Patterns and Severity of Crown Dieback in Young Even-Aged Stands of European Ash (*Fraxinus excelsior* L.) in Relation to Stand Density, Bud Flushing Phenotype, and Season

Remigijus BAKYS[1], Rimvydas VASAITIS[1] and Jens Peter SKOVSGAARD[2]

[1]Department of Forest Mycology and Plant Pathology, Uppsala BioCenter and [2]Southern Swedish Forest Research Centre, Swedish University of Agricultural Sciences, Alnarp, Sweden

Abstract

Bakys R., Vasaitis R., Skovsgaard J.P. (2013): **Patterns and severity of crown dieback in young even-aged stands of european ash** (*Fraxinus excelsior* L.) **in relation to stand density, bud flushing phenotype, and season**. Plant Protect Sci., **49**: 120–126.

The extent and temporal pattern of crown damage (attributed to *Hymenoscyphus pseudoalbidus*) in even-aged stands of *Fraxinus excelsior* in relation to bud flushing phenotype, stand density, and season was investigated. Data were collected in 2007 in four statistically designed thinning experiments located in 12–15-years old plantations of ash in Denmark. The study included 21 plots of four contrasting, residual stand densities: (1) 1700–5500 trees/ha (unthinned control plots), (2) 1500 trees/ha, (3) 500 trees/ha, and (4) 100–150 trees/ha. Assessments included estimation of flushing phenotype in May, followed by evaluation of severity of crown damage (percentage of crown killed) in June and September. Simultaneously, for each tree, the presence or absence of crown wilt and dead tops were recorded. The seasonal pattern of disease severity (average crown damage) was similar in all stands, and disregarding stand density the extent of tree crown damage increased significantly towards the end of the growing season ($P < 0.005$). Disease severity was the worst in unthinned plots, but otherwise unrelated to stand density. Late-flushing trees were most severely affected ($P < 0.001$). The observed patterns of disease severity are probably associated with ecological features of the pathogen that still remain largely unknown.

Keywords: *Hymenoscyphus pseudoalbidus*; crown damage; fungal pathogen; phenology of trees

The dieback of European ash (*Fraxinus excelsior* L.) began in the mid-1990s in East and Central Europe and since then it has spread throughout almost whole Europe (Bakys *et al.* 2009). The disease has resulted in large-scale dieback of *F. excelsior* in most of its distribution range, and currently the disease is reported to occur in more than 20 countries (Timmermann *et al.* 2011). Other ash species in Europe are also susceptible to the dieback, including *F. angustifolia* Vahl (Schumacher *et al.* 2007; Kirisits *et al.* 2010), *F. ornus* L. (Kirisits *et al.* 2009), and several exotic species (Drenkhan & Hanso 2010). Infected trees show wilt of leaves, necroses and cankers on shoots, branches and stems, followed by gradual dieback of crowns (Kowalski 2006; Schumacher *et al.* 2010; Skovsgaard *et al.* 2010). The involvement of the hyper-virulent fungal pathogen *Hymenoscyphus pseudoalbidus* V. Queloz, C.R. Grünig, R. Berndt, T. Kowalski, T.N. Sieber & O. Holdenrieder (Queloz *et al.* 2010), whose anamorphic stage is known as *Chalara fraxinea* T. Kowalski (Kowalski 2006), has been explicitly confirmed to be the primary agent of ash dieback (Bakys *et al.* 2009; Kowalski & Holdenrieder 2009).

In Denmark, ash dieback was first observed in 2002 (Skovsgaard 2002, personal notes), but remained incorrectly diagnosed (Thomsen 2005; Thomsen & Skovsgaard 2006) until 2007 (Thomsen *et al.* 2007). Currently, the disease affects populations of *F. excelsior* all over the country (Skovsgaard *et al.* 2010).

Numerous field surveys on ash dieback have been conducted in various parts of Europe, yet information on the relationship between the phenology of *F. excelsior* and its susceptibility to the disease is scarce. Moreover, local ecological conditions of a forest site that may influence disease development (e.g. stand density) remain largely unknown. For example, in Denmark ash dieback symptoms were found to be more severe on trees of average or below-average size (Skovsgaard *et al.* 2010). In Austria, *F. excelsior* growing in dense stands was found more susceptible to the disease, probably due to water deficiency (Cech & Hoyer-Tomiczek 2007). Following drought during April and early May, i.e. prior to flushing, *F. excelsior* in Denmark was less susceptible on a well-drained site than on a moist site with high, but retreating levels of ground water (Skovsgaard 2008). These findings illustrate that environmental factors possibly predispose ash trees to the disease, or, vice versa, favour the dispersal and infections of *H. pseudoalbidus*.

The objectives of our study were: (1) to assess the relationship between stand density and dieback severity, (2) to investigate the susceptibility of *F. excelsior* trees to dieback in relation to their bud flushing (budburst) phenotype, and (3) to compare relative disease severity in stands at the beginning and the end of the growing season.

MATERIAL AND METHODS

Experimental stands.The four experimental stands used for this study are located along the east coast of Jutland, Denmark (Figure 1): experiment No. 1423 in Haderslev Vesterskov (on former farmland), No. 1424 in Sebberup Skov (on former farmland), No. 1535 at Visborggaard (ancient forest land), and No. 1425 in Sæbygaard Skov (ancient forest land). The stands were planted in 1992–1995
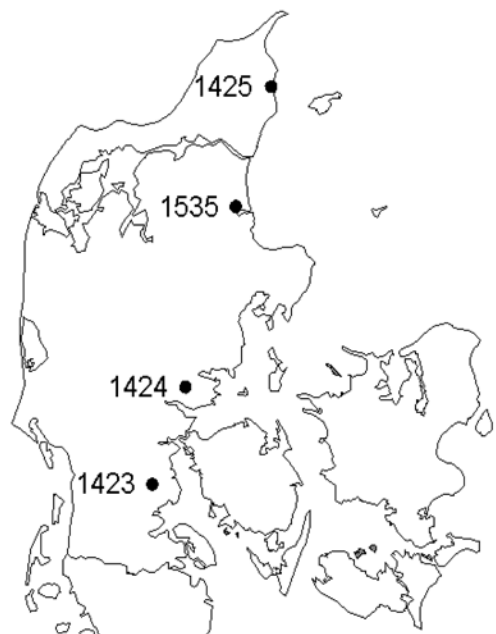

Figure 1. Location of the four *F. excelsior* thinning experiments in Denmark

with two- to four-year-old saplings of *F. excelsior*. All experimental sites consisted of: (1) 5, 5, 4, and 7 plots and (2) 908, 770, 780, and 722 trees, respectively (Table 1). During 2005–2007 experimental plots were installed and thinned to four contrasting stem densities: (1) 1700–5500 trees/ha (unthinned control plots, no thinning, no removal of dead trees), (2) 1500 trees/ha, (3) 500 trees/ha, and (4) 100–150 trees/ha. Thinning was primarily from below and only few trees were thinned based on phyto-sanitary considerations.

In all stands ash dieback has been observed at least since 2006, and *H. pseudoalbidus* was consistently isolated from symptomatic shoots (Johansson *et al.* 2009b). A total of 3180 trees were evaluated for crown dieback: 1945 trees in seven unthinned plots of 1700–5500 trees/ha, 777 trees in six plots with a residual stem density of 1500 trees/ha, 397 trees in six plots of 500 trees/ha, and 61 trees in two plots

Table 1. Characteristics of four experimental stands of declining *F. excelsior*

Stand density (trees/ha)	No. of plots (trees) in experimental stands			
	1423	1424	1535	1425
1700–5500	1 (682)	2 (558)	1 (247)	3 (458)
1500	2 (132)	1 (107)	1 (357)	2 (181)
500	2 (94)	1 (81)	1 (139)	2 (83)
100–150	–	1 (24)	1 (37)	–
Total	5 (908)	5 (770)	4 (780)	7 (722)

of 100–150 trees/ha. Within each plot, each tree was individually numbered to allow monitoring of their conditions on an individual basis.

Evaluation of tree flushing and dieback. The evaluation of timing of bud flushing was carried out during May 14–17, 2007. The state of flushing was rated for the whole crown, based on the most advanced state and using a categorical scale of eight degrees (0–7): (0) buds in winter state (no bud stretching or flushing visible), (1) buds stretching, (2) leaves emerging on 1–50% of buds, (3) leaves emerging on 51–100% of buds, (4) leaves unfolding and petiole visible on 1–50% of buds, (5) leaves unfolding and petiole visible on 51–100% of buds, (6) leaves completely unfolded on 1–50% of buds, (7) leaves completely unfolded on 51–100% of buds**.**

Subsequently, all trees were monitored twice to check for incidence and severity of dieback symptoms (during June 5–10 and September 17–21, 2007). Three dieback-related symptoms (two of which were also used by Skovsgaard *et al.* 2010) were evaluated for each tree: (1) crown damage rate, i.e. percentage of the dead part of a crown, (2) presence or absence of dead tops in the crown (dieback of the upper part of the crown), (3) presence or absence of wilting foliage in the crown. The crown damage rate was scored visually at intervals of 10%, while evaluating the percentage of dead crown. Trees with only epicormic shoots were considered to be dying and were scored as 100% damaged. Wilt of foliage was recorded, whenever dark discolouration or premature shedding of leafs was spotted in the primary crown or on epicormic shoots.

Statistical analysis. The differences in crown damage among plots of different stem densities, the relationship between flushing phenology and crown damage, and the seasonal change in crown damage rate were estimated using One-Way Analysis of Variance (ANOVA) with a confidence level of 95% and multiple comparisons between all pairs of means using Tukey's test. Chi-square analyses were used to assess the significance of differences between presence of the symptoms of wilt and dead tops among trees in relation to stand density and flushing phenotype and to assess the seasonal impact on the presence of dead tops and foliar wilting. All calculations were carried out using MinitabTM (Minitab Inc., State College, USA) statistical software package.

RESULTS

The symptoms of dieback were evenly distributed throughout all examined stands, without any apparent disease centres or clusters of apparently healthy trees.

The highest rate of crown damage (30.1% in June, 53.4% in September) was observed in plots of high stem density (unthinned control plots) followed by those of lower stem density (Table 2). The average crown damage in the unthinned plots was significantly greater than that in the 1500 trees/ha and 500 trees/ha plots in both June and September ($P < 0.001$). Differences in crown damage rate between any other density classes were not significant, either in June or in September ($P \geq 0.07$).

The highest proportion of trees with wilt was found in low density plots (Table 2). The seasonal increase in wilt symptoms was consistent across density classes and independent of stand density ($P < 0.005$). In June the incidence of wilt was

Table 2. Analysis of phenotypic damage symptoms on *F. excelsior* for four different stand density classes

| Stand density (trees/ha) | Number of trees | Percentage of trees with symptoms | | | | | |
| | | wilt* | | dead tops* | | average crown damage** | |
		June	September	June	September	June	September
1700–5500	1945	20.4[a]	58.0[a]	42.4[a]	49.2[a]	30.1[a] ± 0.62[a]	53.4[a] ± 0.72[a]
1500	777	21.5[ab]	50.7[a]	40.9[a]	46.8[a]	24.7[b] ± 0.88[b]	45.9[b] ± 1.25[b]
500	397	29.0[b]	47.1[a]	43.8[a]	46.1[a]	23.1[b] ± 0.98[b]	39.4[b] ± 1.53[b]
100–150	61	37.7[b]	63.9[a]	49.2[a]	54.1[a]	28.4[ab] ± 2.31[ab]	46.2[ab] ± 3.59[ab]
Total/Mean	3180	21.1	54.9	42.3	48.3	27.8 ± 0.46	49.7 ± 0.58

*means in the column followed by the same letter are not significantly different according to the χ^2 test at $P < 0.05$; **values in the column followed by the same letter are not significantly different by Tukey's test at $P < 0.05$; data for crown damage are averages ± SE

Table 3. Phenotypic damage symptoms of *F. excelsior* depending on state of bud flushing

Bud flushing state	Number of trees	Percentage of trees with symptoms					
		wilt*		dead tops*		average crown damage**	
		June	September	June	September	June	September
0	188	17.6[ab]	65.4[a]	67.6[a]	67.6[a]	55.3 ± 2.37[a]	70.2 ± 2.19[a]
1	451	14.8[b]	42.8[a]	30.4[bc]	42.8[b]	22.8 ± 1.07[be]	36.4 ± 1.35[b]
2	673	21.4[ab]	53.5[ab]	43.7[b]	49.0[a]	29.0 ± 1.01[dc]	48.5 ± 1.25[c]
3	394	19.0[ab]	40.1[b]	27.2[c]	33.8[b]	19.5 ± 1.06[e]	39.0 ± 1.58[b]
4	845	27.0[a]	64.4[a]	51.1[ab]	57.3[a]	31.6 ± 0.86[d]	56.6 ± 1.05[d]
5	573	25.3[a]	58.8[a]	39.9[bc]	49.4[a]	26.1 ± 0.95[bc]	52.3 ± 1.35[cd]
6	56	28.6[ab]	58.9[ab]	39.3[abc]	58.9[ab]	24.2 ± 3.2[bcde]	53.4 ± 4.51[cd]

*means in the column followed by the same letter are not significantly different according to the chi-squared test at $P < 0.05$;
**values in the column followed by the same letter are not significantly different by Tukey's test at $P < 0.05$; data for crown damage are averages ± SE

significantly lower in the unthinned plots than in those of 500 trees/ha and 100–150 trees/ha ($P \leq 0.002$). Otherwise, no statistically significant differences were found between classes of stand density in relation to the presence of wilt.

The highest proportion of trees with dead tops was found in low density plots (Table 2). The num-ber of trees with dead tops increased significantly in the growing season in unthinned plots and in plots of 1500 trees/ha ($P < 0.05$), whereas no such development in dieback was observed in plots of 500 trees/ha or 100 trees/ha.

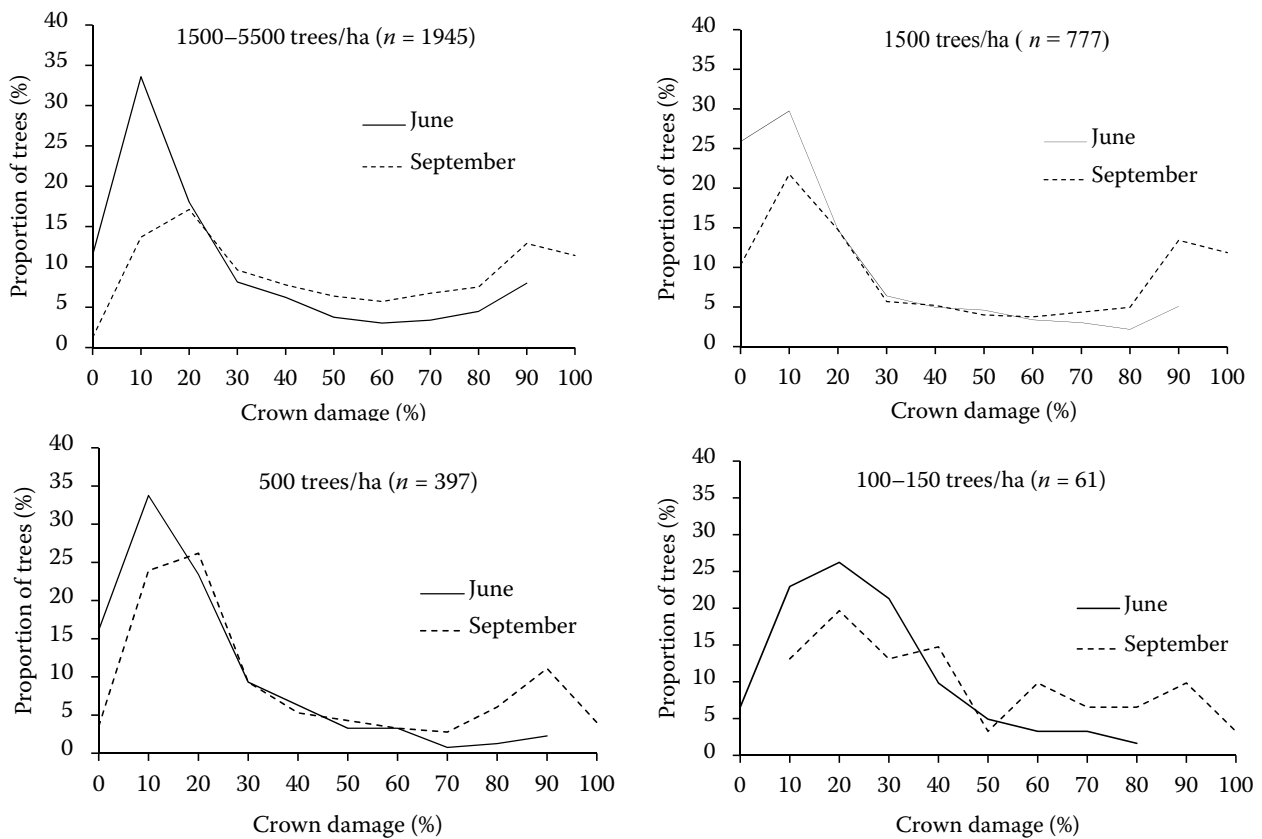In relation to bud flushing the average crown damage varied from 19.5% to 55.3% in June and

Figure 2. Distribution of monitored trees according to the crown damage, assessed over the period June–September 2007

from 36.4% to 70.2% in September (Table 3). Trees of flushing state 0 had more crown damage than other trees in both June and September ($P < 0.001$). Substantial seasonal increases in wilt symptoms and dead tops were observed in trees representing all bud flushing states, except state 0 for which the frequency of dead tops remained stable (Table 3).

The average rate of crown damage varied from 24.7% to 30.1% in June and from 39.4% to 53.4% in September (Table 2), indicating a significant seasonal increase in crown damage for all stand density categories ($P < 0.001$). The percentage-point increase went down slightly with decreasing stand density. The distribution of trees across rate of crown damage changed in a seasonal consistent pattern for all stand density classes (Figure 2). More specifically, the share of trees with little crown damage decreased from June to September, whereas the share of trees with much crown damage increased.

DISCUSSION

In this study we did not find clear evidence that stand density influenced the development of dieback in young, even-aged stands of *F. excelsior*. However, ash dieback was more severe in unthinned control plots than in managed plots. To some extent, this may be due to the absence of the sanitary effects of thinning and to the proximity of trees in unthinned stands. In contrast, wilt symptoms and dead tops related to infection from *H. pseudoalbidus* were more frequent in heavily thinned plots. Increased shading, moisture, and proximity of plant tissues as well as the overall allocation of limited resources to demands other than resistance in dense, single-species stands may render plants highly susceptible to fungal pathogens (Niemelä *et al.* 1992; Garcia-Guzman & Dirzo 2001).

As a light demanding tree species, *F. excelsior* should be thinned regularly to remain vigorous. Mainly due to deficiency of light, the competitiveness of individual ash trees in dense stands decreases considerably (Dobrowolska *et al.* 2011). This and the results of the present study suggest that competition for the light has likely resulted in lower vigour of *F. excelsior* trees and consequently contributed to a more severe disease development in the plots characterised by the highest tree density.

It has been suggested that ascospores of *H. pseudoalbidus* are the primary source of infection which

presumably takes place mainly through leaf petioles, while conidia of the fungus are not involved directly in the infection process (Kirisits *et al.* 2009; Kirisits & Cech 2009). There is evidence of huge loads of spores produced by ascomata in the litter from June to October (Kirisits & Cech 2009; Timmermann *et al.* 2011). This would correspond well with the high incidence of wilting foliage in our study, both in June and September. One possible explanation could be that it takes more time for the fungus to kill the thicker branches at the bottom of the crown.

The development of ash dieback, as many other plant diseases, probably depends on the host's seasonal morphogenesis, including the times of bud flushing and leaf emergence. For *F. excelsior* the timing of the bud flushing is influenced by environmental factors and the most important of these are spring temperatures and winter chilling (Vitasse *et al.* 2011). Nevertheless, the timing of bud flushing cannot be explained by environmental factors alone. For *F. excelsior*, as for many other wind-pollinated tree species in the temperate zone, there are large clonal and within-population variations and a strong heritability in the phenology of bud flushing (Pliūra & Baliuckas 2007).

In our study, bud flushing phenology exhibited a strong correlation with dieback susceptibility, whereas the proportion of crown damage was much higher on dormant rather than on flushing trees. Our results demonstrated that it is unlikely that the severity of the disease significantly influences the timing of bud flushing, because a substantial number of heavily damaged trees were flushing during the May evaluation. This indicates that late-flushing phenotypes of *F. excelsior* are likely to be more susceptible to *H. pseudoalbidus* infection. This is in good accordance with a similar study, where a strong correlation between flushing phenology and the health of *F. excelsior* was recorded (Pliūra & Baliuckas 2007): damaged trees flushed later than healthier trees. In a study of the relationship between genetic variation in *F. excelsior* and its susceptibility to *H. pseudoalbidus*, late flushing trees and late leaf shedding ones were more susceptible to the disease (McKinney *et al.* 2010).

Based on the evidence of sporulation in June (Kirisits & Cech 2009), it may be hypothesized that high incidence of the disease on late-flushing phenotypes of *F. excelsior* can be explained by considerably greater susceptibility of young leaves to infection by *H. pseudoalbidus*. The phenom-

enon of leaf age-related susceptibility to biotic stress, when susceptibility of plant leaves decreases with increasing leaf age, is well described (Koch & Mew 1991; Barth *et al.* 2004). However, to clarify the relationship between leaf senescence and susceptibility to dieback of *F. excelsior*, more investigations are required on the biology and infection routes of *H. pseudoalbidus*.

Besides genetically controlled host resistance and seasonal patterns of sporulation of *H. pseudoalbidus*, higher susceptibility of late-flushing phenotypes to ash dieback can probably be explained by the seasonal activity of *H. pseudoalbidus* in infected tissues of the host at relatively low temperatures, while trees are dormant. Such activity of *H. pseudoalbidus* has been recorded weeks before and after the growing season of *F. excelsior* (Johansson *et al.* 2009a). If the fungus follows such a life cycle, the seasonal activity of *H. pseudoalbidus* is likely to be one of the most important factors influencing the susceptibility of late-flushing phenotypes of *F. excelsior*.

References

Bakys R., Vasaitis R., Barklund P., Ihrmark K., Stenlid J. (2009): Investigations concerning the role of *Chalara fraxinea* in declining *Fraxinus excelsior*. Plant Pathology, **58**: 284–292.

Barth C., Moeder W., Klessig D.F., Conklin P.L. (2004): The timing of senescence and response to pathogens is altered in the ascorbate-deficient *Arabidopsis* mutant vitamin c-1. Plant Physiology, **134**: 1784–1721.

Cech T., Hoyer-Tomiczek U. (2007): Aktuelle Situation des Zurücksterbens der Esche in Österreich. Forstschutz Aktuell, **40**: 8–10.

Dobrowolska D., Hein S., Oosterbaan A., Wagner S., Clark J., Skovsgaard J.P. (2011): A review of European ash (*Fraxinus excelsior* L.): implications for silviculture. Forestry, **84**: 133–148.

Drenkhan R., Hanso M. (2010): New host species for *Chalara fraxinea*. New Disease Reports, **22**: 16.

Garcia-Guzman G., Girzo R. (2001): Patterns of leaf-pathogen infection in the understory of a Mexican rain forest: Incidence, spatiotemporal variation, and mechanisms of infection. American Journal of Botany, **88**: 634–45.

Johansson S., Stenlid J., Barklund P., Vasaitis R. (2009a): Svampen bakom askskottsjukan – biologi och genetik. Fakta Skog, **3**: 1–4.

Johansson S. B.K., Vasaitis R., Ihrmark K., Barklund P., Stenlid J. (2009b): Detection of *Chalara fraxinea* from tissue of *Fraxinus excelsior* using species-specific ITS primers. Forest Pathology, **40**: 111–115.

Kirisits T., Cech T.L. (2009): Beobachtungen zum sexuellen Stadium des Eschentriebsterben-Erregers *Chalara fraxinea* in Österreich. Forstschutz Aktuell, **48**: 21–25.

Kirisits T., Matlakova M., Mottinger-Kroupa S., Cech T.L., Halmschlager E. (2009): The current situation of ash dieback caused by *Chalara fraxinea* in Austria. SDU Faculty of Forestry Journal, Serial: A (Special Issue): 97–119.

Kirisits T., Matlakova M., Mottinger-Kroupa S., Halmschlager E., Lakatos F. (2010): *Chalara fraxinea* associated with dieback of narrow-leafed ash (*Fraxinus angustifolia*). Plant Pathology, **59**: 411.

Koch M.F., Mew T.W. (1991): Effect of plant age and leaf maturity on the quantitative resistance of rice cultivars to *Xanthomonas capestris* pv. *oryzae*. Plant Disease, **75**: 901–904.

Kowalski T. (2006): *Chalara fraxinea* sp. nov. associated with dieback of ash (*Fraxinus excelsior*) in Poland. Forest Pathology, **36**: 264–270.

Kowalski T., Holdenrieder O. (2009): Pathogenicity of *Chalara fraxinea*. Forest Pathology, **39:** 1–7.

McKinney L.V., Nielsen L.R., Hansen J.K., Kjær E.D. (2010): Presence of natural genetic resistance in *Fraxinus excelsior* (Oleraceae) to *Chalara fraxinea* (Ascomycota): an emerging infectious disease. Heredity, **106**: 788–797.

Niemelä P., Lindgren M., Uotila A. (1992): The effect of stand density on the susceptibility of *Pinus sylvestris* to *Gremmeniella abietina*. Scandinavian Journal of Forest Research, **7**: 129–133.

Pliūra A., Baliuckas V. (2007): Genetic variation in adaptive traits of progenies of Lithuanian and western European populations of *Fraxinus excelsior* L. Baltic Forestry, **13**: 28–38.

Queloz V., Grunig C.R., Berndt R., Kowalski T., Sieber T.N., Holdenrieder O. (2010): Cryptic speciation in *Hymenoscyphus albidus*. Forest Pathology, **41**: 133–142.

Schumacher J., Wulf A., Leonhard S. (2007): Erster Nachweis von *Chalara fraxinea* T.Kowalski sp. nov. in Deutschland – ein Verursacher neuartiger Schäden an Eschen. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, **59**: 121–123.

Schumacher J., Kehr R., Leonhard S. (2010): Mycological and histological investigations of *Fraxinus excelsior*

nursery saplings naturally infected by *Chalara fraxinea*. Forest Pathology, **40**: 419–429.

Skovsgaard J.P. (2002): Field notes for survey of potential stands to establish thinning experiment 1518 in oak and 1423 in ash. Unpublished.

Skovsgaard J.P. (2008): Asken har det værre. Skoven, **40**: 276–277.

Skovsgaard J.P., Thomsen I.M., Skovgaard I.M., Martinussen T. (2010): Associations among symptoms of dieback in even-aged stands of ash (*Fraxinus excelsior* L.). Forest Pathology, **40**: 7–18.

Thomsen I.M. (2005): Frostskader i ask. Videnblade Skovbrug, **8.2–1**: 1–2.

Thomsen I.M., Skovsgaard J.P. (2006): Toptørre i ask: klimaskade eller svampeangreb? Skoven, **38**: 408–411.

Thomsen I.M., Skovsgaard J.P., Barklund P., Vasaitis R. (2007): Svampesygdom er årsag til toptørre i ask. Skoven, **39:** 234–236.

Timmermann V., Børja I., Hietala A.M., Kirisits T., Solheim H. (2011): Ash dieback: pathogen spread and diurnal patterns of ascospore dispersal, with special emphasis on Norway. EPPO Bulletin, **41**: 14–20.

Vitasse Y., Francois F., Delpierre N., Dufrene E., Kremer A., Chuine I., Delzon S. (2011): Assessing the effects of climate change on the phenology of European temperate trees. Agricultural and Forest Meteorology, **151**: 696–980.

Corresponding author:

Dr Remigijus Bakys, Swedish University of Agricultural Sciences, Uppsala BioCenter, Department of Forest Mycology and Plant Pathology, P.O. Box 7026, SE-75007 Uppsala, Sweden; E-mail: remigijus.bakys@slu.se