canopy disturbance can suppress AG regeneration directly through competition, allelopathy, limited seedling germination and growth (Runkle, 1990; Gillman *et al*., 2003; Royo and Carson, 2006), thus stalling succession for decades (Schnitzer *et al*., 2000). However, the proportion of AG and UG varied little (Fig. 3), suggesting similar competitiveness of the species under altered conditions following the disturbance. This might be also explained by a quite rapid growth of AG that reached the canopy, hence shaded the understory, as observed for *A. platanoides* and *T. cordata* and *U. glabra* in the Limbaži, Viļaka and Ķemeri3 sites, respectively.

Our results showed that the changes of species composition and density after the *F. excelsior* dieback have been occurring with different rates in relation to the local conditions. Therefore it is difficult to generalize further transformation of *F. excelsior* stands. The temporal stability of AG and UG composition and density suggested similar competitiveness of the species, contraindicating the formation of shrub land. Although the dieback of canopy trees has been progressing, increase of *F. excelsior* AG in the later observation period suggested improvement of regeneration, and hence there is a chance that *F. excelsior* could remain as an admixture species in these stands in the future. Still, monitoring of the stands is necessary to assess further recovery.

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KRŪMU STĀVA IZMAIŅAS PARASTĀ OŠA AUDŽU DESTRUKCIJAS REZULTĀTĀ LATVIJĀ

Pēc *Fraxinus excelsior* L. audžu destrukcijas, šajās audzēs novērotas straujas pameža un paaugas (krūmu stāva) sugu sastāva un biezuma izmaiņas. Šajā pētījumā sugu sastāva un biezuma izmaiņas krūmu stāvā pētītas 15 ilglaicīgajos parauglaukumos (katrs 235,6 m^2) 2005., 2010. un 2015. gadā. Katrā parauglaukumā uzskaitītas visas krūmu stāva sugas un nomērīts to augstums. Sukcesionālās izmaiņas krūmu stāvā novērtētas, izmantojot detrendēto korespondentanalīzi. Kopā uzskaitītas 11 pameža un 20 paaugas sugas. Būtiska krūmu stāva biezuma palielināšanās novērota tikai 2015. g., galvenokārt pieaugot pamežam — *Corylus avellana* L.*,* *Padus avium* Mill. un *Lonicera xylosteum* L. Paaugā lielākais biezums konstatēts *Ulmus glabra* Huds.*,* *F. excelsior* un *Acer platanoides* L.; turklāt *A. platanoides* un *F. excelsior* biezums 2015. gadā palielinājās. Novērotā sukcesija norāda, ka katrai slimības skartajai audzei raksturīga individuāla attīstība atkarībā no palikušās un blakus esošās audzes sastāva.

QUALITY ASSESSMENT OF EUROPEAN ASH *FRAXINUS EXCELSIOR* L. GENETIC RESOURCE FORESTS IN LATVIA

Ilze Pušpure[1,2], Linda Gerra – Inohosa[2], Natālija Arhipova[2]
[1]Latvia University of Agriculture
[2]Latvian State Forest Research Institute 'Silava'
ilze.puspure@silava.lv; linda.gerra@silava.lv, natalija.arhipova@silava.lv

Abstract

In Latvia, two European ash (*Fraxinus excelsior*) genetic resource forests (GRF) have been designated in Skrīveri and Bērvircava. However, as the degree of damage of ash by the pathogenic fungus *Hymenoscyphus fraxineus* has increased, many stands have died and many do not comply anymore with minimum requirements for GRF. The aim of the present research was to evaluate and examine the quality of ash GRFs. In each forest unit, one 20×20 m plot was established, and the first (E3) and second (E2) layer projective cover of each species was determined in August 2014. All undergrowth and advance regeneration species were counted and the degree of ash damage by *H. fraxineus* was determined along a diagonal 25×1 m transect in each plot. In total, 101 plots were established. Quality evaluation was carried out according to the basic criteria for selection of GRF as well as based on criteria cited in other research. Their variation was established by using principal component analysis. Ash in layer E3 was better maintained in Skrīveri, but ash regeneration there was worse, as well as a higher degree of damage by *H. fraxineus* was observed. Ash regeneration density is significantly influenced by its cover on layers E3 and E2 as well as by tree species dominating the growth. The most valuable GRFs have been maintained in Skrīveri where 50% of units were rated of average quality and few of bad quality. In Bērvircava, 56% of forest units were of bad and very bad quality.

Key words: Descriptors, natural regeneration, ash dieback, European Forest Genetic Resource Program.

Introduction

The objective of genetic resource forests (GRF) is to maintain the genetic diversity and gene pool of a concrete tree species; and additionally they also have potential economic, environmental, scientific and social value (The State of…, 2014). These forests can provide for adaptation and evolutionary processes of the forest and trees growing in it; they also foster stand productivity (The State of…, 2014).

Within the framework of the European Forest Genetic Resource Program (EUFORGEN), 59 European ash (*Fraxinus excelsior*) gene reserve forests and protected forest areas have been designated in Europe, two of them are situated in Latvia (Pliûra and Heuertz, 2003). The EUFORGEN database indicates that the forest areas are managed for wood production and/ or multiple uses or services (Pliûra and Heuertz, 2003), but several genetic resource forest units are also specified as woodland key habitats, therefore economic activity in these forest units is restricted. In Bērvircava, selective cutting is allowed in three forest units for research purposes.

Ash GRFs are threatened by the ever-increasing infection rate with the pathogenic fungus *Hymenoscyphus fraxineus* which leads to ash dieback. In the last 20 years, the disease has spread widely throughout Europe and it has been found in more than 20 countries. The Baltic states were among the first where ash stand destruction was identified at the beginning of the 1990s (Stener, 2013), but the disease was confirmed in Latvia in 2007 (Kenigsvalde et al., 2010).

Prior to ash dieback, European ash was the most common hard-wood tree species in Latvia and one of the few tree species that regenerated well naturally (Laiviņš and Mangale, 2004). Natural regeneration is one of the basic GRF requirements (Koskela et al., 2013). At present, European ash comprises only 14 582 ha (0.5%) of the total forest area. The increasing degree of ash damage can lead to serious ecological consequences to European biodiversity and forest ecosystems (Pautasso et al., 2013). In Britain, 953 species are connected with ash stands, and the existence of 44 species fully depends on ash (Mitchella et al., 2014). Notwithstanding the extended history of the disease, further development of ash stands cannot be foreseen, because it greatly depends on persistence of natural resistance and further host organism and pathogen interaction over a longer period of cohabitation (McKinney et al., 2011). Therefore genetic resource forests (GRF) stands have become scientifically important forest stands and they are genetic reserves of European ash.

Pan-European minimum requirements for dynamic gene conservation units stipulate that each GRF stand must have a management plan which, based on systemic surveys, is updated every 5 or 10 years (Koskela et al., 2013). In the present study all ash GRF units were surveyed. As ash dieback increases, it becomes evident that ash GRF minimum requirements stipulating that the minimum size of a genetically viable population is 50, preferably 500 to 1000 trees and 400 reproducing trees per hectare (Koskela et al., 2013), cannot be fulfilled in many forest units. At present, EUFORGEN have not established requirements for re-evaluation of the existing stands, so the study is based on the aforementioned requirements for new GFR stand selection. In Latvia, the features of genetic

Table 1

Ash GRF criteria for division into quality classes

Ash quality and descriptor class	Primary descriptors			Secondary descriptors				
	Ash projective cover, %		Ash regeneration, specimens per hectare [1]	Second and undergrowth layer cover, % [2]	Spruce projective cover E3, E2, %	Degree of *H. fraxineus* damage, %	Broad leaved projective cover E3, %	Competition of other species, number [3]
	E3	E2						
1.	> 17	> 11	> 6001	0 – 30	0 – 0.5	0 – 10	6	0 - 15
2.	13 - 16	4 – 10	3001 - 6000	31 – 50	1 – 3	11 – 25	4 - 5	16 - 35
3.	8 – 12	1 - 3	1001 - 3000	51 – 70	4 – 8	26 – 60	3	36 - 60
4.	4 – 7	0.5	801 - 1000	71 – 80	9 – 15	61 – 99	1 - 2	61-100
5.	0 - 3	0	0 – 800	> 81	> 16	100	0	> 101

[1] The number of ashes counted in transect has been recalculated to hectares.
[2] Total projective E2 cover.
[3] Other regeneration growths and undergrowths counted in transect.

forests, namely descriptors, have been developed within the framework of the research 'The long-term maintenance and sustainable use of Latvian forest tree genetic resources' (Gailis et al., 2008), but the descriptors have not been adopted and are not used in practice.

The objective of the research was to survey both GRF ash stands in Latvia, determine their present state and to give a quality evaluation according to a framework of five classes. As ash cover has decreased dramatically, one of the tasks set by the research was to determine the factors that influence the generation of new ash advance regeneration. Whereas Latvian descriptors have been established for Norway spruce (*Picea abies*) genetic resource forests, one of the objectives of the research was to identify and analyse the features that best characterize European ash forests.

Materials and Methods

The research was carried out in August 2014 in two ash genetic resource forests, which are situated in the central part of Latvia: Skrīveri region (Skrīveri, geographical location: X 564257, Y 6281096) and Jelgava region (Bērvircava, geographical location: X 486817 , Y 6251196). Skrīveri forest area is 155.2 ha, on which 28 GRF units were surveyed. In Bērvircava forest 73 units covering 239.6 ha were surveyed. In both regions there were ash stands of different age, with ash being the principal species with wych elm (*Ulmus glabra*), aspen (*Populus tremula*), alder (*Alnus glutinosa*), and silver birch (*Betula pendula*) admixture; in several units ash was the admixture species for birch, spruce etc. forests. Both regions are characterized by fertile development conditions, mostly *Aegopodiosa*, on rare occasions *Dryopteriosa* or *Mercurialiosa* ameliorated forest types, characterized by various broad leaved species.

In each unit one 20×20 m plot was established, and the projective cover percentage of the first (E3, above 7.0 m) and second (E2, 0.5 – 7.0 m) layer of each species was determined. In each plot, all advance regenertion and undergrowth species (up to 7.0 m) were counted and degree of ash damage by *H. fraxineus* (Table 1) was determined in the framework of five classes along a diagonal 25×1 m transect. *H. fraxineus* damage has been assessed according to the visually determined degree of damage of the tree crown and trunk. The final rating was assigned based on the number of woody plants in each damage class. If there were no new specimens found in the transect, the degree of damage was not established. Twenty eight units were surveyed in Skrīveri and 73 units in Bērvircava.

GRF formation criteria were used as the basis for evaluation of forest quality (Koskela et al., 2013), supplemented by the descriptors listed in the report by A. Gailis et al. (2008). After a complex evaluation, each region was given a quality rating in classes from 1 to 5. The number of classes was determined based on the number of descriptor classes in the research made in Latvia (Gailis et al., 2008). The descriptors are divided into two groups: primary (if all descriptors were given equal rating – quality class could be established without taking into account other descriptors), corresponding to GRF formation basic requirements, and secondary – derived from A. Gailis et al. (2008) (Table 1). Criteria class values have been established according to median, quartile, minimum and maximum values of the evaluations obtained in all samples. In ash regeneration and E3 covering class division, Pan-European minimum requirements have been taken into account, stipulating that for an ash stand to be qualified as a GRF at least 50 mature trees and 400 saplings per hectare are required (Koskela et al., 2013). Based on these rules, it was supposed that the minimum number of advance regeneration ash trees has to be above 800 trees per hectare to qualify as a GRF (Table 1).

Tree level dominance structure has been established according to the principle of dominant and codominant species (Simpson, 2006), where the dominant species comprise ≥ 50% of the total covering or at least exceeds the covering of other species by 20%, and the codominant species is 25 – 50% of the total cover. Vegetation description and growth inventory was done during the 2014 growing season.

In characterizing unit similarities and factors having influence upon them, the detrended correspondence analysis (DCA) method was used. In determining which factors best characterize fluctuation, principal component analysis (PCA) was carried out, the second matrix being basic criteria for GRF selection. Thus criteria were obtained that most precisely characterized forest unit quality; they were taken into account upon determining quality classes. The canopy dominant species' influence on advance regeneration and undergrowth content was analysed by using the DCA method. In determining gradient importance, a randomization test was used for both methods.

The correlation between the degree of ash damage by *H. fraxineus* and the dominant tree species, forest type, new ash advance regeneration density (in classes), shrub layer cover and ash cover in E3 layer, was determined by the general linear model (GLM) method. Analysis of variance (ANOVA) was used to determine whether the number of ash in advance regeneration has been significantly influenced by the forest type and dominant tree species. For the evaluation of ash advance regeneration density, tree and shrub layer projective cover as well as correlations between the number of other species listed in advance regeneration and undergrowth, Pearson and Kendall correlation analysis was carried out. By using the Kendall ratio, it was determined if there was a correlation between shrub layer cover and the number of ash trees in layers E3 and E2. The analyses were carried out at α = 0.05. Statistical analyses were made using PC-ORD 6 (Peck, 2010) and R computer programmes.

Results and Discussion

In the majority of GRF units, ash trees were found in admixture with other broad leaved species, complying with ecological requirements (Ahlberg, 2014). In Skrīveri the E3 layer was dominated by birch (29% of units), small-leaved lime (*Tilia cordata*) and elm (both 14%), whereas in Bērvircava it was dominated by ash (27%) and aspen (21%) (Figure 1). In Latvia the following species are recommended as ash satellite species: birch, alder, grey alder (*Alnus incana*) and pedunculate oak (*Quercus robur*) (Sakss, 1997). Apart from these species, lime and elm are also emphasised in Europe (Dobrowolska et al., 2008), although ash trees in elm forests in Latvia have seldom regenerated, based on previous research (Laiviņš and Mangele, 2004). In Skrīveri, the most common broad leaved tree species are the typical *Aegopodiosa* forest site type species - lime, elm and ash. Analysis of variance indicated that ash regeneration density in Skrīveri has been significantly influenced by the dominant species in layer E3 (p = 0.001) and forest type (p = 0.03), whereas in Bērvircava none of these factors was decisive. In the framework of this research, ash was regenerated best in *Aegopodiosa* and *Mercurialiosa* mel. forest site types, namely, in habitats that are rich in nutrients, biologically active,

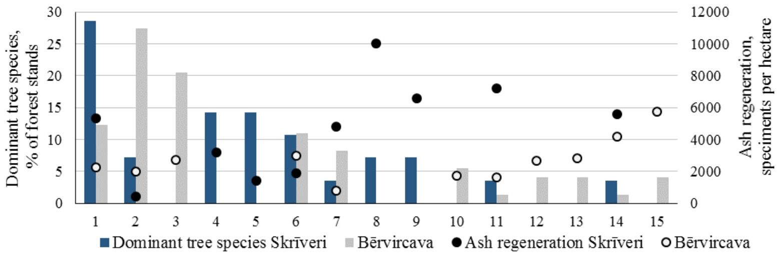

Figure 1. Dominant tree species and ash saplings density: 1 – *Betula pendula*; 2 – *Fraxinus excelsior*; 3 – *Populus tremula*; 4 – *Tilia cordata*; 5 – *Ulmus glabra*; 6 – *Alnus incana*; 7 – *Picea abies*; 8 – *Betula pendula/Ulmus glabra*; 9 – *Fraxinus excelsior/Ulmus glabra*; 10 – *Alnus incana/Fraxinus excelsior*; 11 – *Alnus incana/Betula pendula*; 12 – *Betula pendula/Populus tremula*; 13 – *Betula pendula/Fraxinus excelsior*; 14 – *Alnus glutinosa*; 15 – *Quercus robur.*

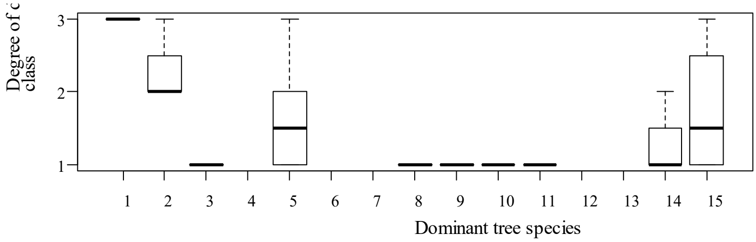

Figure 2. Ash advance regeneration damage degree intensity in stands with different dominant species in Skrīveri: 1 – *Alnus* glutinosa; 2 – Alnus *incana;* 3 – *Alnus incana/Betula pendula*; 4 – *Alnus incana/Fraxinus excelsior*; 5 – *Betula pendula*; 6 – *Betula pendula/Fraxinus* excelsior; 7 – Betula *pendula/Populus tremula*; 8 –*Betula pendula/Ulmus* glabra; 9 – *Fraxinus excelsior*; 10 – *Fraxinus excelsior/Ulmus* glabra; 11 – *Picea abies*; 12 – *Populus tremula*; 13 – *Quercus robur*; 14 – *Tilia cordata*; 15 – *Ulmus glabra.*

moist and seasonally excessively moist, but it was not as successful regenerating in *Dryopteriosa*, as has previously been mentioned in other research carried out in Latvia (Laiviņš and Mangele, 2004; Kenigsvalde et al., 2010).

Better and more regular ash regeneration was observed in Skrīveri, where, in the framework of this research, the greatest number of ash specimens were found in birch/elm (10 000 trees ha^{-1}) and grey alder/birch (7 200 trees ha^{-1}) stands (Figure 1). In ash/elm mixed stands, 6 600 trees ha^{-1} were counted. In Bērvircava the best ash regeneration was observed in oak (7 400 trees ha^{-1}) and alder (4 200 trees ha^{-1}) stands. Ash regeneration below ash stands was not characteristic of any region, but it was higher in Bērvircava (2 000 trees ha^{-1}) (Figure 1).

GLM analysis showed that in Skrīveri the new ash advance regeneration degree of damage was statistically significantly different between stands with various dominant species. In Skrīveri the highest degree of damage of new ash trees was found in alder stands (p = 0.0004) (Figure 2). The second class damage degree was found in grey alder, but a higher degree of damage was also found in birch and elm stands. In Bērvircava ash trees damage degree was much lower and statistically did not differ between stands with various dominant species.

In DCA both locations were firstly divided by their different geographical position, because they are situated in differing geobotanical regions. In DCA all gradients were important and they showed that areas differed in canopy composition, and it had significant impact on ash regeneration density, as mentioned in research by M. Laiviņš and D. Mangale (2004). The DCA first variable was best characterized by grey alder and aspen (DCA1) (p = 0.02), the second by spruce (DCA2) (p = 0.03), and the third by ash together with oak (Figure 3).

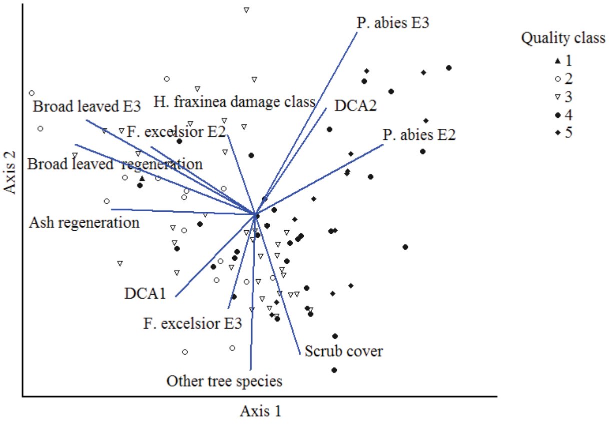

Figure 3. Ash forest quality criteria in PCA analysis.

Ash regeneration density had a statistically significant correlation with a number of woody plants listed in advance regeneration in Bērvircava ($p = 0.03$, $\tau = 0.184$), confirming the previous research in Latvia which showed that regeneration of ash and other woody plants most often takes place in canopy and thinned out places (Sakss, 1997). In Bērvircava two tree species dominated the advance regeneration composition – grey alder (55% of the total number of species, 3 704 trees ha^{-1}) and ash (34%, 2 290 trees ha^{-1}) (Figure 4). Formation of pronouncedly mixed stands with elm (58%, 4 243 trees ha^{-1}), ash (29%, 4 214 trees ha^{-1}), lime (17%, 2 514 trees ha^{-1}) and Norway maple (*Acer platanoides*) (16%, 2 329 trees ha^{-1}) is typical of Skrīveri. Species composition corresponds to the species listed in ash clear-cuts in Lithuania, only the cover was different: birch (in Lithuania – 32.9%), grey alder (32.4%), aspen (11.2%), elm (6.4%) and ash (4.3%, but 13 941 trees ha^{-1}) (Lygis et al., 2014). The results of this research indicated that pure ash stands are not formed by means of natural regeneration, but in both locations ash is maintained as an admixture species. In research by Laiviņš and Mangele (2004) it is foreseen that ash will be maintained in tree layer as a permanent admixture only on average in 5% of broad leaved forests. In Skrīveri the same species regenerate that dominate layer E3 (lime, elm), the same correlation has also been observed in Lithuania (Lygis et al., 2014). Whereas in Bērvircava, where the dominant species is ash, undergrowth species regenerate - hazel (*Corylus avellana*) and bird cherry (*Padus avium*), indicating the beginning of a pronounced process of shrub overgrowth.

In Skrīveri three species were dominant in undergrowth: cherry (39%, 1 071 trees ha^{-1}), hazel (33%, 914 trees ha^{-1}) and fly honeysuckle (*Lonicera xylosteum*) (21%, 586 trees ha^{-1}), but in Bērvircava - cherry (63%, 12 433 trees ha^{-1}) and hazel (20%, 3 945 trees ha^{-1}) (Figure 4). Although hazel is considered a valuable ash forest species (Loidi, 2004), together with cherry they comprised more than 50% of all species composition in both areas, thus leaving little room for growth to other species. The correlation indicated that as the shrub layer cover increases, the density of new ash trees decreases, but statistically it was not significant. Research carried out in Denmark (Bakys et al., 2013) showed that there is greater degree of ash damage in thinned stands, but it is not correlated with ash tree density. In the present research, identical results were obtained, although in Skrīveri it was found out that a higher degree of damage (class 2) can be established in stands with denser shrub cover (Figure 5), in several plots, the degree of damage reached class 3 and 4 in units with greater ash density, but the correlation was not significant. On average, the degree of damage was lower than indicated in the Lithuanian research (Lygis et al., 2014), because 80.8% were rated as class 1,while in Lithuania only 29.3% were considered visually healthy (Lygis et al., 2014). It could not be established that the degree of ash damage is significantly influenced by ash cover in the tree layer, but in stands with greater ash cover, more often a higher regeneration growth degree of damage was found.

GLM analysis showed that statistically the new ash advance regeneration degree of damage does not differ significantly among forest types. The lowest new ash advance regeneration's degree of damage was established in *Mercurialiosa* mel. forest site type ($p=1.26E-05$), where it was not higher than class 1 in any of the units. The highest degree of damage was established in *Dryopteriosa* forest site type (most often class 2). Although there is no united opinion in Europe on whether there is a correlation between ash degree of damage and the ecological conditions in the

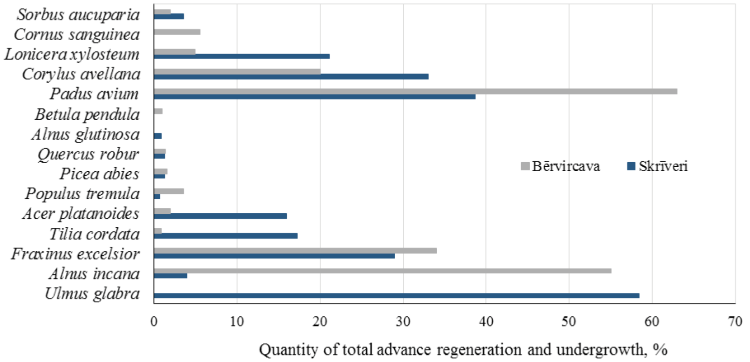

Figure 4. Species composition in advance regeneration and undergrowth.

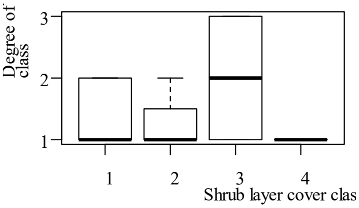

Figure 5. Ash degree of damage depending on shrub layer density in Skrīveri.

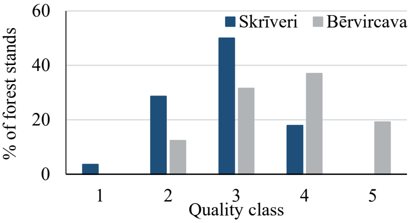

Figure 6. Ash GRF quality evaluation in classes.

locations (Bakys et al., 2013), this research shows that there is a higher degree of damage by *H. fraxineus* in moist circumstances than indicated in A. Gross's research (Gross et al., 2014).

According to the basic criteria for GRFs, the Skrīveri plots corresponded to higher quality classes. In both locations ash regeneration density was statistically significantly influenced by the total canopy cover ($p = 0.02$, $\tau = 0.16$) confirming that ash during its first years of life is ombrophile (Dobrowolska et al., 2008). In research by R. Harmer et al. (2005) it was concluded that the number of ash seedlings was positively related to the number of parent trees, which was also confirmed during this research ($p = 0.001$, $\tau = 0.22$). The number of ash trees in any of the regions was not influenced by DCA1 variable grey alder/ aspen ($p>0.05$), but it negatively influenced the number of spruce in layers E3 and E2. It was more pronounced in Bērvircava ($p = 0.02$, $\tau = -0.2$), confirming that spruce in Latvia is not recommended as an ash satellite species because it decreases soil productivity and creates a strong root system which outcompetes ash (Sakss, 1997). Ash regeneration was strongly influenced by the number of broad leaved species in layer E3 ($p = 0.005$, $\tau = 0.21$) emphasizing the importance of mixed stands (Ahlberg, 2014).

The PCA of all criteria showed that without GRF basic criteria ash stand variation is also well characterized by spruce cover in layers E3 and E2, the total cover of layer 2 and undergrowth layer, DCA1, the number of broad leaved species in layer E3 and other criteria (Figure 3). However, the correlations of these variables with ash regeneration density indicated that DCA1 and the number of broad leaved species do not have a significant impact. Evaluating all criteria (Table 1) better forest condition was found in Skrīveri, where 50% of units were rated with the quality class 3 and there were no evaluations of the lowest class 5 (Figure 6). In Bērvircava, 19% units were rated with class 5 and a relatively similar number of units were rated with class 3 and 4. In several units ash trees have not been maintained neither in layer E3 nor layer E2. In both GRFs, the new ash is mostly young and it is difficult to foresee its further development as well as changes in the evaluation of stands.

Conclusions

1. In the beginning, 12 quality indicators were set for GRF quality evaluation, out of which during the research only 9 were recognized as significant, 3 of them (ash projective cover in canopy and midstorey, ash regeneration density) are considered primary, others have a secondary importance.
2. In the majority of stands, ash is not the dominant species anymore, but a statistically significant correlation remains, namely, ash regenerate best in stands with greater ash projective cover in the canopy, although the number of specimens is comparatively small (2000 trees ha^{-1} in Skrīveri, 400 trees ha^{-1} in Bērvircava).
3. In stands where ash was the dominant species prior to ash dieback, intense shrub overgrowth has started, indicating that change of species is significantly influenced by dominant tree layer species which is typical of *F. excelsior* stands.
4. Ash stand destruction in both regions developed differently. Although ash is better maintained in Bērvircava, the overall state of GRF stands was recognized as worse there, and 19% of units do not comply with GRF criteria anymore.

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Natural Regeneration of Common Ash in Young Stands in Latvia

ILZE PUŠPURE*, ROBERTS MATISONS, MĀRIS LAIVIŅŠ, TĀLIS GAITNIEKS AND JURĢIS JANSONS

Latvian State Forest Research Institute (LSFRI) Silava, Rigas str. 111, Salaspils, Latvia, LV2169

** Corresponding author: ilze.puspure@silava.lv, tel. +371 29351372*

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Abstract

Due to the dieback caused by ascomycete *Hymenoscyphus fraxineus*, common ash (*Fraxinus excelsior* L.) regeneration currently occurs only naturally and is crucial for existence of the species. Hence, in this study, we assessed the success of the natural regeneration and health condition of common ash in 90 diverse young stands. Additionally, the age structure of ash advance growth (saplings and seedlings) was characterized in four plots, initially dominated by ash. Ash was abundant in the advance growth in the studied plots with the mean density of 4185 ± 401 trees ha^{-1}. Ash advance growth density and health condition decreased with increasing height and age. From the 7533 accounted regeneration ashes, 75% were considered as healthy, 15% damaged and 10% were already dead. Ash regeneration density was the highest and the degree of *H. fraxineus* damage was the lowest in young stands on drained mineral soils. The best ash health condition was found in the densest stands with increased number of advance growth and undergrowth individuals. The highest ash mortality (ca 20%) was found in pure young stands. In the young stands, which were previously formed by ash, regeneration density was relatively low (4319 ± 592 trees ha^{-1}), but the mortality intermediate (ca. 10% of all trees). In contrast, in the stands dominated by black alder and birch, the density of ash advance growth was higher – 7300 ± 6300 and 6933 ± 2711 trees ha^{-1}, respectively, but the number of dead ash was lower (ca. 5%). Ash appeared more susceptible to the disease in the dense and unmanaged stands, as the health condition of ash regeneration was positively related to the number of tendings. A significant correlations between diameter, age and height of ash was observed, yet the analysis of the dimension showed, that the ash regeneration after harvesting and/or dieback has been occurring at different rates.

Keywords: *Fraxinus excelsior*; advance growth; establishment; young stand; ash dieback; natural succession; hemiboreal forest zone

Introduction

Intensive dieback of common ash (*Fraxinus excelsior* L.) caused by the ascomycete fungus *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz, Hosoya, comb. nov. has been observed in Europe since the mid-1990s (Kowalski 2006). The Baltic countries were among the first regions, where the ash dieback was described (Bakys et al. 2009, Stener 2013); yet in Latvia, the disease was confirmed only in 2007 (Kenigsvalde et al. 2010). According to the national inventory conducted in 2009, one third of all ash forest in Latvia has been lost (Kenigsvalde et al. 2010). At present, common ash forms ca. 0.5% (14582 ha) of the total forest area, of which ca. 25% are young stands. Due to the rapid spread of the dieback (Kirisits and Freinschlag 2011, Pliūra et al. 2011), ash planting has been stopped as economically non-sustainable (Kirisits et al. 2011, Bakys 2013). Nevertheless, ash has been regenerating naturally in Latvia (Laiviņš and Mangale 2004) and all Europe (FRAXIGEN 2005, Dobrowolska et al. 2011). In the United Kingdom (Wardle 1961) and Sweden (Dobrowolska et al. 2011), ash has been considered as a pioneer species, while in Denmark it is considered as intermediate between the pioneer and permanent component of forest (Ahlberg 2014). In Central Europe, ash has been associated with invasive species (Wagner 1990, FRAXIGEN 2005), while in northern Europe the term "fraxinisation", which represents the successful self-regeneration of ash, has been used (FRAXIGEN 2005). Still, during the last 10 years, the situation has radically changed, e.g. in Sweden, ash is now a Red listed species (Gärdenfors 2010). Therefore, the assessment of the natural regeneration pathways of ash is necessary to improve the management of existing stands aiming to maintain existence and better health condition of the species by applying silvicultural activities.

In many European countries, the damaged ash stands are transformed every year and certain part of those territories is left for the natural regeneration, subjecting ash to competition with other species that causes stress. In rich and moist sites, the most common competitors to ash advance growth (saplings and seedlings) are the early successional or pioneer species such as grey alder (*Alnus incana* (L.) Moench), silver birch (*Betula pendula* Roth.) and, in some cases, common aspen (*Populus tremula* L.) (Lygis et al. 2014). Natural regeneration of ash differs amongst site with diverse soil types. Prior to the dieback in Latvia, the best regeneration of ash was observed on mineral and drained mineral soils, especially in stands dominated by ash (Sakss 1958, Laiviņš and Mangale 2004). Due to the different views on the *H. fraxineus* spread and aggressiveness in diverse growing conditions (Kirisits et al. 2011, Stener 2013, Bakys 2013, Bakys et al. 2013), it has been unclear, how the dieback of mature ashes affects its natural regeneration, spread and health condition. At present, there is a large uncertainty about the development of ash forests, as since the onset of the disease, only a few studies dealing with the regeneration of damaged stands have been conducted (Ahlberg 2014, Lygis et al. 2014). Still, the optimistic forecasts suggest that, after a certain period of time, ash should recover from the dieback (Pliūra et al. 2011). The aim of this study was to evaluate the density and health condition of different young stands of common ash in Latvia. We hypothesised that the intensity of the damage was higher in the denser stands on the drained soil types. We also assumed that the susceptibility to damage has been affected by the ash advance growth dimensions.

Material and methods

Study sites, sampling and measurements

Ash regeneration was studied in 90 stands (Figure 1) distributed across Latvia. In Latvia, ash occurs in the mixed forests together with other deciduous trees (e.g., aspen, birch, alder, spruce (*Picea abies* Karst.), etc.); pure stands are rare. Ash is distributed quite frequently, but mainly the stands occur in the central part of Latvia, where soils are fertile and the climate milder (Nikodemus et al. 2009). The climate in Latvia can be classified as transitional maritime to continental, the continentality increases eastwards. Accordingly, the territory can be divided into three regions: the western, central and eastern part of Latvia (Figure 1). In these regions, the mean temperature in January is ca. -1.8, -3.2 and -4.5 °C, but in July ca. 17.4, 18.2 and 17.9 °C, respectively. The mean precipitation in July in these regions is about 748, 619 and 665 mm, respectively.

The young stands of common ash, where in previous rotation ash formed ≥ 40% of standing volume, with the age of 5 to 40 years and the size ≥ 1 ha, were selected from the State Forest Service inventory database. All age groups (distinguished by the step of ten years) were presented by 19–25 plots (Table 1). Stands corresponded to four soils types, mostly dry mineral (46 plots) and drained mineral (26 plots). The studied sites have undergone up to four tending events (mostly once or twice).

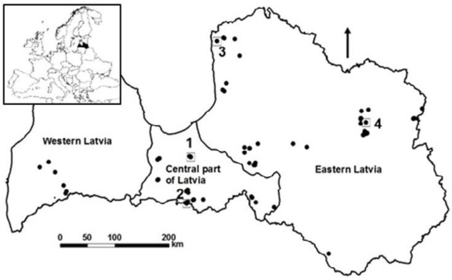

Figure 1. The location of study sites. Dots indicate the studied common ash regeneration plots. Squares denote the plots where stem discs have been collected for the estimates of age (1 – Plakanciems, 2 – Bauska, 3 – Ainaži, 4 – Lejasstradi)

Table 1. Classes of ash height, age and *Hymenoscyphus fraxineus* damage

Class	Age years	Height cm	Degree of ash damage%	Damage visual characteristics
I	< 10	< 50	0 - 10	Tree looks healthy or slightly damaged individual leaves.
II	11 - 20	50 - 100	11 - 25	Damaged several pages, some necrosis of the bark.
III	21 - 30	101 - 150	26 - 60	Fully damaged / dead separate branch; damaged part of the foliage; necrosis of the bark on large areas.
IV	31 - 40	151 - 200	61 - 99	Completely broken up dead part of the crown; partially damaged the entire crown; live separately branches in secondary crown.
V		201 - 250	100	Tree completely dead.
VI		251 - 300		
VII		> 300		

Within each young stand, one 2 × 100 m sampling plot was established along the longest diagonal of the forest district. Within each plot, all trees were accounted and their height was determined with the precision of 0.5 m (Table 1). All species were divided in two groups: advance growth containing all tree species, and undergrowth (shrub species). For each ash, degree of *H. fraxineus* damage was recorded according to five classes described in Pušpure et al. (2015) (Table 1).

To assess the relationship between ash height, diameter, age and the degree of damage, four of the studied young stands (two six-year old and two eight-year old), were selected (Figure 1, indicated by squares). In each stand, one 2 × 100 m sampling plot was established. In the sampling plots, all ashes were sampled, the cutting was done at the soil level and their height was measured with the precision of one cm. From each stem, a sample at its base (stem disc) was taken. The degree of *H. fraxineus* damage was recorded according to the five classes as described above. The number of felled trees per stand ranged from 108 to 160; at least 10 ashes in each height class were sampled. In the laboratory, stem discs were grinded and tree-rings were counted under a microscope.

Growth inventory in the study stands was conducted from mid-June to September 2015 when the damage of *H. fraxineus* was clearly visible and identifiable (Lygis et al. 2014). Ash samples were collected in August 2015. Dominant canopy species in previous rotation were determined according to the national inventory 2015; mostly they were ash (48% of all plots), ash with silver birch (14%) and grey alder (7%) admixture, spruce (8%) ect. The dominance of species in the advance growth was distinguished according to Simson (2006) (the dominant species comprises ≥ 50% of the total number of advance growth individuals and exceeds other species by 20%; the codominant species comprises 25–50% of all individuals). Soil types were distinguished according to data from the National State Forest Service inventory. Peat soils were considered if thickness of peat layer exceeds 30 cm.

Data analysis

To assess the effect of region and species composition on ash regeneration, generalized linear models (GLM) were applied. Differences in ash density according to soils and stand age (classes) were determined by the generalized linear mixed models (GLMM). The region (western, central and, eastern part of Latvia) as well as site was included in the models as the random factors. In both models, Gaussian distribution with "log" function was used. The models were based on the mean values for sampling plots. The GLMM method was also used to determine the factors (ash height and age, soil type, ash density, dominant species in advance growth, number of tending events) affecting ash health condition. For the tested factors, the central part of Latvia, aspen and dry mineral soils were chosen as the reference levels, to which other levels were compared. Such reference levels were chosen as the largest ash forests occur in the central part of Latvia, ash grows best on the mineral soils (Sakss 1958) and its health condition is the best in the stands with admixture of aspen. The significance of the GLMM was evaluated using the Likelihood ratio test (West et al. 2006). The relationships between ash density and height (classes from I to VI, Table 1), between health condition and height, and between the number of undergrowth and advance growth species were quantified by a bootstrapped (Johnson 2001) Pearson correlation analysis. The relationships between the ash diameter, age and height, were evaluated using a linear model. The differences in ash diameter, height and age between the sites and health classes were assessed by one-way ANOVA. The mean values of the gradation classes were compared using the Tukey HSD post-hoc test. The distributions of the dimensions of trees were compared by the chi-square test. All analyses were calculated at the significance level $\alpha = 0.05$ in the program R v. 3.1.2 (R Core Team 2014) using libraries "lme4" (Bates et al. 2014) and "lmerTest" (Kuznetsova et al. 2015).

Results

Species composition and ash regeneration

In total, 11 advance growth and 23 undergrowth species were accounted in the studied young stands, which had the mean density of 18410 ± 1040 trees ha^{-1}. The proportion of the advance growth and undergrowth individuals was 48.4 vs. 51.6. The undergrowth was dominated by two species – bird-cherry (*Padus avium* Mill.) (55% of total number of species, 5163 ± 638 shoots ha^{-1}) and hazel (*Corylus avellana* L.) (15%, 1399 ± 172 shoots ha^{-1}) (Figure 2). The advance growth density differed greatly and ranged from 1050 to 22900, with the mean value of 7150 ± 558 trees ha^{-1}. The highest advance growth density was observed for ash with 4185 ± 401 (ranging from 50 to 17750) trees ha^{-1} followed by grey alder (1620 ± 321 trees ha^{-1}), silver birch (681 ± 114 trees ha^{-1}) and aspen (687 ± 134 trees ha^{-1}) (Figure 2).

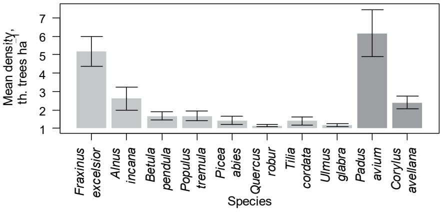

Figure 2. Mean density of the main understorey (advanced- and undergrowth) species

Ash advance growth density was similar amongst the regions (p-value = 0.06), age groups (p-value = 0.29), soil types (p-value = 0.58) and stands with different canopy species in the previous rotation (p-value = 0.06) (Figure 3a-d), yet some tendencies were observed. Ash density decreased with the increasing ash height and age. The density in the age group I was 5405 ± 951 trees ha^{-1}, while in the group IV 3036 ± 486 (Figure 3b). Although the correlation between ash advance growth density and ash height (up to three meters) was not significant (r = -0.08, p-value = 0.30), the highest density occurred in the height group I – 1857 trees ha^{-1}, but in the following groups, it decreased three time.

The highest number of ash was observed in the stands on the drained peat (mean 4584 ± 917 trees ha^{-1}), dry mineral (4275 ± 565 trees ha^{-1}) and drained mineral soils (4269 ± 846 trees ha^{-1}), but the lowest in the stands on wet mineral soil (1880 ± 331 trees ha^{-1}) (Figure 3c). The GLM analysis showed that the ash advance growth density was not significantly (p-value = 0.59) affect by the species composition. Yet the highest density of ash advance growth occurred in the stands where black alder (7300 ± 6 300 trees ha^{-1}) and birch (6933 ± 2711 trees ha^{-1}) were the main species, but the lowest (less than 2000 ash trees ha^{-1}) in stands dominated by lime and aspen (Figure 3d).

Incidence of Hymenoscyphus fraxineus in ash undergrowth

Of the 7533 accounted young ashes, 75% (5644 trees.) were considered as healthy, 15% (1134 trees) were damaged to varying degree, while 10% (755 trees) were dead. The degree of damage differed significantly (p-value < 0.001) among the regions (Figure 3a). The best ash health condition was observed in the central part of Latvia, where 78% of ash was healthy and 8% was dead, but the worst – in the western part of Latvia, where only 49% were healthy and 27% of ashes were already dead.

The degree of damage increased with age and height of ashes that was confirmed by the GLMM analysis (Figure 3b, 4). Significant correlation (r = 0.28, p-value < 0.001) was observed between disease intensity and height of ash. Up to 3 m height, 81% of ashes were healthy, but 4% were dead, while above the height of 3 m, these numbers were 54% and 33%, respectively. The age of young ash also had a significant (p-value < 0.001) effect on the occurrence of the disease. In the age group I, 81% of all ash trees were healthy, but in the group IV, it decreased to 58%, while the amount of dead trees was 3% and 24%, respectively.

The incidence of *H. fraxineus* damage differed significantly (p-value < 0.001) among the stands on different soils (Figure 3c). Similarly to ash density, health condition was the best in stands on the dry mineral and drained soils, e.g. 79% of ashes on drained mineral soils and 76% on dry

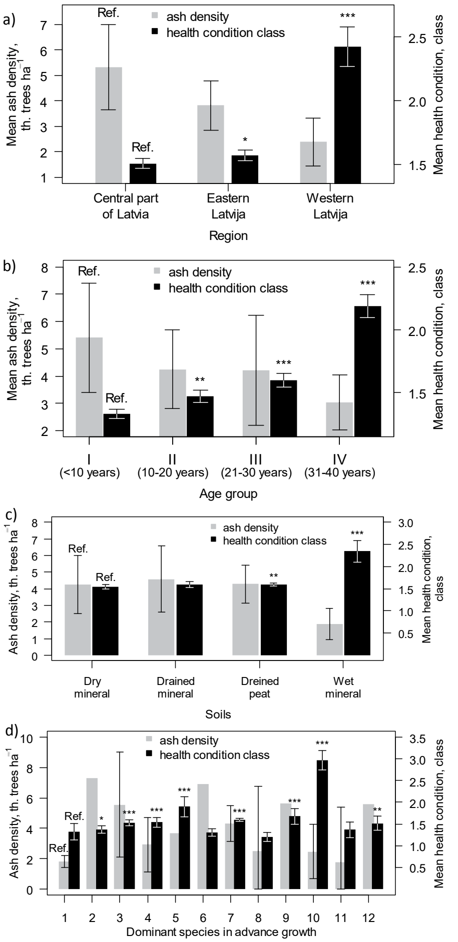

Figure 3. The mean density and health condition of common ash amongst the regions (a), age classes (b), soil type (c) and dominant species in advance growth (d) of Latvia. The asterisks indicate the differences from the central part of Latvia (a), youngest age class (b), dry mineral soil (c) and *Populus tremula* (d) used as the reference level (Ref.). Significance codes: * - p ≤ 0.05, ** < 0.01, *** - p ≤ 0.001. Dominant species in advance growth: 1 – *Populus tremula*, 2 – *Alnus glutinosa*, 3 – *A. incana* ,4 – *A. incana/F. excelsior*, 5 – *Acer platanoides*, 6 – *Betula pendula*, 7 – *F. excelsior,* 8 – *F. excelsior/B. pendula*, 9 – *Picea abies*, 10 – *P. abies/F. excelsior*, 11 – *Tilia cordata*, 12 - *T. cordata/F. excelsior*

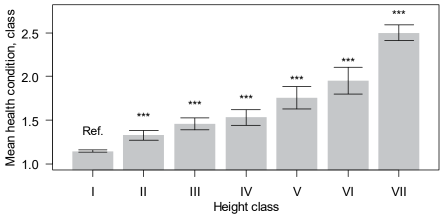

Figure 4. Ash health conditions in he studied young stands depending on tree height compared to the smallest height class, used as the reference level (Ref.). Significance code: * - $p \leq 0.05$, ** < 0.01, *** - $p \leq 0.001$

mineral soils had minimal or no symptoms. The highest degree of the damage of ashes was observed in stands on the wet mineral soils, where only 54% of trees were healthy and 27% were dead.

Although the relationship between ash density and ash health condition differed among sites, a significant (*p*-value < 0.001) negative logarithmic relationship between the mean health condition class and ash density was observed (Figure 5). Ash density was < 2000 tree ha^{-1} when the mean health class exceeded 4.3 (Figure 5). Analogically, negative correlations were observed between the ash density and the density of advance growth and undergrowth density, r = - 0.23 and - 0.24, respectively. The highest ash mortality was observed in the pure stands where 20% of ash was dead. In contrast, in stands where ash was in the admixture, its health condition was better and 82–95% of ash trees were healthy and only 1% was dead.

Health condition of ash significantly differed (*p*-value < 0.001) among the young stands with diverse dominant species (Figure 3d). The main species in the advance growth composition in the plots where ash (53% of the plots), grey alder (10%), grey alder/ash (9%) and spruce/ash (6%), yet the greatest *H. fraxineus* damage was observed in young stands formed by spruce/ash (40% of ash were dead), maple (*Acer platanoides* L.) (14%) and spruce (12%), but the lowest in ash/birch (87% of ash were healthy), birch (87%) and aspen (89%) young stand.

Health condition of the young stands was influenced by management. The intensity of ash dieback differed significantly (*p*-value < 0.001) among the stands with different number of tending events performed. The best ash health condition was in the young stands, which were tended four times, as the mean value of disease class score was 1.12, but it gradually increased with the decreasing number of tending events reaching 1.70 for untended stands. All differences among stands with different number of tending events were strictly significant (*p*-value < 0.001).

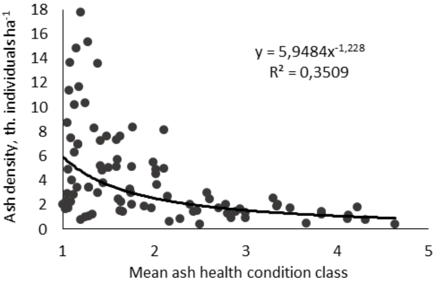

Figure 5. The relationship between the health condition and density of the common ash in the studied plots

Advance growth dimensions

The mean height of ash did not differ significantly (*p*-value = 0.57) among the studied four plots (regions), but the mean diameter (*p*-value = 0.001) and age of ash did (*p*-value < 0.001) (Table 2), although the mean age of ash in the first three height classes (up to 150 cm) was five years. A significant (*p*-value < 0.001) linear relationship between the diameter and height of ash was observed (Figure 6). The relationship between ash age and height was also significant (*p*-value < 0.001), still during the first 5–8 years, the height of ash increased irregularly and individually as rather high variability was present at each age (Figure 6).

Ash height distribution did not differ significantly between the sites (*p*-value = 0.57), but the diameter distribution differed only between Bauska and Lejasstradi sites (*p*-value = 0.02). In contrast, the age distribution was similar only in the same two sites (Figure 7). Ash height was significantly (*p*-value = 0.002) affected by the disease, but significant differences were observed only between the health classes I and II (*p*-value = 0.005).

Table 2. Ash measurement in stem discs collection plots

	Mean density, ash ha^{-1}	Young stand age, years	Mean age, years	Std. Error	Mean height, cm	Std. Error	Mean diameter, mm	Std. Error
Ainaži	4850	8	6.23	0.26	159.77	9.87	264.74	15.81
Bauska	5100	6	7.38	0.32	178.83	11.53	307.83	18.39
Lejasstradi	14850	6	7.23	0.35	166.02	10.32	211.63	13.97
Plakanciems	11650	8	3.77	0.22	175.30	9.50	266.60	16.19

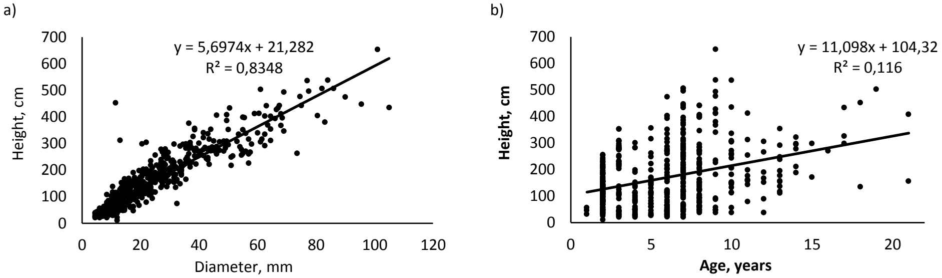

Figure 6. Relationship between the diameter and height (a), and age and height (b) of ash in the studied plots

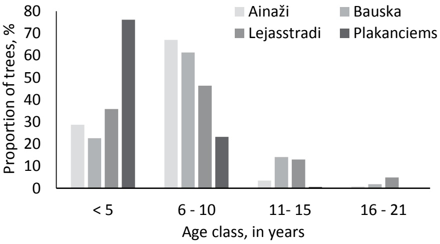

Figure 7. The age distribution of common ash in the understory of the studied plots

The best health condition was observed for the lowest trees. Similarly, ash diameter was affected by the disease (*p*-value < 0.001); smaller diameter trees were healthier. Ash health condition class differed significantly among the age classes (*p*-value < 0.001) and decreased with age.

Discussion

This study showed that the density of advance growth and undergrowth (18410 ± 1040 ashes ha^{-1}) was similar as observed before the decline (15 – 30 th. shoots ha^{-1}) (Sakss 1958), though growing conditions might have altered due to changing climate . The floristic composition of regeneration of ash stands, appeared little affected by the dieback as the same species have been observed before (Sakss 1958) and after (Figure 2; Lygis et al. 2014) the outbreak of the disease. Still, the proportions of regenerations species have been altered. In the studies stands, increased proportion of the undergrowth species was observed (Figure 2), suggesting ongoing changes in the stands. Although ash dieback can facilitate development of the undergrowth species, which consumes nutrients and alter light climate stressing ash (Keer 1998, Givnish 2002, Royo and Carson 2006, Skovsgaard et al. 2010), ash advance growth density was higher in stands with dense undergrowth (r = ca. -0.23). In the stands with the densest undergrowth (> 15 th. individuals ha^{-1}), ash advance growth also had the best health condition, likely due to higher species diversity.

Ash advance growth density (Table 2) was considerably higher than recently observed in the neighbouring Lithuania (Lygis et al. 2014) (4185 ± 401 vs. 599 trees ha^{-1}, respectively), suggesting regional differences in intensity of the dieback. Considering that the disease was spreading from south, better ash health condition in Latvia might be related to longer time available for the adaptation (Pliūra et al. 2011). Yet it was lower than before the outbreak of the disease in Europe, when more intense natural regeneration of ash (150000 young ashes ha^{-1}) occurred (Sakss 1958, Tabari and Lust 1999, Lygis et al. 2014). Nevertheless, Ahlberg (2014) suggested that in Denmark optimal ash advance growth density is 1500 individuals ha^{-1}, when the interspecific competition is the lowest. Ash advance growth density was inversely related to the disease intensity (Figure 5) that might be explained by the increased mortality and differences in the resistance of young ash. Similar relationships due to rapid development of the disease was observed by Enderle et al. (2013).

Ash density decreased with increasing age and height (Figures 3b, 4) as the density of the stands younger than 10 years was ca. 5000 trees ha^{-1}, but at the age of 31–40 years, it was only up to 56% of that density, thus following the reverse-J shape distribution of the natural regeneration. Normally, ca. 40–50% of the recruiting young ashes die annually, but under intensive disturbance, e.g. dieback, mortality can reach up to 85% (Harmer et al. 2005). Ash is a gap specialist, which is shade-tolerant at the sapling phase, but is light-demanding when reaches canopy (Petriţan at al. 2009, Kerr and Cahalan 2004), hence insufficient light conditions decreases its competitiveness with other species (Niemelä et al. 1992, Guzman and Dirzo 2001) and resistance against pathogens (Bakys at al. 2013) thus increasing mortality. Probably, the density of young stands was also decreased by the dieback, as a considerable part of seedlings might be weakened and outcompeted by the herbaceous vegetation (Wardle 1961, de la Cretaz and Kelty 2002), especially in fertile sites (Dobrowolska et al. 2011). Still, the amount of dead seedlings (10% of total number) was lower compared to Lithuania (Lygis et al. 2014), supporting regional differences in health condition of ash. Skovgaard et al. (2010) showed that in a planted stand, small- and medium-sized trees were more susceptible to the disease. However, in this study in the naturally regenerating stands, the opposite was observed as the smaller ashes (height ca. 160 ± 50 cm and diameter ca. 23 ± 7 mm, which comprised 39% of all measured) were the most healthy (Figure 4), but the largest ashes (H > 240 cm, D > 45 mm which comprised 20% of all measured) were the most damaged, suggesting age-related increase in susceptibility to the disease.

Although diverse opinions about the effect of site type on the susceptibility to *H. fraxineus* damage persist in Europe (Bakys et al. 2013), in Latvia, higher susceptibility of ash was observed in the wet sites, as previously shown by Gross et al (2014). Ash is susceptible to prolonged waterlogging (Wardle 1961), hence the most abundant ash regeneration with the best health condition was observed in stands growing in well-drained and dry mineral soils (Figure 3c). The positive effects of drainage system on ash health condition has been emphasized in Denmark (Ahlberg 2014), Germany (Schumacher 2011) and other countries (Dobrowolska et al. 2011) as in the over-moist sites, trees have been more stressed, hence less resistant to disease.

Ash regeneration (mean 8064 trees ha^{-1}) and health condition (82–95% of ash trees were healthy) was better in the mixed stands. In Central Europe, establishment and development of ash seedlings is influenced by the canopy species composition (Götmark et al. 2005). Similarly, lower degree of damaged ash and better increments have been observed in mixed rather than pure stands (Dobrowolska et al. 2011, Schumacher 2011, Stener 2013). The lowest ash sampling mortality was observed in stands where certain satellite species occured (Givnish 2002). Likewise, in this study, the most abundant ash regeneration with the best health condition, was observed in stands formed by black alder and birch 7300 ± 6300 and 6933 ± 2711 trees ha^{-1}, respectively (Figure 3d), as demonstrated previously (FRAXIGEN 2005, Dobrowolska et al. 2011, Ahlberg 2014). Although ash is considered to have the lowest regeneration in sites with acidic humus layer (Tabari et al. 1999, Dufour and Piegay 2008), we found rather high regeneration density also in stands formed by spruce (5650 ± 2650 trees ha^{-1}). This might be related to decreased competition with other broadleaved species likely due to poor light conditions. Yet, ash health condition was considerably lower compared to broadleaved stands (Figure 3d) likely due to stress caused by root competition between ash and spruce (Lei et al. 2012). Although in mixed stands young ash has rapid development (Le Goff and Ottorino 1996, Keer 2004) thus outcompeting others (Rysavy and Roloff 1994, Dobrowolska et al. 2011), the disease might severely decrease its competitiveness. Hence, decreased ash health condition was observed in stands with maple admixture (Figure 3d) pointing to increased competition, as both species have similar growth strategies (Petritan et al. 2009), but the competitiveness of ash (Urbinati and Cillia 1995) has been weakened. In stands where ash was the canopy species, its regeneration density was lower (4319 ± 592 ash trees ha^{-1}) likely due to intraspecific competition.

High site-specificity of the increments of ash has been observed, as ash dimensions had a wide range within each of the four studied sites (Table 2), suggesting the plasticity of the species. The management of young stands had an effect not only on the height and diameter, but also on the health condition of ash advance growth. In the stands that have undergone several tendings, young ashes had a higher stem diameter and best health condition (Table 2, Figure 5), as the highest susceptible to disease has been observed in dense and unmanaged stands (Cech and Hoyer-Tomiczek 2007, Skovsgaard et al. 2010, Bakys et al. 2013). After thinning, the competition amongst ashes is decreased, thus minimizing biotic (competition) and abiotic (increased moisture) stresses (Niemelä et al. 1992, Guzman and Dirzo 2001). Hence, thinning might be recommended as one of the means to improve ash condition (Guzman and Dirzo 2001, Niemelä et al. 1992, FRAXIGEN 2005) also in Latvia. Though, excessive tending can also promote the disease (Bakys et al. 2013).

Conclusions

Our study showed that after 15 years since the initiation of the ash dieback, natural regeneration has been taking place in sufficient quantities. The floristic composition of advance growth and undergrowth species in the declining ash stands have remained similar with pre-dieback stands, yet the proportion of undergrowth species has increased, apparently altering the succession. It is expected that ash regeneration would continue on dry or drained sites, where the species was more abundant and their health condition was the best. Still, at present, 75% of the studied young ash trees are healthy, but mostly they are two to six years old, hence further monitoring is necessary. Considering current health condition and regeneration density, ash will apparently could remain as an admixture species in rich sites.

Acknowledgements

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Health Condition of European Ash in Young Stands of Diverse Composition

ILZE MATISONE*, ROBERTS MATISONS AND ĀRIS JANSONS
Latvian State Forest Research Institute ‘Silava’, Riga str. 111, Salaspils, Latvia, LV2169
** Corresponding author, e-mail: ilze.matisone@silava.lv, tel.: +371 29351372*

Matisone, I., Matisons, R. and Jansons, Ā. 2019. Health Condition of European Ash in Young Stands of Diverse Composition. *Baltic Forestry* 25(1): 59–62.

Abstract

During the recent decades, the ascomycete *Hymenoscyphus fraxineus* has been spreading across Europe causing dieback of European ash (*Fraxinus excelsior*) and threatening the existence of the species. Still, several studies have suggested positive effect of stand diversity on ash health condition. The aim of this study was to evaluate the effect of stand composition and structure on the health condition of ash in young stands in Latvia. Among the tested stand properties, number of shrub species and tree height were the main factors affecting ash health condition, yet their effect differed regionally. In the eastern part of Latvia, richness of shrub species in forest stands showed positive relationship with ash health, while the taller trees displayed worsening health condition. In the western part of Latvia, the relationships were inverse. Such differences in the biotic relationships might be related to the differences in climate, hence spread and development of the pathogen, and/or genetically determined susceptibility of different populations of ash.

Keywords: *Fraxinus excelsior*; *Hymenoscyphus fraxineus,* species diversity; dieback; mixed stands; populations

Introduction

Since the 1990s, dieback of European ash (*Fraxinus excelsior* L.), caused by the ascomycete *Hymenoscyphus fraxineus* (T. Kowalski), has been marching across Europe, devastating most of the stands and threatening the existence of the species (Pautasso et al. 2013, Gross et al. 2014). Accordingly, numerous efforts have been made to understand the mechanisms of the dieback and to improve the resistance of ash stands (Skovsgaard et al. 2017). The fungus affects trees, irrespectively of size and age, subjecting them to infestation by the secondary agents and decreasing their lifespan severely (Bakys et al. 2009, Schumacher et al. 2010); still, some studies have shown that ash has suffered less damage in mixed, compared to pure stands (Dobrowolska et al. 2011, Schumacher 2011, Stener 2013, Pušpure et al. 2017). Higher resilience of European ash in the mixed stands has been related to chemical, physical, and biological barriers limiting the spread of the primary and secondary pathogen(s) (Loreau et al. 2001, Jactel et al. 2005, Pautasso et al. 2005, Kosawang et al. 2018). Additionally, some population-related differences in the susceptibility of European ash to the pathogen suggest genetic aspect of the resistance (Pliūra et al. 2015). They, however, are modulated by weather conditions (Papic et al. 2018), hence by the dynamics of development of the pathogen (Hietala et al. 2013, Dvorak et al. 2016), thus adding complexity to the species-host interactions.

The aim of this study was to assess the effect of composition and structure of young stands on the health condition of European ash in the hemiboreal conditions in Latvia. We hypothesised that the health condition of ash was related to species richness of woody plants within a stand, yet the effect might differ regionally (between metapopulations, or due to the climate).

Material and Methods

Study sites and measurements

In total, 35 young stands (26–36 years old), where European ash formed >30% of standing volume and had area ≥1 ha, were selected from the national forest inventory database. The stands were scattered across the western and eastern part of Latvia and represented two local provenance regions (Hewitt 1999, Jansons and Baumanis 2005; Figure 1), differing by the tree growth responses to the environment. The stands were growing in hemiboreal lowland conditions (altitude <250 m a.s.l.) on mesotrophic silty soils (*Oxalidosa* type); the topography of the sites was flat. The climate was temperate, yet it was milder in the western part of Latvia. The mean annual temperature (± standard deviation) in the western and eastern part of Latvia was 7.8±1.8 and 6.2±1.5 °C, respectively. In the western part of Latvia, the mean monthly temperature ranged from 2.1±2.6 to 18.1±0.4 °C, and, in the eastern part of Latvia, from 3.8±2.2

to 16.8±0.5°C in January and July, respectively. The mean annual precipitation in the western and eastern part of Latvia was 690±78 and 740±71 mm, respectively.

In each stand, a 2×100 m sampling plot was established along the longest diagonal of forest patch, and height of all trees within it was measured with the 0.2 m precision. For each ash tree, the degree (percentage) of *H. fraxineus* damage was recorded according to five grades, where “1” represented trees with slight or no damage, and “5” represented recently dead trees (Pušpure et al. 2015). The survey was made by the same person in August 2017.

Data Analysis

The relationships of ash health condition with the structure and composition of stands were assessed by the mixed ordinal regression. Tree was considered as observation and sampling plots were used as random intercepts. In the models, 1) height of trees, 2) mean height of trees in a plot, 3) number of tree, 4) shrub species in a plot, 5) forest type, 6) region (the western or eastern part of Latvia; Figure 1), 7) proportion of ash within a plot, 8) stand density, 9) dominant species of a plot, 10) species diversity in a plot (Shannon diversity index), and their combinations were tested as the linear predictors of ash health conditions. Considering that development of the pathogen is affected by climatic factors (Hietala et al. 2013, Dvorak et al. 2016), mean air temperature, precipitation sum and standardized precipitation-evapotranspiration index (Vicente-Serrano et al. 2010) for the July–August and May–August periods (Dvorak et al. 2016) in 2017 were also tested. Gridded climatic data were used (Harris et al. 2014). The best combination of predictors was determined by manual stepwise-forward selection; up to seven factors were tested simultaneously. The performance of the models was assessed by the Akaike information criterion (AIC) and conformity with biological realism; the significant predictors were tested for multicollinearity (Fox and Weisberg 2011). The distribution of tree and shrub species between the western and eastern part of Latvia was compared by the χ^2 test. Data analysis was conducted in R v. 3.5.1 (R Core Team 2018), using packages ‘ordinal’ (Christensen 2018) and ‘car’ (Fox and Weisberg 2011).

Results

The studied stands were formed by 7–15 tree and shrub species. The most common tree species, besides European ash, were *Alnus glutinosa*, *A. incana*, *Corylus avellana*, *Padus avium*, *Picea abies*, *Tilia cordata*, and *Betula* spp. The distribution of the most common species significantly (p-value < 0.01) differed between the western and eastern part of Latvia; the abundance of *A. glutinosa*, *C. avellana*, *P. abies*, and *T. cordata* was higher in the eastern, while the abundance of *A. incana*, *P. avium*, as well as European ash was higher in the western part of Latvia. The density of the studied stands ranged from 4,400 to 74,900 trees ha^{-1}, and ash formed 40–100% of all trees. The mean height of all trees in the studied stands ranged from 1.07 to 4.73 m, yet the mean height of ash trees ranged from 0.45 to 7.54 m; trees were ca. 65% higher in the eastern part of Latvia. The mean health grade of ash ranged from 1.0 to 4.1 indicating diverse levels of dieback among the stands, yet the mean health grade was higher (worse health condition) in the eastern than in the western part of Latvia, 2.4 and 1.7, respectively.

Among 156 combinations of the factors tested, the combination of tree height and number of shrub species in a stand were the best predictors of ash health condition, yet both their effects were highly significantly (p-value < 0.01) interacted by the provenance regions (Table 1). The next best model displayed notably weaker performance (AIC was 5201 vs. 4727 of the best model). Although the regions differ by climate, the tested climatic factors were excluded during the selection process, likely as in 2017, the differences between the regions were small (e.g. 0.3 °C for July-August temperature). In the stands in the western part of Latvia (Figure 1), the number of shrub species was positively related to the health grade of European ash (Figure 2A), indicating worse health condition in more diverse stands. The opposite was observed in the eastern part of Latvia, and the effect of shrub species richness there was even stronger. Nevertheless, tree height showed positive relationship with the health grade of ash, particularly in the eastern part of Latvia, implying that health condition was worse for the larger trees (Figure 2B). Only slight effect (the regression line was almost horizontal) of tree height was observed in the western part of Latvia, where the mean tree height was lower. Some models, in which the effect of the dominant species of stand showed a p-value of ca. 0.06,

Table 1. Description of the best mixed ordinal regression model predicting health condition of European ash in young mixed stands based on stand and tree properties

Fixed effects estimates			
	Estimate	Std. error	*z*-value
Number of shrub species	-0.68	0.32	-2.12
Region (western)	-5.76	1.78	-3.23
Tree height	0.17	0.02	6.68
Number of shrub species × region (western)	1.09	0.38	2.89
Region (western) × tree height	0.09	0.03	3.07
Threshold coefficients			
Health grade	Estimate	Std. error	*z*-value
1 vs. 2	-2.38	1.40	-1.71
2 vs. 3	-0.11	1.39	-0.08
3 vs. 4	0.41	1.39	0.29
4 vs. 5	0.68	1.39	0.49
Random effects			
	Variance	Std. error	
Stand (intercept)	1.321	1.149	
Model analysis of deviance table, type II test			
	Likelihood ratio, χ^2	Degree of freedom	*p*-value
Number of shrub species	0.14	1	0.71
Region	0.48	1	0.49
Tree height	245.04	1	< 0.001
Number of shrub species × region	9.12	1	<0.01
Region × tree height	9.43	1	<0.01

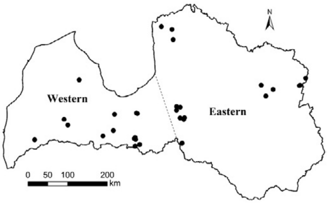

Figure 1. Location of studied stands of European ash. Broken line denotes two parts of Latvia (western and eastern) with differing tree growth (provenance regions)

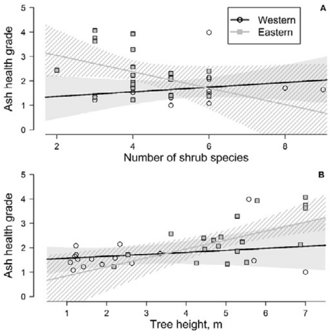

Figure 2. The relationship between health condition (health grade) of European ash in young mixed stands and number of shrub species (A), and tree height (B) in the western and eastern parts (provenance regions) of Latvia. The polygons (envelopes) denote confidence intervals

suggested that the presence of *P. abies* tended to worsen health of ash (not shown).

Discussion

The richness of shrub (woody) species in stand had effect on health condition of European ash in young stands (Figure 2A), as observed previously (Dobrowolska et al. 2011, Schumacher 2011, Stener 2013, Skovsgaard et al. 2017). The richness of shrub species can be related to fertility, as well as history, hence structural diversity of stands (Fescenko et al. 2014). The explicit regional differences in the effect of shrub species richness on ash health condition (Table 1, Figure 2) might be related to the climatic conditions, as suggested by Papic et al. (2018). In the western part of Latvia, warmer climate, apparently, facilitated development of *H. fraxineus* (Kowalski and Bartnik 2010, Dvorak et al. 2016) in longer term, suppressing growth of ash, as hinted by shorter tree height, and marginal effect of tree height on ash health grade (Figure 2B). Under such conditions, the admixture species, apparently, competed with ash decreasing its vigour, thus explaining positive linkage with the health grade (Figure 2A).

Under cooler climate in the eastern part of Latvia, which is less favourable for *H. fraxineus* (Kowalski and Bartnik 2010), the diversity of shrub species had positive effect on the health condition of ash (Figure 2A), likely acting as mechanical barriers (in mixed stand, ash tress are scattered and the composition of debris is diverse), hindering spread and development of the inoculum of the pathogen (Jactel et al. 2005, Pautasso et al. 2005). Higher species richness also can provide more habitats for antagonists of the disease (Kosawang et al. 2018), acting as biological barriers. Although ashes suffered more damage, they were also taller, indicating better growing conditions under more continental climate, as previously shown by Papic et al. (2018). This suggests, that higher diversity of woody species in a stand might be applied for the improvement of survival of ash as previously suggested by Givnish (2002), at least in the eastern part (provenance region) of Latvia. Unfortunately, considering the numbers of sampled stands and admixture species, it was impossible to determine, which shrub species had the strongest effect on ash health condition. Still, the presence of *P. abies* in stand tended to worsen ash health condition (not shown), likely due to the competition for light, soil, as well as soil acidification (Lei et al. 2012). Also, the understory under *P.abies* is poor, providing weak barriers for the spread of the disease (Pautasso et al. 2005).

Alternatively, regional differences in the relationships of health condition of ash with stand diversity and tree height might be related to the two populations of European ash that colonised post-glacial Eastern Europe (Hewitt 1999), hence genetic components of the resistance to dieback is appeared (Pliūra et al. 2015). Diverse stand composition might be mentioned as another cause of regional differences in health grade. Still, the positive relationship between height and health grade of ash (Figure 2B) suggested cumulative effect of the pathogen as the trees grow.

Conclusion

The hypothesis was verified only partially, as linkage between the richness of shrub species, growth, and health status of ash was observed. The observed relationships suggested that management for higher diversity might be applied to decrease the effects of dieback under more continual climate in the eastern part of Latvia. The negative effect of richness of woody species under milder and warmer climate in the western part of Latvia suggested that decrease of ash health conditions is still expectable due to the warming. Accordingly, efforts to preserve ash stands should be primarily focussed in the areas with more continental climate.

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Latvijas Valsts mežzinātnes institūts “Silava”
Rīgas iela 111, Salaspils, Salaspils novads
Tālr.: 67942555
E-pasts: inst@silava.lv
www.silava.lv

Vāka noformējumam izmantotās fotogrāfijas autors: Ilze Matisone
Maketa izstrāde: Ilva Konstantinova

Iespiests: SIA “Latgales druka”
Baznīcas iela 28, Rēzekne
Tālr./fakss: 64625938