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Pathogen-induced defoliation of *Pinus sylvestris* leads to tree decline and death from secondary biotic factors

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ABSTRACT

The contribution of non-lethal pathogenic attacks to tree death is still unclear. Manion's theory of the spiral of decline predicts that tree decline and death occurs because of a sequence of predisposing, inciting and contributing events. To understand whether pathogens can act as predisposing or inciting factors, we tested whether a sequence of non-lethal pathogen attacks causing crown defoliation could lead to a chronic decline in tree health and predispose trees to die. Scots pine (*Pinus sylvestris*) trees predisposed or escaping (non-predisposed) a first outbreak by the pathogen *Gremmeniella abietina* (predisposing event) were compared in terms of survival and susceptibility to secondary pests (contributing event) after a second *G. abietina* outbreak (inciting event). Four years after the inciting event, mortality among predisposed trees was up to five times higher than among trees escaping the first epidemic. Predisposed trees were twice as susceptible to secondary attacks by the common pine shoot beetle (*Tomicus piniperda*). Ten years after the inciting event, severely predisposed trees had not been able to restore their crowns and still showed stagnated growth. This study showed that pathogen-induced defoliation can act as predisposing and inciting factors for tree death, reducing the capacity of trees to survive short- or long-term stressing events, such as bark beetle attacks. We also showed that tree decline can result from a combination of predisposing and inciting events caused by pathogens.

1. Introduction

Increased tree mortality is a phenomenon of global concern involving complex interactions among biotic and abiotic factors (Allen et al., 2010, 2015; McDowell et al., 2011; Cohen et al., 2016). Owing to its complexity, tree mortality, especially when combined with biotic factors, is still difficult to predict (Camarero et al., 2015; Trumbore et al., 2015). Pathogen population increases co-occur with tree mortality events, although the way pathogens contribute to tree death is still the subject of debate (McDowell et al., 2013; Oliva et al., 2014; Aguadé et al., 2015). Although tree death can be caused directly by some aggressive pathogens, in most cases, mortality is caused by a combination of different factors, including pathogens, acting at different time scales and causing impacts of variable intensity (Oliva and Colinas, 2007; Sangüesa-Barreda et al., 2015). As postulated by Manion (1981), the mortality process is triggered in predisposed trees by a short-term disturbance (inciting event) that makes them vulnerable to secondary biotic agents, that give the *coup de grâce*, eventually killing the tree. Manion's framework emphasizes the role of non-lethal attacks that may leave no apparent sign on trees but that can compromise the capacity of trees to cope with future stressors. However, data on how much non-lethal attacks by pathogens can predispose or incite tree death is scarce since previous studies have mainly focussed on pathogens acting as contributing factors (Cherubini et al., 2002; Marcais and Bréda, 2006). Recent studies have suggested that root rot pathogens alter carbon pools in the tree and, hence, affect the capacity of trees to cope with abiotic stressors such as drought (Aguadé et al., 2015; Camarero et al., 2015). However, the long-term effects of non-lethal pathogenic attacks have only been predicted at a theoretical level (Oliva et al., 2014).

Over the past decades, severe outbreaks by foliar pathogens have been recorded worldwide affecting large forested areas (Zwolinski et al., 1990; Davidson et al., 2005; Woods et al., 2005; Stone et al., 2008; Capretti et al., 2013). These outbreaks have resulted in severe crown defoliations, after which some trees died

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directly, whereas others perished some years later as they became susceptible to attacks by secondary biotic agents, as predicted by Manion's framework. These events exemplify the need to predict not only direct tree mortality caused by pathogens, but also mortality occurring in the long term following non-lethal pathogen attacks. The ascomycete *Gremmeniella abietina* (Lagerb.) M. Morelet is a representative example of a shoot and bud pathogen that causes extensive defoliation in the Northern hemisphere (Nevalainen, 1999; Capretti et al., 2013). This ascomycete causes canker and shoot dieback on Scots pine (*Pinus sylvestris* L.) in boreal and temperate forests in a two-year cycle (Hellgren and Barklund, 1992). Normally damage is limited to a share of the current buds and the previous year's shoots and needles; however, when weather conditions are favourable to the fungus, the infection extends over the branches, causing extensive cankers and defoliation in the entire crown. Pine trees heavily damaged by *G. abietina* are typically attacked by the common pine shoot beetle (*Tomicus piniperda* L.) (Sikström et al., 2005). In general, this insect pest attacks fallen or weakened trees during the spring when adults bore entrance holes. If the trees are vigorous enough, they can exude resin to flush beetles out of the entrance holes, but if they are unable to mount a rapid and strong-enough response, the beetles can establish in the phloem where they mate (Annila et al., 1999). *T. piniperda* beetles carry vascular wilt pathogens that trigger tree defences, thus exhausting starch reserves in the trunk, and contribute to weakening the tree (Lieutier et al., 2009). Larvae feed on the phloem, further extending the damage, and eventually killing the tree. During the summer the brood emerges, creating exit holes (brood emergence holes), and matures by feeding on pine shoots. Site fertility has also been suggested to act as a predisposing factor for *G. abietina*. Higher damage on *P. sylvestris* planted in more fertile so-called 'spruce sites' than in 'pine sites' has been recorded in Sweden (Witzell and Karlman, 2000), though whether the observed differences were due to fertility or other confounding factors remains unresolved.

Large outbreaks by shoot, bud and foliar pathogens are normally associated with periods of favourable weather for the pathogens, so-called conducive conditions, which enable inoculum to build-up (Oliva et al., 2013). In Sweden, this was the case in 1999 and in 2001, when two severe *G. abietina* epidemics affected 484 000 ha of Scots pine (*Pinus sylvestris* L.) forest (Wulff et al., 2006). Both attacks were preceded by two years of optimal conditions for the pathogen: two cool and wet growing seasons (1998 and 2000) and two long and mild winters (1998/1999 and 2000/2001). The epidemics started in 1999 with attacks on trees growing at elevations over 300 m in the area of Bergslagen in the mid-western Sweden. Two years later in 2001, new and more severe attacks struck in the central part of southern and northern Sweden affecting a much larger area. However, major epidemics caused by *G. abietina* are not an isolated case. Worldwide outbreaks by foliar pathogens, such as *Phaeocryptopus gaeumannii*, *Dothistroma septosporum*, *Diplodia sapinea* or *Phytophthora ramorum* are occurring along with unusual weather conditions (Woods et al., 2005; Venette and Cohen, 2006; Stone et al., 2008; Fabre et al., 2011). The predicted increase of these types of large epidemics in connection with the predicted global change in weather patterns (Woods et al., 2005; Desprez-Loustau et al., 2007; Sturrock et al., 2011) highlights the need to predict the short- and long-term impact of these attacks on trees.

In this study, tree mortality after the *G. abietina* epidemics observed in Sweden was used to quantify the role of pathogen-induced defoliation as a predisposing or as an inciting event during the life of a tree. From a physiological perspective, tree death occurs when trees cannot mobilize resources to repair damaged tissues or to sustain living tissues (Waring, 1987; McDowell et al., 2011). Defoliation is therefore a dramatic event for evergreen tree species, particularly if the buds are also affected, because evergreen species tend to store larger quantities of nutrients and carbon in their needles compared with that stored in the leaves of deciduous species (Krause and Raffa, 1996). Defoliation can also have long-lasting effects on carbon and nutrient reserves, affecting the tree's capacity to cope with other pests and pathogens (Christiansen and Ericsson, 1986; Roitto et al., 2009; Galiano et al., 2010) and, therefore, defoliation effects may act similarly as a predisposing factor as defined in Manion's decline model (Oliva et al., 2014). Defoliation can also act as an inciting factor if defoliation occurs in trees that are already predisposed (Oliva et al., 2014). In this study, the occurrence of two sequential attacks affecting the same stands enabled us to investigate how pathogen-driven defoliation increases the chances of trees to die after a second pathogen attack. By following the trees for 10 years, we were also able to study whether the trees that survived both outbreaks were able to resume their growth and restore their crowns, or if they entered a spiral of decline as predicted by Manion's theory. Pathogen defoliation is difficult to replicate experimentally by, for instance, artificially defoliating trees because the effects of artificial defoliation are often weaker than those caused by biotic factors (Quentin et al., 2010), which makes our data valuable. Data on sequential attacks by defoliating pathogens is lacking, even though this situation occurs widely in nature, where trees are normally subjected to a constellation of biotic stressors as they perish (Oliva and Colinas, 2007; Camarero et al., 2015; Sangüesa-Barreda et al., 2015).

The aims of this study were: (i) to quantify the capacity of pathogen-induced defoliation to act as a predisposing factor and to accelerate tree mortality after an inciting event as predicted by Oliva et al. (2014); and (ii) to test whether the sequence of predisposing and inciting events leads to tree decline as predicted by Manion (1981). We undertook a survey of the fate of *P. sylvestris* trees affected by one or two *G. abietina* outbreaks. After the first epidemic, trees with different degrees of defoliation and trees that escaped defoliation where marked in three locations. Most of those trees were again severely defoliated during a second epidemic. This setup enabled us to compare trees that had been defoliated once (i.e. those that escaped the epidemic in 1999 but were defoliated in 2001) and those that had been defoliated twice (i.e. in 1999 and again in 2001). Ten years after the attack, surviving trees were assessed in terms of tree growth and defoliation, and whether predisposition was associated with symptoms of decline. One of the experimental locations included a fertilization treatment in some of the plots, which also allowed us to test the effects of fertilization on *G. abietina* damages.

2. Material and methods

2.1. Field measurements

This study is based on data collected from an experiment established during the two *G. abietina* epidemics that struck the region of Bergslagen in Sweden in 1999 and 2001. After the first epidemic in 1999, three experimental sites were established in 2000 with six or nine plots of 30 × 30 m (Table 1). In each plot, five severely defoliated Scots pine trees (80–90% defoliation), five moderately defoliated trees (60–70% defoliation) and five healthy trees that escaped the epidemic (<20% defoliation) were marked. In 2001, the plots were struck by the second *G. abietina* outbreak. After the second epidemic, the trees were classified as having a severe (>70% defoliation), moderate (40–70% defoliation) or low level of defoliation (<40%) (defoliation assessment described later on). The trees that were defoliated during the first epidemic were considered to be predisposed (Fig. 1) when the second outbreak took

Table 1
Site information, number of *Pinus sylvestris* trees monitored between 1999 and 2010, and number of living trees with tree-ring measurements performed in 2010.

Site	Location	Year of plantation	Site index [a]	Number of plots	Number of trees monitored (H/M/S) [b]	Trees with tree-ring measurements (H/M/S)
Ljungkullen	60°10′N 14°28′E	1969	24	6	30/29/29	18/6/7
Fagerberget	60°20′N 14°21′E	1966	22	9	43/41/40	35/19/6
Bekens	60°19′N 14°13′E	1963	24	9	41/40/39	41/21/13

[a] Site index represents the maximum height (m) in an ideal stand at 100 years.
[b] The number of trees in each defoliation class after the first *Gremmeniella abietina* outbreak are shown separately: 'H', healthy trees that escaped the outbreak; 'M', moderately defoliated trees; 'S', severely defoliated trees.

place. The second outbreak was considered to be an inciting event (Fig. 1).

All trees were monitored during the summer each year from the beginning of the experiment in 2000 until 2004. The trees were measured again in 2010. Throughout the years, a note was made of whether the trees were alive or dead, and the level of defoliation was assessed in the living trees. Defoliation was calculated by determining the proportion of green needles that had been lost in the upper third of the crown relative to a fully foliated crown. The level of defoliation was assessed by two people standing opposite one another on either side of the tree using binoculars in order to assign the tree to one of 10 defoliation classes (0 < 10%, 10 < 20%, 20 < 30%, 30 < 40%, 40 < 50%, 50 < 60%, 60 < 70%, 70 < 80%, 80 < 90%, 90–100% defoliation). The tree diameter at breast height was measured at the beginning of the experiments and in 2010. The presence of entrance holes created by *Tomicus piniperda* L. on the lower part of the stem (0.2–2.0 m above ground) was noted between 2001 and 2004. The presence of brood emergence holes was assessed in 2003 and 2004. Crown recovery was calculated for each tree by subtracting the level of defoliation in 2010 from the level of defoliation in 2001, and dividing it by the level of defoliation in 2001. Over 10 years, some of the marked trees were lost (28 out of 360), as they were wind felled, broken or thinned. These trees were excluded from the analysis (Table 1).

The different plots in the study were subjected to thinning treatments of different intensity, although because of the absence of effects on survival (results not shown, control vs. thinned stands $p = 0.45$), plots were treated as replicates (blocks within the location). At one of the locations (Bekens), three out of the six plots were fertilized with Skog-Vital® (2 × 500 kg/ha), which contains all the mineral nutrients except for nitrogen. The fertilizer was applied in autumn 2000 and then again in spring 2001.

2.2. Dendrochronological analysis of surviving trees

In 2010, wood cores were taken with an increment borer from surviving trees marked in the experimental plots in 2000 (one core per tree). The cores were mounted on wooden strips, polished with sand paper and then digitized. The yearly ring widths were measured using the programmes CooRecorder and CDendro (http://www.cybis.se/forfun/dendro). Annual basal area increments (BAI) were calculated based on the growth of the annual rings recorded between 1990 and 2010. All the trees showed the same decreasing BAI pattern until 2002, one year after the second epidemic, when BAI started increasing until 2007. Not all trees showed an increase in BAI in the same year. Thus a variable called "time until BA growth recovery" was calculated as the number of years from 2001 until the year when the BAI was higher than the BAI of the year before. Growth recovery was mostly linear until 2007, although differences in the steepness of the recovery could be observed. A second variable called "rate of BA growth recovery" was calculated as the rate (slope) of the growth recovery from the year the trees started to recover growth until 2007. The rate was obtained by regressing the BAI for each tree versus year. The cumulated basal area growth of trees between 2001 and 2007 was also compared.

2.3. Experimental design and statistical analyses

We compared survival between trees that were predisposed and trees that escaped the first epidemic (non-predisposed trees).

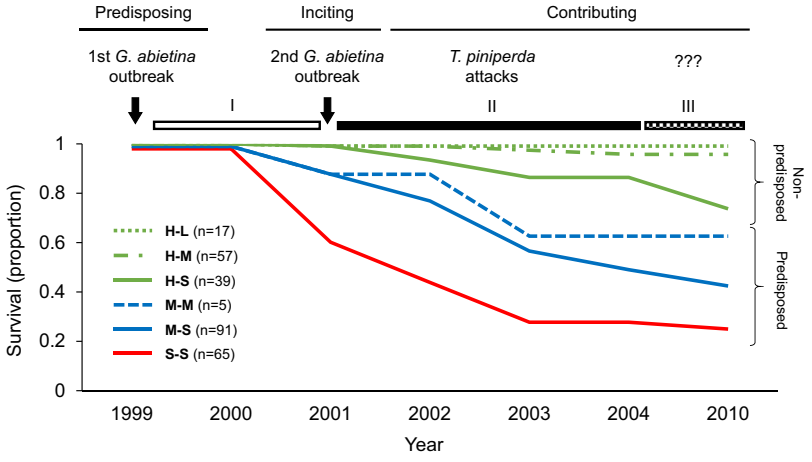

Fig. 1. Experimental design and factors involved in mortality during two *Gremmeniella abietina* attacks in Sweden. Mortality was categorized as either direct mortality (I) caused by the pathogen (empty bar), or indirect mortality caused by secondary pests, such as *Tomicus piniperda* (II), or by other unknown factors (III) (filled bars). Between 2004 and 2010, data on the number of secondary pests or pathogens were not collected. In 2000, *Pinus sylvestris* trees surviving the first outbreak were classified as severely defoliated (S), moderately defoliated (M) or healthy (H) (indicated by the first letter). After the second outbreak, trees were classified as severely defoliated (S), moderately defoliated (M) or lightly defoliated (L) (indicated by the second letter). Number of trees (*n*) in each class is indicated.

Survival was partitioned into three different phases (Fig. 1). Survival was calculated from the inciting event until 2010; however, it was also calculated separately for the different mortality periods (1999–2001, 2001–2004 and 2004–2010). The different mortality periods were investigated to differentiate the different sources of mortality: direct mortality caused by *G. abietina*, typically observed the year after trees were attacked, and indirect mortality occurring several years after. When calculating mortality for a given period, the proportion of dead trees referred to those alive at the start of that period. Single tree survival was modelled using the GLIMMIX procedure in SAS/STAT (version 9.4) as a binomial variable and by using *logit* as a link function (Schabenberger and Pierce, 2002). The model included the two factors and their interaction: "Defoliation before outbreak", with levels "healthy", "moderate" and "severe" and "Defoliation after the outbreak" with levels "low", "moderate" and "severe". The model included the site as a blocking factor. The plot within the site was also included as a blocking factor unless the model fit was compromised. Comparisons were made using the protected least squares method at $P < 0.05$. Defoliation comparisons were carried out in a similar way to those for survival but the percentage of lost green needles was modelled instead. Overdispersion was corrected based on deviance (Schabenberger and Pierce, 2002). Crown recovery and dendrochronological variables were analysed in the same way as for survival but a normal distribution was assumed.

The bark beetle *T. piniperda* was considered a contributing factor, and therefore the probability of colonization was compared between trees with different defoliation levels before and after the second outbreak (inciting event). Percent successful colonization indicated the probability of *T. piniperda* to reproduce (presence of brood emergence holes) on trees that were attacked (presence of entrance holes). Insect damage data were missing in some tree/year/location combinations. To cope with missing data, when analysing the proportion of attacked trees in which *T. piniperda* was able to proliferate (brood emergence holes), we classified trees as: (i) not being attack when the absence of entrance holes was confirmed in all years (i.e. no missing data in any year) and (ii) considered positive in cases with one record of attack from 2001 to 2003. All other trees with missing data were excluded from the analysis.

The effect of fertilization on survival was modelled as previously described for other variables but only data from the location Bekens was used. The model included the factor "treatment", with two levels (control and fertilization), the factor "Defoliation before outbreak" and their interaction.

3. Results

In 1999, an unprecedented *G. abietina* outbreak affected central Sweden, where healthy, moderately defoliated or severely defoliated Scots pine were marked (total of 332 trees) (Table 1). In 2001, a second epidemic affected the stands and trees, including those that had escaped the first epidemic, were attacked. Following the course of these events, we considered the first outbreak to be a predisposing event affecting some of the trees, and the second outbreak to be an inciting event affecting all the trees.

After the predisposing event (first infection) and during the next ten years, mortality mostly affected trees that had been severely or moderately defoliated during the first outbreak (77% and 56% mortality from 2000 to 2010, respectively) (Fig. 1). Within that period, predisposition contributed largely to the mortality occurring after the inciting event, but so did the intensity of the second outbreak. Ten years after the first outbreak, mortality had only affected severely defoliated trees during the inciting event (second infection). Among those severely defoliated trees, those that had been previously predisposed by a moderate or a severe defoliation of the crown had a lower survival rate (49% and 40%, respectively) ($P < 0.0001$) than those that had escaped the first outbreak (93%) (Fig. 2a).

Throughout the years, mortality could be assigned to three events: direct mortality owing to *G. abietina* defoliation after the first attack (until 2001), mortality after the second attack (2001–2004), part of which could be attributed to the secondary pest *T. piniperda*, and finally, mortality and tree decline between 2004 and 2010 (Fig. 1). For all events, the role of the predisposing and inciting events was evaluated. For instance, direct mortality after the first attack (1999–2001) was particularly noticeable on severely defoliated trees (40% mortality vs. 13% mortality on moderately defoliated trees, $P < 0.0001$). Healthy trees were classified as those that had escaped the first attack; almost none of the trees among this group had succumbed to mortality by the end of the study (<1%).

Between 2001 and 2004, mortality only occurred among the severely defoliated trees after the second outbreak. However, when considering those severely defoliated trees after the inciting event, mortality was almost five times higher ($P = 0.0002$) among predisposed trees (48%) than among non-predisposed trees (10%). The secondary pest *T. piniperda* was responsible for almost half (49%) of the observed mortality during this period. The entrance holes of *T. piniperda* were observed in trees with low, moderate and severe levels of defoliation. Attacks were more frequent in 2001

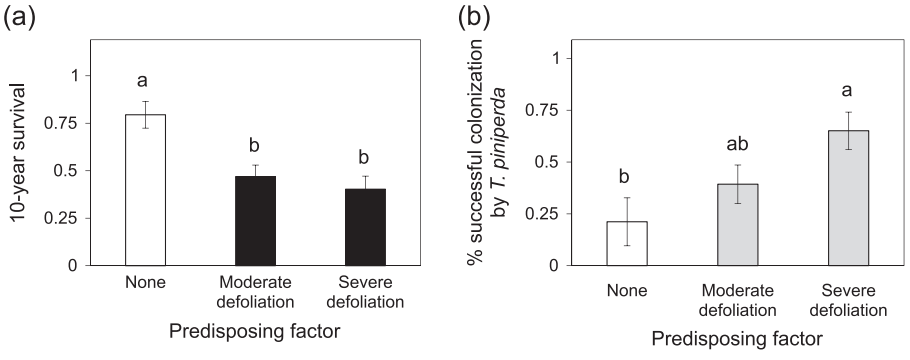

Fig. 2. Proportion of (a) surviving trees and (b) colonization of *Tomicus piniperda* on *Pinus sylvestris* trees severely defoliated after a major outbreak of *Gremmeniella abietina* depending on whether they were attacked by the same pathogen two years before (predisposed; shaded bars) or if they had escaped the outbreak (non-predisposed; white bars). Crown defoliation after the first outbreak was 0–20% in healthy trees and was classified as 'none', 60–70% for trees classified as 'moderately defoliated', and 80–90% for trees with severe defoliation. In (b), only trees with entrance holes were considered. Different letters above the bars indicate significant differences based on Tukey's significance differences (HSD) at $P < 0.05$. Bars show the standard error.

(66% of trees attacked) and became less frequent in 2002, 2003 and 2004 (7%, 4% and 7% of trees attacked, respectively). The level of defoliation prior to the inciting event was associated with the capacity of *T. piniperda* to successfully colonize severely defoliated trees after the second outbreak ($P < 0.0001$) (Fig. 2b). Colonization on severely defoliated trees that were predisposed was more than double that on non-predisposed trees (52% vs. 22%).

Between 2004 and 2010, mortality was low and affected mainly trees severely defoliated during the second outbreak (12% vs. 0% for both moderately and slightly defoliated trees) (Fig. 1). Mortality between 2004 and 2010 no longer correlated with the defoliation level after the first outbreak ($P = 0.82$) or with the intensity of the second outbreak ($P = 0.99$).

The growth of surviving trees 10 years after the second epidemic was largely associated with the level of defoliation after the inciting event in 2001. Generally speaking, growth was declining in the years before the outbreak, after which it rose abruptly until 2007, when it started to slow down again (Fig. 3a). Until 2007, the cumulated growth of trees was highly dependent on the level of defoliation that occurred during the second outbreak ($F = 11.87$, $P < 0.0001$) (Fig. 3b). Surviving trees that had suffered severe defoliation after the second outbreak showed a smaller increase in their basal area than those that experienced moderate or low levels of defoliation (25.5, 40.7 and 62.4 cm^2, respectively).

When looking at the number of years needed for trees to start increasing growth, both the inciting and predisposing defoliation had an effect ($F = 12.9$, $P < 0.0001$ and $F = 7.20$, $P = 0.001$, respectively) (Fig. 3c). Trees severely defoliated in both attacks took the longest to increase in growth again (4.5 years vs. 1.0 years for healthy trees with low levels of defoliation after the second outbreak). When growth recovery started, trees severely defoliated before and after the second outbreak increased growth at a much lower rate than healthy or moderately defoliated trees during the first outbreak, even when they were also severely defoliated during the second outbreak (0.26 $cm^2\ year^{-2}$ vs. 1.3 and 1.1 $cm^2\ year^{-2}$, respectively) (Fig. 3d). Most importantly, the rate of growth increase of trees severely defoliated twice was not different from 0 ($P = 0.32$), indicating stagnation, while recovery occurred in all other defoliation classes. The rate of growth recovery was dependent on the level of defoliation that had occurred before the second outbreak ($F = 6.48$, $P = 0.002$).

Both defoliation after the predisposing event ($P = 0.0005$) and defoliation after ($P < 0.0001$) the inciting event affected the level of defoliation among the surviving trees in 2010 (Fig. 4a). Ten years after the inciting event took place, trees that were healthy or moderately defoliated during the first outbreak had started to restore their crowns (Fig. 4b). However, this was not the case for trees that had suffered severe levels of defoliation twice: the crown recovery rate for these trees was not significantly different from 0 ($P = 0.32$), indicating a chronic failure to restore their crown.

The effect of fertilization was only studied at one location (Bekens). A smaller proportion of the severely defoliated trees growing in the fertilized fields survived two *G. abietina* attacks compared with those growing in the control plots (12.2% vs. 53.6%, $P = 0.039$). However, fertilization did not significantly affect moderately defoliated trees ($P = 0.34$) (Fig. 5).

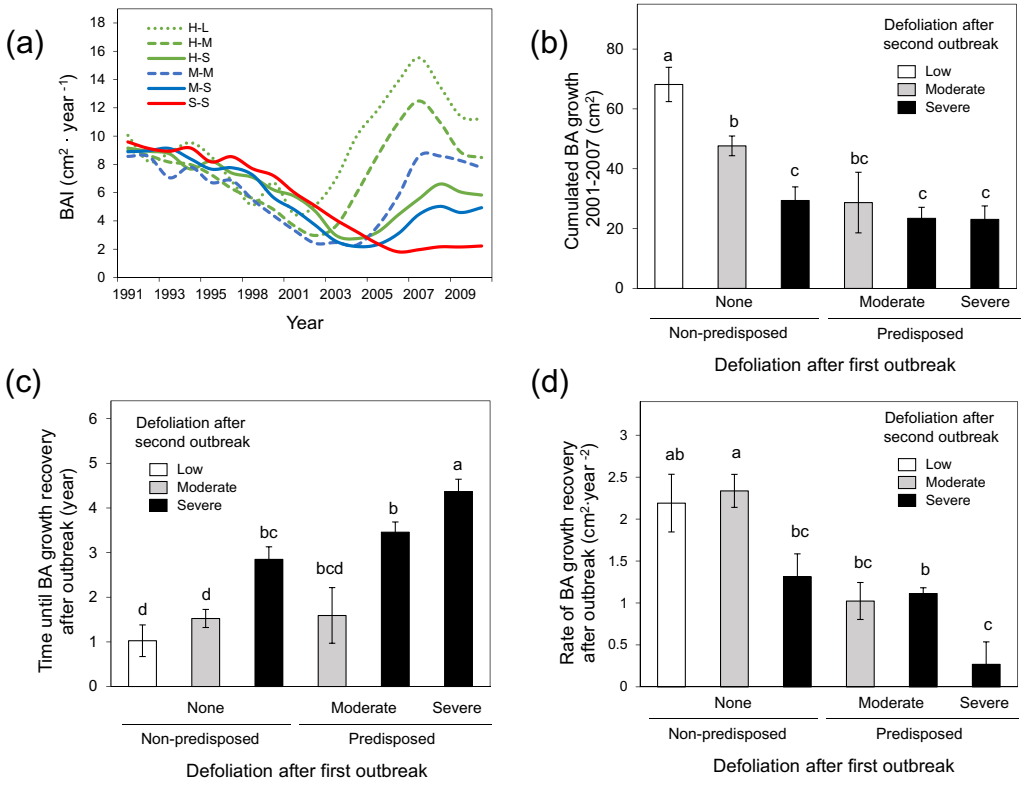

Fig. 3. (a) Basal area increment (BAI) patterns among surviving *Pinus sylvestris* trees according to the defoliation before and after two major outbreaks of *Gremmeniella abietina* in 1999 and 2001; the two events were considered predisposing and inciting events, respectively. Crown defoliation after the first outbreak was 0–20% for healthy trees classified as 'none', 60–70% for trees classified as 'moderately defoliated', and 80–90% for trees with severe defoliation. Crown defoliation after the second outbreak was classified as 'low' when less than 40% of the crown was defoliated, 'moderate' when 40–70% of the crown was defoliated, and as 'severe' when more than 70% of the crown was defoliated. The letters H, M, S indicate healthy, moderately defoliated, and severely defoliated trees, respectively, after the first outbreak of *G. abietina* (first letter), and M and S indicate moderate and severe defoliation, respectively, after the second outbreak (second letter). (b) The cumulated growth differences between defoliation classes. (c) The number of years needed to recover growth after the second outbreak. (d) The rate of growth recovery. Different letters above the bars indicate significant differences based on mean comparisons using Tukey's HSD comparisons at $P < 0.05$.

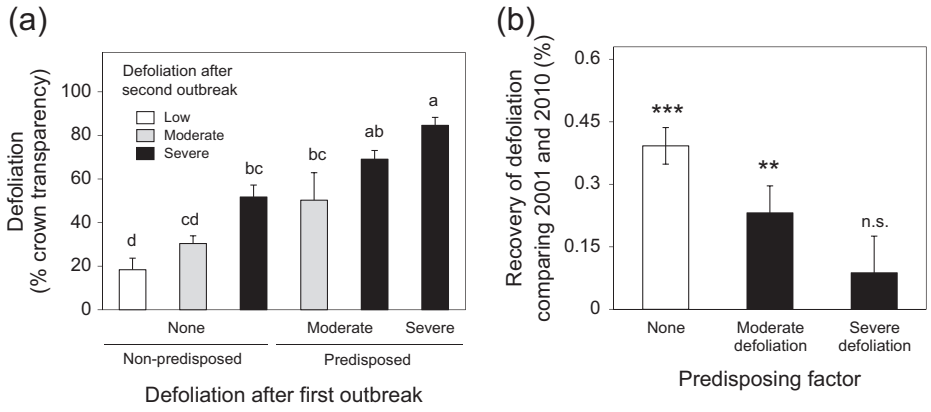

Fig. 4. (a) Average defoliation of surviving trees in each defoliation class according to defoliation levels in 2000 after the first attack (predisposing event), and in 2001 after the second attack (inciting event). Crown defoliation after the first outbreak was 0–20% for healthy trees classified as 'none', 60–70% for trees classified as 'moderately defoliated' and 80–90% for trees with severe defoliation. Crown defoliation after the second outbreak was classified as 'low' when less than 40% of the crown was defoliated, 'moderate' when 40–70% of the crown was defoliated, and as 'severe' when more than 70% of the crown was defoliated. Different letters above the bars indicate significant differences based on HSD Tukey's mean comparisons at $P < 0.05$. (b) Recovery of defoliation relative to defoliation in 2001. Positive values indicate crown recovery expressed as the percent increase of crown density between 2001 and 2010. Significant differences from 0% (no-crown recovery) are indicated: $^{**}P < 0.001$; $^{***}P < 0.0001$; non-significant (n.s.), $P > 0.05$.

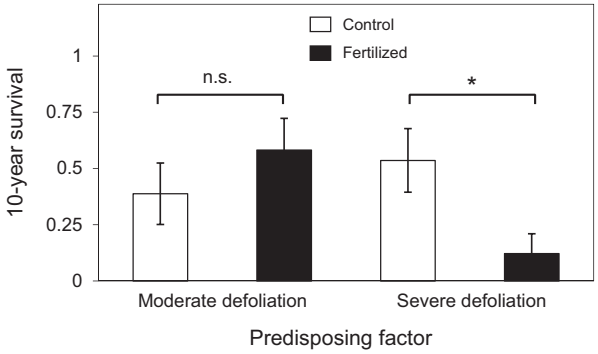

Fig. 5. Effects of N-free mineral fertilizer application on the 10-year survival of *Pinus sylvestris* trees subjected to two *Gremmeniella abietina* attacks that had suffered moderate (60–70%) or severe (80–90%) crown defoliation after the first attack (predisposing factor). Healthy trees were excluded from this comparison because they all survived. Significant differences using a *t*-test between treated and untreated plots are indicated: $^{*}P < 0.05$; non-significant (n.s.), $P > 0.05$.

4. Discussion

Theory predicts that the contribution of a pathogen attack to the death of a tree depends on the timing of the attack relative to previous stresses suffered by the host tree (Manion, 1981; Oliva et al., 2014). Accordingly, in this study, we showed that tree mortality after a *G. abietina* epidemic was dependent on the history of stress suffered by the tree before the attack. Our results indicated that the level of defoliation after a pathogen attack was only a good predictor of the likelihood of survival if combined with data on damage suffered in a previous outbreak. The combination of both predisposing and inciting pathogen-induced defoliations determined the capacity of trees to cope with later attacks of a secondary pest such as *Tomicus piniperda*. Ours represents a good example in which tree death could be attributed to pathogens acting as predisposing and inciting factors. Previous studies have reported cases of pathogens acting as contributing factors to tree death (Cherubini et al., 2002; Marcais and Bréda, 2006); however, little has been reported about the capacity of pathogens to act as predisposing and inciting factors of tree death and tree decline (following Manion's terminology).

In our study, we assumed that some trees escaped a first *G. abietina* epidemic by chance. However, phenotypic or genetic differences could explain the escape and thus also determine why those trees were less likely to die after the second attack. We find little support for this hypothesis in our data, since those putatively resistant trees were largely damaged during the second outbreak (Fig. 1) i.e. they did not escape the second outbreak. Furthermore, the fact that *P. sylvestris* displays a quantitative (dose dependent) resistance and not a qualitative resistance against the *G. abietina* (Hansson, 1998), also argues against damage differences between trees being determined by genetic differences and supports the effect of chance. In any case and in order to control for possible biases, we compared trees within the same defoliation level after the second attack e.g. severely defoliated trees, thus excluding those that also escaped the second outbreak. Because of these putative caveats, we did not make any claims about whether predisposition increased the risk of suffering a second attack.

4.1. Predisposition reduced tree survival after the inciting event

A key result of this study was to be able to quantify the amplification effect that pathogen-induced defoliation can have on tree mortality. After the first outbreak, immediate mortality due to pathogen defoliation was very high, and was of the same magnitude as mortality occurring years later in conjunction with secondary agents (Fig. 1). In the following years, mortality first affected trees that had been both predisposed and subjected to a second attack. However, after 2004, mortality could no longer be attributed to the predisposition level. One could speculate that when the most predisposed trees died, the defoliation caused by the second outbreak started to operate as a predisposing factor itself on surviving trees. Our findings therefore support the implicit statement within Manion's theory that the previous history of stressing events is needed in order to predict the contribution of an event to tree death. The level of defoliation after the second outbreak alone was a poor predictor of the likelihood of death in the following years. Within the same defoliation class, predisposed trees were three to five times more likely to die than non-predisposed trees. Defoliation has often been suggested to be a good estimator of tree mortality at large scales (Dobbertin and Brang, 2001), however our results suggest that predictions of tree death based on the level of defoliation should be treated with caution at local scales.

4.2. Predisposing and inciting events lead trees to decline

Our study has shown that the combination of predisposing and inciting events can lead trees to decline. The combination of two defoliations contributed to the growth stagnation and chronic defoliation of the trees (Fig. 4a). Defoliation is a dramatic event for evergreen trees such as *Pinus sylvestris* and has a large impact on their carbon reserves (Krause and Raffa, 1996). In this study, we showed how pathogen-induced defoliation impaired the capacity of trees to recover from a second defoliation. Growth recovery is strongly dependent on the capacity of trees to restore their crown. As we observed, trees under these conditions show higher mortality rates than normal (Bigler and Bugmann, 2004). In a recent study, Galiano et al. (2011) showed how crown recovery from drought-induced defoliation was mainly driven by carbon accessibility. Trees that were unable to restore their crown could not resume C assimilation and, hence, depleted their reserves until they died. In our study, we can only speculate about the mechanisms of tree death. However, we know that insect-defoliation has an impact on C reserves (Krause and Raffa, 1996; Annila et al., 1999), which could explain the observed growth stagnation. In our study, growth stagnation and defoliated crowns were still observed 10 years after the inciting event, highlighting the long-term consequences of a sequence of damaging events.

4.3. A sequence of predisposing and inciting factors increased tree susceptibility to secondary pests

Predisposition affected the capacity of trees to defend themselves against secondary agents after the inciting event. Mortality only occurred among the severely defoliated trees in the second outbreak, which suggests that the combination of a predisposing and an inciting event seem to be a prerequisite for death, as predicted in Manion's model. The secondary insect pest *T. piniperda* was responsible for 48% of mortality after the second epidemic, mainly affecting severely defoliated trees before the outbreak. Trees in all classes of defoliation were attacked following the second defoliation, possibly owing to the release of attracting substances (Schroeder, 1988). However, for trees within the same defoliation class, it was clear that colonization was much higher on predisposed trees. As before, we can only speculate on the mechanisms behind the high mortality associated with predisposition. The tree's defence against *T. piniperda* and its fungal symbionts relies on mounting carbon-expensive defences (Långström et al., 1992), most of which rely on carbon stored as reserves (Guérard et al., 2007). C depletion of reserves driven by a lack of assimilation could explain the inability of the trees to prevent secondary attacks (Marcais and Bréda, 2006). As shown in other studies, colonization after one defoliation event only occurred in very damaged trees with more than 97% defoliation or between 90 and 100% defoliation (Cedervind et al., 2003; Sikström et al., 2005). However, the amount of mortality associated with *T. piniperda* observed after two defoliation events by *G. abietina* was higher than the mortality reported for Scots pine trees following two consecutive sawfly defoliations (Annila et al., 1999). Besides defoliation-associated costs, cambial tissue damage and bud death caused by *G. abietina* may imply extra-compartmentalization and repair costs, which could pose additional difficulties for recovery (Oliva et al., 2014).

4.4. Increased mortality associated with fertilization might relate to needle age-class shifts

In one location, we observed that in fertilized plots, severely defoliated trees experienced higher tree mortality rates than in the control plots, supporting previous observations in Sweden (Witzell and Karlman, 2000). The observed mortality increases associated with fertilization are difficult to interpret, although we speculate that they may relate to fertilization-induced changes in branch architecture. Fertilization decreases the number of needle cohorts but does not affect foliated branch length (Balster and Marshall, 2000). Considering that *G. abietina* kills the previous-year's needles, we speculate that the impact of defoliation could be higher for fertilized trees because relatively more tree foliage would be killed compared with trees growing on unfertilized plots. Another explanation could be that the fertilized trees had a different chemistry in their needles, as previously shown for fertilized red pine (*Pinus resinosa*), which was less resistant to the pathogen *Sphaeropsis sapinea* (formerly *Diplodia pinea*) compared with unfertilized trees (Blodgett et al., 2005). The finding is contrary to the common notion that vigorous trees are more resistant to disease attacks and, in this case, may be because of the particular biology of the pathogen.

4.5. Final remarks

Current challenges to tree health are compromising the capacity of large forested areas worldwide to act as C sinks (Trumbore et al., 2015). Predicting tree mortality is therefore an urgent matter on the research agenda of the scientific community working to increase our understanding of the impact of predicted global climate changes (McDowell et al., 2011). Biotic agents have been recognised to play a role in tree mortality processes (McDowell et al., 2008), though rarely incorporated in mortality models e.g. drought induced mortality. In this study we have shown the magnitude and the duration of the effects of non-lethal pathogen attacks on tree mortality. Our long-term monitoring study enabled us to dissect mortality patterns over a period of 10 years after two severe epidemics of *G. abietina*. The epidemics occurring in Sweden are just an example of the many outbreaks currently affecting forested areas worldwide that are predisposing trees to subsequent pathogen attacks and, therefore, increasing the mortality rate. The projected increase in pathogen attacks requires explicitly accounting for predisposition within mortality models.

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