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SHORT COMMUNICATION

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Monitoring of long-term tolerance of European ash to *Hymenoscyphus fraxineus* in clonal seed orchards in Sweden

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Abstract

The invasive alien pathogenic fungus *Hymenoscyphus fraxineus* causing ash dieback (ADB) has devastated European ash (*Fraxinus excelsior*) populations across Europe. Breeding for resistance is the most feasible measure to reduce future losses of ash, and the presence of resistance, albeit at low level, has been demonstrated in numerous genetic trials around Europe. This study is a continuation of the inventories tracking the vitality status of different clones, which started in 2006 at two ash seed orchards in southern Sweden. A new inventory conducted in the summer of 2021 revealed that the ten clones previously identified as the most tolerant to ADB based on periodic surveys from 2006 and onwards still remain the most tolerant, while susceptible clones continued to decline and are completely disappearing from the orchards. Browsing caused mortality in some of the most tolerant clones in one of the orchards during the last assessment period. Despite the animal damage, the stable resistance observed in tolerant clones over a 15 years period forms a solid basis for the continuation of the breeding programme where good candidates are selected for further study.

KEYWORDS
disease type, disease type—shoot disease, fraxinus, host genus—dieback

1 | INTRODUCTION

Hymenoscyphus fraxineus (T. Kowalski Baral, Queloz & Hosoya) is an invasive fungal pathogen originating from East Asia where it is seemingly non-pathogenic on native ash in its natural distribution range (Cleary et al., 2016). The fungus is now widespread throughout most of Europe and has dramatically reduced the host population size of European ash (*Fraxinus excelsior* L.) in most countries. Since ash is known as a keystone species in temperate broadleaved forests and has huge importance for biodiversity, the continuous loss of ash is expected to have negative ecological impacts of other vulnerable ecosystem elements including a large number of species that are highly dependent on ash in the landscape (Hultberg et al., 2020).

Tolerance against the disease has been recorded in several European ash populations where healthy trees have been observed to survive well among the affected ones, showing relatively little dieback damage (McKinney et al., 2014 and references within). The high vitality of such trees has also been observed over longer periods.

Estimations of genetic heritability show that between 30 and 50% of the variation in tolerance to ADB may be explained by genetic components of the host species (Plumb et al., 2020). In Sweden, Stener (2013, 2018) surveyed two clonal seed orchards 5, 10 and 15 years after the first report of ADB in the country and

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substantiated other European investigations reporting a large genotypic variation in susceptibility to ADB. The numerous reports showing the genetic basis of resistance to ADB across Europe suggest that selective breeding can mitigate the disease impact (McKinney et al., 2014; Stener, 2013, 2018).

The main aim of this study was to investigate the stability of vitality scores on *F. excelsior* clones in two seed orchards in southern Sweden, with a special emphasis on the performance of tolerant clones initially identified during earlier surveys. Knowledge about stability of resistance is needed to validate the assumption of the current breeding programmes that have been initiated in European countries.

2 | MATERIALS AND METHODS

Two seed orchards located at Snogeholm (55°32′N, 13°32′E, 50 m) and Trolleholm (55°57′N, 13°12′E, 100 m) in southern Sweden were established in 1992 and 1995, respectively, to produce seeds for commercial forestry purposes. The selection criteria for the clones were growth and stem quality properties aiming to increase production of valuable ash timber. There were 100 and 106 clones planted in Snogeholm and Trolleholm, respectively. The clones came from 27 stands from southern Sweden between latitudes 55°41′N and 58°02′N. The number of ramets per clone was between 40 and 60 at Snogeholm and up to 10 at Trolleholm.

Assessments of damage caused by ADB were initially conducted in September 2006 and in the middle of August in 2007 at Snogeholm, and thereafter in 2010, 2011 and 2016 at both Snogeholm and Trolleholm sites, as reported by Stener (2013, 2018). There was also an inventory of living ramets done in Trolleholm in 1996, one year after planting.

In summer of 2021, assessments of damage caused by ADB were made according to the protocol of Stener (2018). Tree vitality was scored using a scale from 1 to 9, where 1 indicated low vitality and 9 very good vitality. The crown damage was scored as a percentage of crown dieback where class 0 indicated no damage, class 1 indicated low damage (up to 10% of the crown exhibited dieback), and class 9 indicated very serious damage (up to 90% of the crown exhibited dieback) (e.g. Figure 1a).

In addition, the occurrence of damage caused by fallow deer and wild boar was recorded for all trees in Snogeholm. The type of damage was scored as 0—no damage, 1—damage to the bark (layers of periderm removed) and 2—damage to the vascular cambium (layers of cambial tissue removed exposing underlying sapwood). Estimates of damage severity were calculated as the per cent circumference of girdling, in 10% classes (0%–10%, 11%–20% and so on).

In the analysis, the focus was on the overall long-term dynamics of ADB symptoms including all clones in orchards as well as on mortality of individuals among the ten most tolerant or most susceptible clones previously selected by Stener (2013, 2018). The selection of tolerant, intermediate and susceptible clones was based on the genetic breeding values known as best linear unbiased predictor (BLUP) estimated for vitality and crown damage jointly for both orchards. For any tolerant tree that died, the cause of mortality was investigated to determine whether it was due to ADB or other factors. The data from all available inventories are presented as descriptive statistics and in figures.

3 | RESULTS AND DISCUSSION

The cumulative mortality for all clones between the first and last assessment was 68% at Trolleholm (11 years after the first inventory in 2010) and 89% at Snogeholm (15 years after the first inventory in 2006), giving an average mortality rate of approximately 6% annually. The rate of mortality was similar over the years in both orchards (Figure 1b). Not surprisingly, the relative mortality rate between 2016 and 2021 decreased in comparison with the previous assessment period (2011–2016) as many susceptible genotypes had already succumbed to the disease and the ongoing natural selection favours only the most tolerant individuals. At the last assessment conducted in 2021, it was observed that 22 clones (21% of all planted) at Trolleholm and 8 clones (8% of all planted) at Snogeholm had died. Of these clones that died, three were common to both orchards.

In the year 2021, for all clones, the mean number of living ramets per clone was 3.3 ± 2.2 and 2.3 ± 1.8 in Snogeholm and Trolleholm seed orchards, respectively. Considering tolerant clones only, the mean number of living ramets per clone was twofold higher; 6.5 ± 3.4 in Snogeholm and 4.5 ± 1.4 in Trolleholm.

In the most recent assessment period (2016–2021), the absolute number of dead trees among the ten most tolerant clones previously identified by Stener (2013, 2018) varied between orchards. In the summer of 2016, there were 89 and 50 living 'tolerant' ramets at Snogeholm and Trolleholm, respectively. In the smaller orchard (Trolleholm), four of the 50 ramets (8%) died in 2016. There, mortality was registered for just two out of ten tolerant clones. In one of those clones, three out of six ramets died and in the other clone just one ramet died. Mortality at Snogeholm was observed in seven of the tolerant clones including the same two clones with observed mortality at Trolleholm. For three clones, just one ramet died. In the most extreme case, eight out of 13 ramets died. In total, 23 of 89 (or approximately 26%) of living 'tolerant' ramets in 2016 died since the last assessment (Figure 1c).

In general, the mortality among clones in the tolerant group was associated with smaller tree size. The average diameter of all dead trees in this group was 127 ± 29 mm, while the average of all remaining trees was 195 ± 36 mm regardless of the seed orchard.

The average vitality score of the ten most tolerant clones fluctuated slightly over time (Figure 1d), which may be attributable to temporal variability in damage severity related to variable weather conditions across seasons, especially precipitation that is an important factor for ash vitality. On the contrary, the scores had not shifted more than one grade in the nine-grade scale over the years (Figure 1d). There were some clones that had a score close to the

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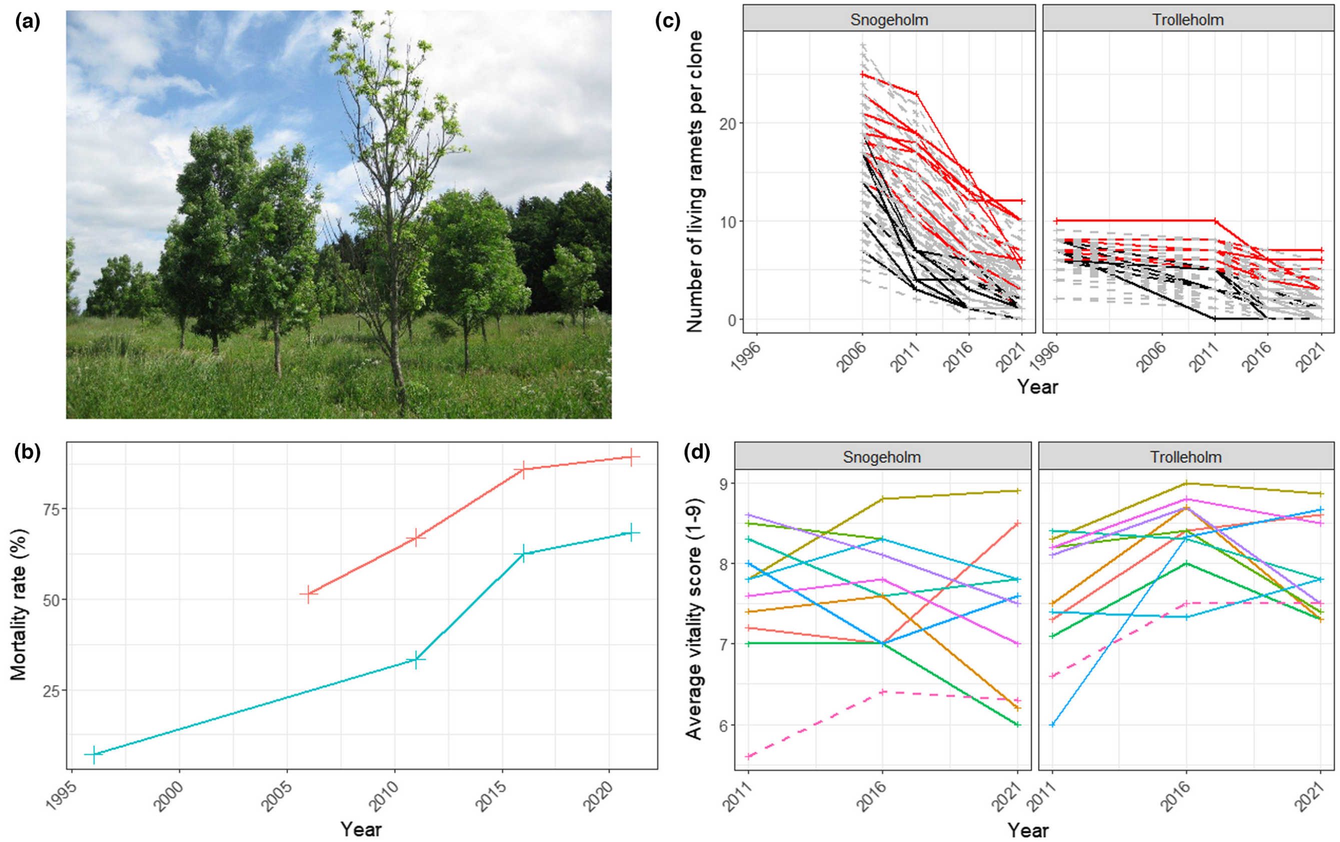

FIGURE 1 (a) Representatives of susceptible (left) and tolerant (right) ash clones at Trolleholm seed orchard, (b) Changes in mortality rate over time from the first assessments in Snogeholm (red) and Trolleholm (blue) seed orchards, (c) the number of living ramets of the ten most tolerant clones (red and solid lines), the ten most susceptible (black and solid lines) and clones with intermediate susceptibility (grey and dashed lines) (d) the vitality score of ten best (most tolerant) clones in both seed orchards at the different assessments conducted between 2011 and 2021; here, lines of different colours represent different clones; the dashed line shows the average vitality score of all living clones with intermediate susceptibility.

average score for other intermediate living clones. Some within-clone variation was evident across phenotype groups. In 2021, the mean vitality scores of the remaining intermediate/susceptible clones were relatively high in both seed orchards (6.2 and 7.5 at Snogeholm and Trolleholm, respectively) compared with the ten most tolerant clones. However, the average number of living ramets for these intermediate/susceptible clones was three with 42 clones having only one or two living ramets, which had a great effect on the calculated mean.

Some variation may have also occurred between assessment years due to subjectivity in the assessment that was conducted by different people. Among the 23 'tolerant' ramets that died between 2016 and 2021 at both seed orchards, 16 were characterized as having highest vitality scores (8 or 9) in 2016.

From the time of the first available inventory, cumulative mortality of ramets belonging to the group of ten most tolerant clones was 66% and 39% in Snogeholm and Trolleholm, respectively. Most of this mortality should be attributed to ADB. However, at Snogeholm, other causes, namely fallow deer and wild boar, damaged large portions of the main stem causing direct mortality by girdling, but also indirectly contributed to mortality by inducing stress over several years of continuous browsing. Animal damage was observed on 84% (n = 296) of trees at Snogeholm after the opening of the fence in 2018. Of those, about 41% (n = 144) had only minor bark damage with removal of the periderm and some inner phloem, while 43% (n = 152) had severe damage to the stem with complete removal of the bark and vascular cambium. Exposed wounds were up to 1 m in length on the stem, affecting approximately 40% of stem circumference. In the group of ten most tolerant clones, for 14 ramets (out of 23 that were recorded to have died during the period 2016–2021), animal damage was determined to be the main reason for mortality. No browsing damage was observed at Trolleholm. In some parts of the seed orchard at Snogeholm, natural regeneration filled in gaps created by the loss of ash trees. In some cases, the naturally regenerated trees have caused significant competition to ash, overshadowing its tree crowns. Some stand management activities have been carried out at both seed orchards to remove this competing vegetation and at the same time some ash trees, which were deemed to have poor vitality could also have been removed.

Between 2006 and 2016, 89% (160 out of 180) of ramets representing the 10 most susceptible clones according to Stener (2013, 2018) died; 103 at Snogeholm and 57 at Trolleholm. Of the 20

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susceptible ramets remaining in 2016, 9 died during the last assessment period and only 11 ramets remained, three susceptible clones with each two ramets and five clone with just one ramet each.

The long-term monitoring of the oldest genetic trial of European ash in Sweden indicate that there is considerable within-clone variation in tolerance to ADB. At both Snogeholm and Trolleholm, there was continued mortality of clones previously identified as being genetically superior due to low severity of damage caused by ADB, albeit at a very low level compared with susceptible clones.

Careful monitoring of other mortality agents should be implemented in future assessments to improve tolerance assessments. In forests in Sweden, species of *Armillaria* are opportunistic on the roots of diseased ash trees, causing decay and increasing tree susceptibility to windthrow. Observations from annual field surveys in ash forests in Sweden during the last 10 years suggest the incidence of root infection caused by *Armillaria* spp., and tree mortality as a result of girdling of mycelial fans at the root collar and/or subsequent windthrow is increasing. Both seed orchards in this study are, however, located on former agricultural land where *Armillaria* spp. are less prevalent and the main contributing mortality factor appears to be animals. Contributing agents of mortality other than ADB represent a potential problem for the breeding programme where long-term tolerance stability to ADB is needed to secure the establishment of a tolerant breeding population.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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