



Seasonal variation in Norway spruce response to inoculation with bark beetle-associated bluestain fungi one year after a severe drought

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ABSTRACT

In 2018, up to 4 million m³ Norway spruce was killed by the spruce bark beetle *Ips typographus* in Sweden. The event was unique for Sweden, in terms of both affected volume and the fact that it was triggered by severe drought stress, not by ample availability of relatively defenseless storm-felled trees. The outbreak continued in 2019 and 2020, each year with twice as many trees killed as in 2018. The aim of this study was to quantify seasonal variation and potential lag-effects in tree defense capacity the year after a severe drought stress. Inoculation with a bark beetle-associated bluestain fungus, repeated four times with one-month-intervals between May and August 2019, were carried out at three field sites with spruce provenances of Swedish and East European origin representing early and late bud burst, respectively.

All sites had experienced moderate to severe drought stress in 2018, and site-specific defense capacity correlated positively with the cumulative precipitation two months before inoculation. Sites with two-month precipitation levels <100 mm had larger necrotic lesions in the phloem following inoculation, an indication of lower tree defense capacity. Lesion size did not differ between provenances, and all trees were able to confine fungal infection successfully.

There were some seasonal differences in necrotic lesion size, with the sites Skårnsnäs and Norberg having significantly larger lesions in June than in May, and site Lugnet having large lesions also in May. Lesions were generally smaller in July and August than in June.

The cross-sectional area and number of traumatic resin ducts was measured in sapwood samples from one site, Lugnet, to quantify an additional aspect of tree defenses. The area of resin ducts produced in May and June were larger than that in July and August. This is in line with a positive correlation between lesion area and resin duct area, indicating that a stronger fungal infection following inoculation in spring triggered a stronger induced defense response. The East European provenances had more resin ducts than Swedish provenances, but the area of resin ducts did not differ significantly between provenances.

1. Introduction

The spruce bark beetle (*Ips typographus*) is a major forest pest in Europe and the risk of beetle outbreaks is strongly influenced by climatic factors. Beetle-induced mortality of Norway spruce (*Picea abies* (L.) H. Karst) trees averaged 150 000 m³ per year in south Sweden for the period 1990–2010 (Marini et al., 2017). However, in 2018 the beetles killed 3–4 million m³ spruce, and 7 million m³ in both 2019 and 2020 (Wulff and Roberge, 2021). This extreme mortality was triggered by the warm and dry summer in 2018, leading to the completion of two beetle

generations and rapid population build-up. Historically, bivoltine development of the spruce bark beetle has rarely been observed in Sweden (Öhrn et al. 2014), but is expected to occur much more frequently in a warmer climate (Jönsson et al., 2012).

Norway spruce has evolved multiple defenses to resist insect and pathogen attack. These include preformed constitutive defenses and induced defenses that are activated in response to attack (Franceschi et al., 2005). Anatomical structures such as resin ducts in the phloem and sapwood are important in both lines of defense. Resin ducts are filled with terpenoid resin, a key chemical defense in many conifers

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which dose-dependently inhibits bark beetle colonization (Zhao et al., 2011a). Traumatic resin ducts in the sapwood are induced in response to insect attack, pathogen infection or abiotic stress (Krokene, 2015). To overcome tree defenses the spruce bark beetle engages in mass-attacks synchronized by aggregation pheromones (Bakke et al., 1977; Franceschi et al., 2005). If successful, the aggregated attack leads to massive inoculation of beetle-vectored bluestain fungi forming necrotic lesions in the bark, invading the sapwood and gradually overwhelming the tree's defense capacity (Krokene, 2015).

The 2018 spruce bark beetle outbreak in Sweden was unique, as it was triggered by drought stress, and not by a large supply of storm-felled trees with little to no defense capacity, which is the usual trigger of outbreaks in Sweden (Marini et al., 2013). Severe drought stress has been shown to reduce tree resistance to fungal infection and to trigger bark beetle outbreaks (Netherer et al. 2021, Raffa et al. 2008). The continuation of the outbreak into 2019 may indicate carry-over effects of the 2018 drought stress. Such lag-effects contribute to uncertainties in assessments of how climate change may affect the risk of spruce bark beetle attacks (Jönsson et al., 2012). Quantitative information on lag-effects is lacking because severe natural drought stress events are rare and it is expensive to carry out realistic drought stress experiments in the field.

Norway spruce makes up 42% of the forest volume in Sweden and about 200 million seedlings are planted each year. To promote vigorous trees with high wood quality, selection of suitable seed sources (provenances) has been carried out for >100 years (Myking et al., 2016). Tree phenology is a key selection character, as the timing of shoot elongation and wood formation determines the risk of frost damage, since provenances with later bud burst are less exposed to spring frost damage (Hannerz, 1999). East European provenances are commonly selected, as they in general have later bud burst and increased growth compared to local provenances in southern Sweden (Persson and Persson, 1992; Werner and Karlsson, 1982). Tree defense capacity has not been selected for *per se*, even though there is a genetic component to tree resistance to bluestain fungi (Steffenrem et al., 2016). A key concern about selecting for resistance is that physiological tradeoffs between growth and defense may lead to a negative correlation between growth and tree defense capacity (Herms and Mattson, 1992; Krokene et al., 2012). The timing of budburst in relation to the flight activity of the spruce bark beetles may thus influence tree defense capacity in spring.

A general, seasonal variation in resistance to fungal infection has been demonstrated in Norway spruce previously (Horntvedt 1988; Krokene et al. 2012). This variation appears to be related to shoot phenology and could be a result of physiological trade-offs between growth and defense processes (Herms and Mattson 1992; Krokene et al. 2012). Several independent but partially overlapping hypotheses attempt to explain patterns in plant defense allocation. The expanded Growth-Differentiation Balance (GDB) hypothesis incorporates all the other hypotheses into its conceptual framework to predict how plants balance resource allocation to growth-related processes versus defense and other differentiation-related processes (Mattson and Herms, 1992). Since the GDB hypothesis predicts that there is a trade-off in allocation to growth and defense, actively growing trees are expected to be less resistant to attack. When plant resources are allocated to growth, less are available for investment in physical and chemical defenses.

The aim of this study was to quantify potential lag-effects of drought on tree resistance, as 2019 provided a unique opportunity to better understand and predict how climate change may affect spruce vulnerability to bark beetle attacks. We inoculated Norway spruce trees with bluestain fungi on four different occasions throughout the growing season at three field sites. Spruce provenances with different phenology that experienced severe or moderate drought stress in 2018 were inoculated. The size of necrotic lesions in the phloem and traumatic resin duct formation in the sapwood following fungal inoculation provided measures of tree defense capacity. We tested three hypotheses for how environmental and genetic factors influence tree defense capacity:

H1. Site-specific differences: Trees at the site with the most severe drought in 2018 have lower defense capacity in 2019 than trees at sites experiencing moderate drought.

H2. Differences between provenances: Swedish and East European spruce provenances, with a generally early and late timing of budburst, respectively, differ in defense dynamics in spring.

H3. Seasonal differences: Spruce trees differ in defense capacity over the season, with lower defense capacity during periods of active growth.

2. Material and methods

2.1. Study sites and experimental trees

Tree defense capacity was assessed at three field sites in S-Sweden; Skärnsås [56.2°N, 14.3°E], Lugnet [59.6°N, 17.5°E], and Norberg [60.1°N, 15.8°E] (Fig. 1a). All three sites had been influenced by the warm and dry summer in 2018, with total precipitation during April – August being >100 mm below average at Skärnsås and about 50 mm below average at Lugnet and Norberg (Fig. 1b). The 2018 annual temperature sums were around 1800° days (dd) (>5 °C) at Norberg and 2000 dd at Skärnsås and Lugnet, which is about 50% above the 1961–1990 average (Fig. 1c). The Standardized Precipitation Evapotranspiration Index (SPEI) (Begueria et al., 2014) was significantly below normal at all sites and indicated that the drought was more severe at Skärnsås (SPEI –3.5) than at Lugnet and Norberg (SPEI –2.0) (Fig. 1d).

The field sites were established in 2002 as part of one of Sweden's largest test series of Norway spruce seed sources, with 64 provenances and seed orchard crops of south Swedish and Eastern European origin (Lundströmer et al., 2020). At each site, 50 replicates (2 trees × 25 blocks) of each provenance were planted in a randomized block design. Each block, measuring ca. 20 × 24 m, was planted with 2-year-old seedlings in a 2 × 2 m formation in May–June 2002. We randomly selected 10 out of the 25 blocks at each site for this study (i.e. 20 trees per provenance).

Previously collected data on tree phenology, growth, vitality and damage were used in the present study. All trees had been assessed for growth, vitality and damages six and 13 years after planting. In addition, assessments of phenological characters (timing of bud burst and lignification of wood cells indicating growth cessation) made in 2004 has been shown to correlate with the geographical origin of the trees (Lundströmer et al., 2020). Budburst and shoot elongation measurements were scored on all trees according to the Krutzsch-scale (Krutzsch, 1973) in early summer 2004, when the trees were 4 years old (in mid-May at Skärnsås, in late May at Lugnet, and in early June at Norberg). At the time of scoring, virtually all provenances had active bud development, i.e. were above class 0 ('dormant bud') but below class 8 ('all needles more or less scattered, new buds begin to form'). Based on these scores we selected provenances with an early or late spring phenology for comparisons of defense capacity. We selected the provenances Ängelsfors and Bollebygd as representative Swedish provenances with an early spring phenology, and the provenances Istra and Rezekne (both from Latvia) as representative Eastern European provenances with a late spring phenology. The selected provenances were inspected to ensure that they did not have more damages than the average for all provenances.

2.2. Experiments and sampling

Tree defense reactions were assessed by inoculation with a bark beetle-associated fungus, a common method to elicit and evaluate conifer defense responses (Steffenrem et al., 2016). As inoculum we used malt agar colonized by *Grosmannia europhioides* (isolate 7.206.1 sampled August 16 2007 from a Norway spruce tree at Asa experimental forest and research station [57.2°N, 14.8°E], gifted by Dr. Ylva Strid, SVA), as this relatively virulent bluestain fungus is a frequent associate of the spruce bark beetle in Sweden (Zhao et al. 2018). Inoculum was prepared

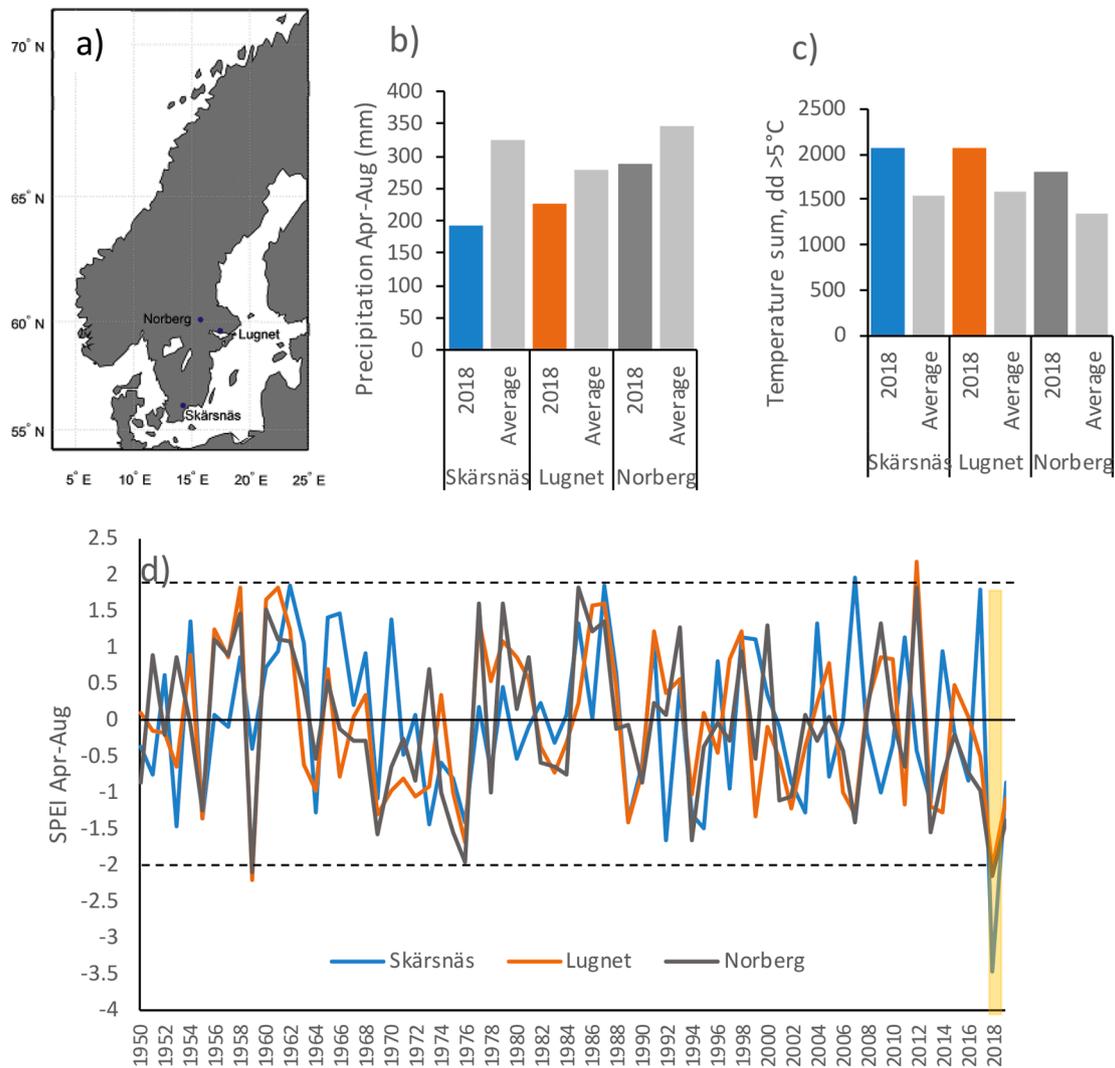


Fig. 1. (a) Map displaying the three sites. (b) Precipitation during April-August (main tree growth period) and (c) total annual degree days (>5 °C) for 2018 and the long time average (1961–1990). (d) Standardized Precipitation Evapotranspiration Index (SPEI) during April-August 1950–2019 (2018 and 2019 highlighted). Gridded precipitation and temperature data are from SMHI <http://luftweb.smhi.se/>. SPEI was derived from <http://spei.cscic.es/map> and is based on z-scores. Black solid line represent the average condition, and values exceeding ± 1.96 (dashed lines) are significantly drier or wetter than the average (95% confidence level).

by growing *G. europhioides* on malt extract agar (MEA, 2%) for approximately three weeks before inoculation into Norway spruce. On the day of inoculation, MEA colonized by *G. europhioides* was mashed into a slush and loaded into syringes without needles for ease of inoculation.

Trees were inoculated on four occasions from May to August (Fig. 2, Table 1). At each field site, we inoculated five trees from each of four provenances every month, in total 240 trees (except four trees that could

not be recovered after inoculation) (Table 2). The north facing side of the tree was inoculated three times about 1.3 m above ground, with 10 cm between inoculation sites (see Fig. 3). Bark plugs were removed with a 4 mm cork-borer and malt agar slush with living *G. europhioides* was injected into the holes. The bark plugs were then put back and biofilm was wrapped around the tree to keep the plugs in place. The first inoculations were made in mid-May, when overwintering spruce bark beetles normally initiate their main brood. The second inoculations in

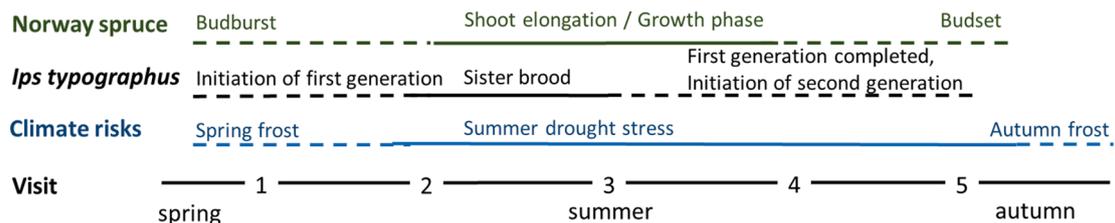


Fig. 2. Schematic representation of Norway spruce shoot phenology (green line), in relation to spruce bark beetle (*Ips typographus*) activity (black line), and climate-related risks for tree damage (blue line). New fungal inoculations were performed at field visit 1–4, and the sampling of previously inoculated trees were carried out at visit 2–5, i.e. inoculation and sampling were coordinated during visit 2–4. Dashed lines represent temporal variability, as influenced by provenance-specific tree phenology and inter-annual variation in temperature conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Timing of field visits, accumulated temperature sums (degree days calculated from January 1, 2019) and monthly precipitation at the field sites used in this study. Meteorological data are from nearby stations of the Swedish Meteorological and Hydrological Institute.

Site	Date of field visit	Degree days (dd, > 5 °C)		Precipitation between visits (mm)
		From Jan 1st	Accumulation between visits	
Skärsnäs	17-May	191	–	58*
	17-Jun	509	318	67
	17-Jul	847	338	37
	19-Aug	1258	411	26
Lugnet	17-Sep	1554	296	47
	20-May	198	–	19*
	18-Jun	496	298	57
	19-Jul	821	325	56
	20-Aug	1229	408	39
Norberg	18-Sep	1528	299	48
	21-May	194	–	19*
	19-Jun	471	277	155
	20-Jul	795	324	66
	21-Aug	1169	374	59
	19-Sep	1406	237	99

* The value for May is the sum for the previous 30-day-period.

Table 2

Number of inoculated Norway spruce trees per field visit, average stem diameters and seasonal variability in necrotic lesion area (mean ± SD) following inoculation with *Grosmannia europhiodes*. Means followed by different letters are significantly different (Tukey’s test following ANOVA, $\alpha = 0.05$; small letters: comparisons within sites between inoculation times; capital letters: comparisons between sites).

Site	Visit	Number of inoculated trees (n)	Stem diameter (mm)		Necrotic lesion area (cm ²)	Necrotic lesion area (cm ²)/tree diameter (mm)
			Total	Growth last 5 years		
Skärsnäs	May	20	79 ± 25	22 ± 6	17.9 ± 5.5 a	0.24 ± 0.08 a
			73 ± 25	24 ± 7	31.1 ± 18.2b	0.43 ± 0.15b
	July	20	83 ± 22	26 ± 7	26.3 ± 10.5 ab	0.32 ± 0.11 a
			73 ± 23	25 ± 11	17.5 ± 4.0 a	0.27 ± 0.12 a
	Total	80	77 ± 24B	23 ± 8B	23.2 ± 12.3 A	0.31 ± 0.14 A
Lugnet	May	19	96 ± 16	44 ± 10	44.2 ± 15.0c	0.47 ± 0.16b
			87 ± 17	42 ± 8	36.7 ± 19.6 cb	0.43 ± 0.21b
	July	20	92 ± 15	40 ± 10	25.7 ± 14.5b	0.27 ± 0.13 a
			93 ± 12	41 ± 7	18.4 ± 9.6 a	0.20 ± 0.11 a
	Total	77	92 ± 15 A	40 ± 11 A	31.4 ± 17.9B	0.34 ± 0.19 A
Norberg	May	20	93 ± 33	32 ± 10	28.6 ± 12.4 bcd	0.33 ± 0.13b
			80 ± 22	29 ± 7	34.4 ± 11.9 dc	0.44 ± 0.13c
	July	19	99 ± 24	36 ± 7	20.8 ± 9.2 ab	0.21 ± 0.08 a
			88 ± 23	30 ± 6	16.8 ± 5.5 a	0.20 ± 0.07 a
	Total	79	90 ± 26 A	30 ± 9 A	25.2 ± 12.0 AB	0.30 ± 0.14 A



Fig. 3. Three necrotic lesions on a Norway spruce tree with the bark pulled aside. The area of the necrotic lesions following *Grosmannia europhiodes* inoculation (light brown zones) was measured on the xylem surface. The white pin in the center of the stem measures 10 mm in diameter.

mid-June represented the timing of any sister broods, and the third and fourth inoculations in mid-July and mid-August represented the timing of a second beetle generation (Fig. 1). The size of necrotic lesions in the bark following fungal infection was measured one month after inoculation. To obtain a quantitative measure of tree defense capacity, the bark on the inoculated part of the stem was removed to expose the necrotic lesions in the inner bark and on the sapwood surface. All lesions were photographed (Fig. 3) and the total area of each lesion on the sapwood surface was measured using ImageJ software (Abramoff et al., 2004).

2.3. Analysis of traumatic resin ducts and 5-year sapwood growth increment

The number and area of traumatic resin ducts (Kane and Kolb, 2010; Rosner and Hannrup, 2004) were quantified from sapwood samples collected in October 2019 at the Lugnet site from a subset of 40 trees (five trees, two provenance classes, and four inoculation times). These trees were representative for all trees and provenances sampled at Lugnet in terms of lesion area. A single sapwood sample was collected from each tree 0.5 m below the inoculation area and 90° counter-clockwise using a 4 mm diameter cork-borer. Sapwood cross-sections were prepared from the cork-borer samples using a sliding freezing microtome and photographed at 50 × magnification (Leica Microsystems CMS GmbH). Traumatic resin ducts were counted over ~2.5 mm tangential width of the cross-sections and total resin duct area was quantified using ImageJ software (Abramoff et al., 2004).

2.4. Data sources, calculations and statistical analysis

Precipitation and temperature data were derived from the closest stations of the Swedish Meteorological and Hydrological Institute (SMHI). Precipitation data came from Olofström (8 km from Skärsnäs), Skjörby (13 km from Lugnet) and Norberg (8 km from Norberg), and temperature data from Hästveda Mo (27 km from Skärsnäs), Enköping Mo (29 km from Lugnet) and Avesta (23 km from Norberg). Temperature data from Avesta was corrected for the difference in elevation between Avesta and Norberg [corrected mean temp_{Norberg} = mean

$\text{temp}_{\text{Avesta}} - (0.065 \text{ } ^\circ\text{C} * 91 \text{ m}_{\text{elevation difference [Norberg -Avesta]}})$. Cumulative temperature sums were calculated as growing degree days (dd) above a threshold of 5 °C, starting from 1 January (Hannerz, 1999), to quantify thermal differences between sites throughout the season.

Variation in soil moisture conditions at the study sites was determined from data from the Swedish Forestry Commission (<https://kartor.skogsstyrelsen.se/kartor/?startapp=skogligagrunddata>). The experimental blocks within sites were classified as relatively moist (ground water level < 1 m deep) or dry (ground water level > 1 m deep). At Skärnsnäs, four out of 10 experimental blocks were situated on a plain surrounded by ditched bogs and had moist soil. Six blocks with dry soil were situated on sloping terrain above the moist blocks. Due to the clear difference between blocks, the effect of soil conditions was analysed for this site. At Lugnet, seven out of 10 blocks were classified as having moist soil conditions. However, since this site was on flat former agricultural land with clay, it had no obvious soil moisture gradient and there were no clear difference between dryer and moister blocks. All 10 blocks at Norberg had relatively dry soil conditions.

To quantify potential differences in stem increment of individual trees over the last 5 years, we calculated the difference between stem diameter measured in September 2019 and stem diameter measured in 2014. To capture genetic components we compared trees from early-flushing Swedish provenances with trees from late-flushing Eastern European provenances. To capture phenotypical components and variation among individuals, budburst data for individual trees were included in the analysis.

Necrotic lesion areas of inoculated trees were analyzed using a three-way ANOVA to compare the three field sites, the two provenance classes with predominately early or late phenology, and the four inoculation times. Two different lesion measures were analyzed: total lesion area per tree (in cm²) and total lesion area relative to stem diameter (in cm²mm⁻¹). By being normalized to tree size the latter measure accounts for the potential importance of tree size for tree defense capacity.

We did a logarithmic regression analysis between necrotic lesion area and precipitation at each study site to assess the effect of cumulative precipitation one to 12 months prior to inoculation. The interval with the best correlation (highest r²-value) was selected for further analysis to separate the effects of timing of inoculation and tree defense capacity. Another logarithmic regression was done to compare tree defense responses between sites with low and high precipitation.

Data on traumatic resin ducts from Lugnet were subjected to a two-way ANOVA to determine if the quantity (number or area) of traumatic resin ducts differed between provenances with early and late phenology or between inoculation times. For comparison between the smaller subset of trees used for resin duct analyses and all trees sampled at Lugnet, necrotic lesion data was tested with a similar ANOVA, to ensure that the subsample was representative of the larger sample. All statistical analyses were performed using JASP Team (2019), JASP (Version 0.9.2).

3. Results

The study sites differed in bioclimatological conditions (Table 1). The temperature sums accumulated between fungal inoculation and measurement of necrotic lesions were in general 20–30 dd lower at Norberg than at the other two sites. The average temperature sums across sites were 298 dd for the period May to June, 329 dd for June to July, 396 dd for July to August, and 277 for August to September. Monthly precipitation in 2019 differed between sites, with Skärnsnäs receiving more rain than the other sites in the spring, and Norberg having a wetter summer than Skärnsnäs and Lugnet (Table 1).

Trees inoculated at Skärnsnäs had significantly smaller stem diameter than trees at Lugnet and Norberg (Table 2). Furthermore, the stem diameter increment during the last 5 years was significantly smaller at Skärnsnäs. At all sites, larger trees tended to have larger necrotic lesions, and the same differences in response were detected between sites, time

of inoculation and provenance classes when tree defense capacity was analyzed in terms of absolute lesion area or normalized lesion area per stem diameter (Table 2 and 3). In the following we therefore focus on absolute lesion areas. At Skärnsnäs and Norberg, lesion size peaked in June (Table 2), whereas lesion size was smaller and about the same in May, July and August. At Lugnet, lesions were largest in May and June, and successively smaller thereafter. The differences in lesion size between Swedish and East European provenances were not statistically significant, and were small in comparison with the differences between inoculation times. At Skärnsnäs, there was a temporal difference in defense capacity between trees growing under different soil moisture conditions (Table 3).

The bud burst data from 2004 confirmed that Swedish provenances on average had earlier bud burst than East European provenances (Fig. 4). There was, however, considerable variation in timing of bud burst among individuals within each provenance class. The proportion of trees categorized as Krutzsch class 1–4 (i.e. having a later bud burst) was 27% for trees of Swedish origin and 39% for trees of East European origin. Early- and late-flushing individuals did not differ in lesion size (Figs. 4 and 5). Only at Skärnsnäs, late-flushing trees tended to develop smaller lesions but the differences were not statistically significant.

The relationship between lesion size and total precipitation during 1 to 12 month periods prior to the inoculation was strongest for the 2-month precipitation sum (r² = 0.63, Fig. 6). The degree of explanation (r²) for the other precipitation sums was between 0.30 and 0.55, but dropped below 0.30 for the 10- to 12-month sums. The 2-month precipitation sum was further analyzed to separate effects of inoculation time and provenance class (Fig. 6c). The analysis indicated that lesions were larger when total precipitation the preceding two months was below 100 mm. In general, the relationship was similar between provenances. There was no correlation between average lesion area for a site and the thermal sum that had accumulated between visits.

At Skärnsnäs, the trees in blocks with moist soil were smaller (mean stem diameter 65 mm ± 3.5, n = 32) than trees in dry-soil blocks (85 mm ± 3.3, n = 48) (t-test, p < 0.001) and they also developed smaller lesions in June and July (Fig. 7). The difference between dry and moist soils was significant when tree defense capacity was calculated in terms of lesion area, but not when defense capacity was expressed as lesion area per stem diameter (Table 3).

In the wood samples collected at Lugnet in October, resin ducts were significantly larger and more numerous in trees inoculated in May and June compare to July and August (Table 4 and 5). East European provenances had more resin ducts than Swedish provenances, with

Table 3

p-values from ANOVA of stem growth and necrotic lesion data from Norway spruce trees inoculated with *Grosmannia europhioides* at three field sites. Data were analyzed in terms of site differences. For one site, Skärnsnäs, a separate analysis was carried out to explore within-site differences in soil moisture conditions. P-values < 0.05 are shown in bold (n = 236).

Variables	df	Lesion area (cm ²)	Lesion area/stem diameter (cm ² mm ⁻¹)
Sites (Skärnsnäs, Lugnet, Norberg)	2	<0.001	0.088
Time of inoculation	3	<0.001	<0.001
Provenance class	1	0.869	0.823
Site * Time of inoculation	2	<0.001	<0.001
Site * Provenance class	27	0.974	0.438
Time of inoculation * Provenance class	18	0.902	0.289
Soil moisture (Skärnsnäs)	1	0.015	0.708
Time of inoculation	3	0.002	<0.001
Provenance class	1	0.939	0.823
Soil moisture * Time of inoculation	3	0.013	0.032
Soil moisture * Provenance class	1	0.993	0.274
Soil moisture * Time of inoculation * Provenance class	3	0.895	0.911

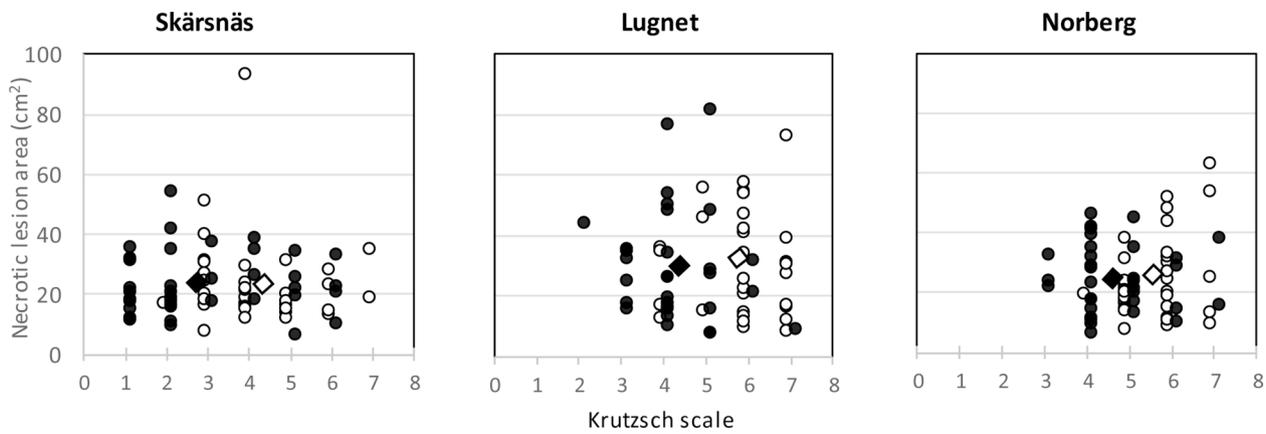


Fig. 4. Individual variation in shoot phenology and necrotic lesion area in Norway spruce trees following inoculation with *Grosmannia europhoides*. The shoot phenology of Swedish (○, predominantly early flushing) and East European (●, predominantly late flushing) provenances was assessed in spring 2004, when trees were 4 years old, using a bud burst scale ranging from 0 to 8, where 0 = ‘dormant bud’ and 8 = ‘all needles more or less scattered, new buds begin to form’ (Krutzsch, 1973). Rhombi indicate mean value of necrotic lesion area and mean Krutzsch-value for Swedish (◇) and East European (◆) provenances.

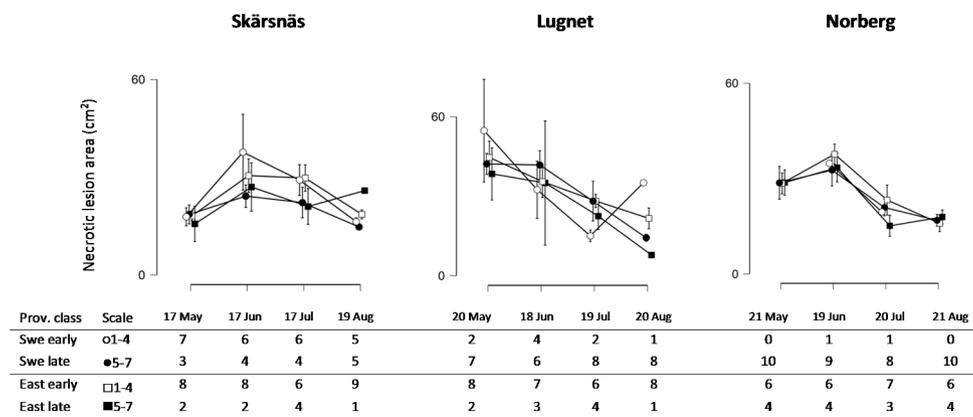


Fig. 5. Seasonal variability in necrotic lesion area in Norway spruce following inoculation with *Grosmannia europhoides* (mean ± SE). The figure panels show the seasonal variation within Swedish (circles) and East European (squares) provenances for both early-flushing (Krutzsch class 1–4) and late-flushing (Krutzsch class 5–7) individuals. The number of sampled trees for each measurement is indicated in the table below the figure panels. See Fig. 4 for a definition of Krutzsch classes.

significant differences between provenance classes for trees inoculated in May and June (Table 5). The relationship between lesion area and resin duct area in individual trees was positive for both provenance classes, but statistically significant only for the East European provenances (Fig. 8).

4. Discussion

In Sweden, the 2018 drought stress event predisposed spruce trees to attack by the spruce bark beetle, with massive tree killing occurring in 2018, 2019 and 2020 (Schroeder and Fritscher, 2020; Wulff and Roberge, 2021). In this field study carried out one year after the drought stress event, we inoculated Norway spruce trees with the bark beetle-associated blue stain fungus *G. europhoides* to study carry-over effects of drought stress on tree defence capacity. Larger necrotic lesions in response to fungal infection are generally considered to be a symptom of lower tree defence capacity. Necrotic lesions differed in size both between sites and between inoculation times in our study. We identified a threshold in tree defence capacity at a precipitation sum of about 100 mm during the last two months before inoculation, with small lesions (about 20 cm² lesion area per tree) at sites with >100 mm precipitation and exponentially increasing lesion sizes at sites with lower precipitation levels (up to 45 cm² lesion area at 50 mm precipitation). We will discuss our results relative to three sets of factors that can influence both tree defence capacity and level of water stress: (1) site-specific

conditions, (2) tree genetics, and (3) seasonal/phenological variability.

4.1. Site-specific differences

Previous studies in Norway spruce have shown that low water availability can alter the strain-specific severity of fungal infestation in spruce trees, leading to larger lesions and increased risk of seedling mortality at dryer sites (Linnakoski et al., 2017; Terhonen et al., 2019). In this study, we detected significant differences in tree defence capacity between sites. However, our first hypothesis of lower defence capacity in trees experiencing the most severe drought was rejected: trees at Lugnet developed the largest lesions following fungal inoculation in May, even though the 2018 drought was most severe at Skärsnäs (Fig. 2). In June, lesion size was more similar across the three sites. Total precipitation over the last two months before inoculation was the best predictor of lesion size ($R^2 = 0.63$), whereas the 12-month cumulative precipitation sum, which included the actual drought period, performed substantially poorer ($R^2 = 0.26$). This indicates that Norway spruce trees have a potential to recover after drought stress, at least up to a base defence level defined by water-dependent defence properties (i.e. resin viscosity and flow). Full recovery of tree defence capacity after drought, including replenishment of non-structural carbohydrate (NSC) stores and repair of drought-damaged xylem, may take substantially longer (Trugman et al., 2018).

NSC availability is important for the production of chemical

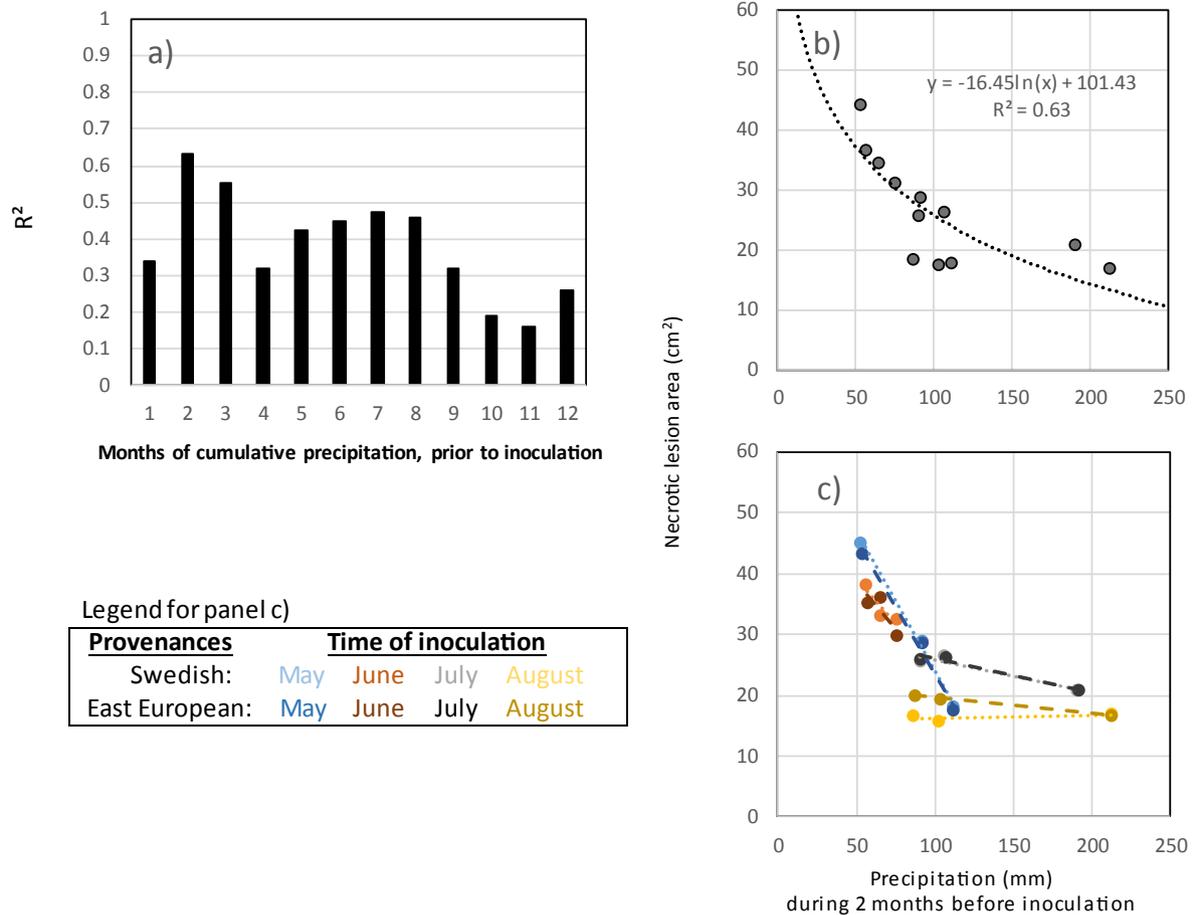


Fig. 6. (a) R^2 -values of logarithmic regressions between necrotic lesion area in Norway spruce following inoculation with *Grossmannia europhoides* and cumulative precipitation over one to 12 month periods prior to inoculation. (b) The logarithmic regression with the highest R^2 -value, corresponding to 2 months cumulative precipitation. Each circle represents the mean lesion area per study site and time of inoculation; $n = 12$ (3 study sites \times 4 inoculation times). (c) Monthly linear regressions of lesion area versus precipitation data from nearby meteorological stations. Lesion data in May was correlated with total precipitation in March-April, lesion data in June was correlated with total precipitation in April-May, etc.. Each circle represents the mean value per study site and time of inoculation, and dotted or dashed lines show the time-dependent regression lines between the three study sites.

defenses. NSCs provide a resource buffer between growing seasons. Severe drought stress can reduce NSC stores because carbohydrates are used to support autotrophic respiration also when photosynthesis is restricted by water limitations (Hartmann and Trumbore, 2016). Such a loss of NSC stores has been indicated by ecosystem modelling (Jönsson and Lagergren, 2018). It has also been observed empirically: the biomass increment of Norway spruce and Scots pine in one growing season is correlated with the net ecosystem exchange of carbon in the previous growing season. Thus, reduced carbon uptake in one season leads to reduced biomass increment the following year (Lagergren et al., 2019). Spruce trees with low NSC stores have been found to prioritize defense, in the form of production of secondary metabolites, over growth and respiration (Huang et al., 2019). The correlation we observed between spruce defense capacity and short-term precipitation may thus rather indicate that water-stressed trees have impaired induction of resin flow (Kolb et al., 2019).

Acute drought leading to tree transpiration deficits has been associated with increased risk of spruce bark beetle attacks, with 35% of Norway spruce stands with a transpiration deficit above 40 mm being attacked (Netherer et al., 2019). Two measurement stations associated with the Integrated Carbon Observation System research infrastructure (ICOS), the Hyltemossa station and the Norunda station, are situated in southern and central Sweden, and trees at both stations were attacked by spruce bark beetles following the 2018 drought. In 2018, both stations showed a decrease in annual net ecosystem productivity (NEP) and

annual evapotranspiration (Lindroth et al., 2020). At Hyltemossa, situated 80 km from Skärnsnäs, the relative precipitation deficit was 0.52 and NEP came close to zero, due to a relatively large decrease in gross primary production (GPP) accompanied by an increase in ecosystem respiration. Norunda, 120 km from Norberg and 170 km from Lugnet, had a relative precipitation deficit of 0.36 and an increase in GPP relative to a normal year. Both the decrease in NEP and increase in ecosystem respiration were thus more pronounced at Hyltemossa than at Norunda, and this is in line with the observation that the 2018 drought stress was more pronounced in southern Sweden (including Skärnsnäs) than in central Sweden (including Norberg and Lugnet).

4.2. Differences between spruce provenances classes

All trees inoculated with the blue stain fungus *G. europhoides* in this study were able to confine the infections and there was no significant difference in lesion size between Swedish and East European provenances. However, we found a general positive relationship between lesion area and resin duct area (Fig. 8), indicating that a stronger local reaction to fungal inoculation triggered a stronger induction of traumatic resin duct formation. This relationship was significant for East European provenances, which also produced more numerous traumatic resin ducts in May and June than the Swedish provenances. The resin duct area, however, did not differ significantly between provenances. Our second hypothesis that Swedish and East European spruce

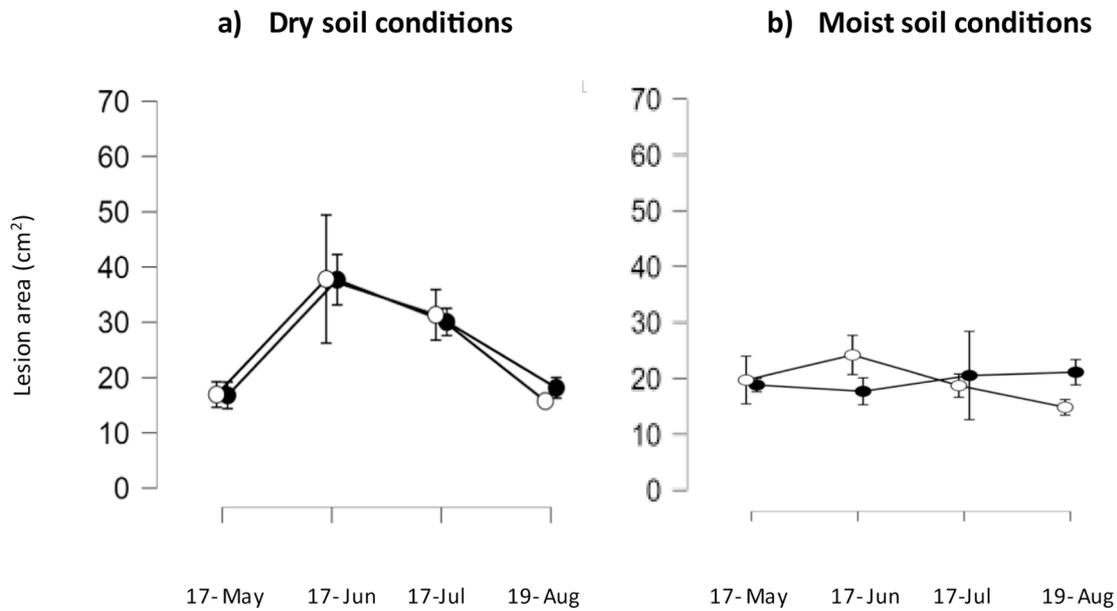


Fig. 7. Seasonal variability in necrotic lesions (mean ± SE) in Swedish (○) and East European (●) provenances of Norway spruce inoculated with *Grosmannia europhoides* at sites with dry and moist soil conditions. (a) Trees growing on dry soil (ground water level < 1 m depth) or on (b) moist soil conditions (ground water level > 1 m depth) in Skårnsnäs (see Table 3b for statistical details).

Table 4

Seasonal variability in area of traumatic resin ducts formed in the last annual ring (2019) of Norway spruce trees following inoculation with *Grosmannia europhoides* (five blocks at site Lugnet, 20 trees per provenance class). For each provenance class, means followed by different letters are significantly different (Tukey’s test following ANOVA, $p < 0.05$).

Time of inoculation	Area resin ducts ($\mu\text{m}^2/\mu\text{m}$ tangential annual ring)	
	Provenance class	
	Swedish	East European
May (n = 5)	18.1 ± 5.8 a	29.8 ± 6.7 a
June (n = 5)	14.8 ± 2.2 ab	25.4 ± 6.1 a
July (n = 5)	4.4 ± 2.5 ab	1.9 ± 0.9b
August (n = 5)	3.4 ± 1.5b	2.6 ± 2.2b
Total n = 20	10.0 ± 2.3	16.0 ± 3.8

Table 5

p-values from ANOVA of number and area of axial resin ducts and necrotic lesions in Norway spruce trees inoculated with *Grosmannia europhoides* (five blocks at site Lugnet, $n_{\text{total}} = 40$). p-values < 0.05 are shown in bold.

Variables	df	Resin ducts		Lesion area
		number	area	
Block	4	0.194	0.209	0.027
Time of inoculation	3	<0.001	<0.001	0.007
Provenance class	1	0.048	0.102	0.324
Time of inoculation *Provenance class	3	0.352	0.180	0.787

provenances differ in defense capacity was thus partly rejected and partly supported. The capacity of Norway spruce trees to respond to infection by increasing the number of resin ducts in the sapwood may be linked to the inherent growth rhythm of the trees. The pattern we observed in May and June may thus reflect differences in growth rhythm between the two provenance classes: East European provenances have a later onset of growth than Swedish provenances (Karlsson, 2009) and produce more traumatic resin ducts as they have a larger degree of freedom to form new structures.

Formation of traumatic resin ducts is part of the induced defense responses of conifer trees. For both Engelmann spruce *Picea engelmannii* (DeRose et al., 2017) and ponderosa pine *Pinus ponderosa* (Kane and

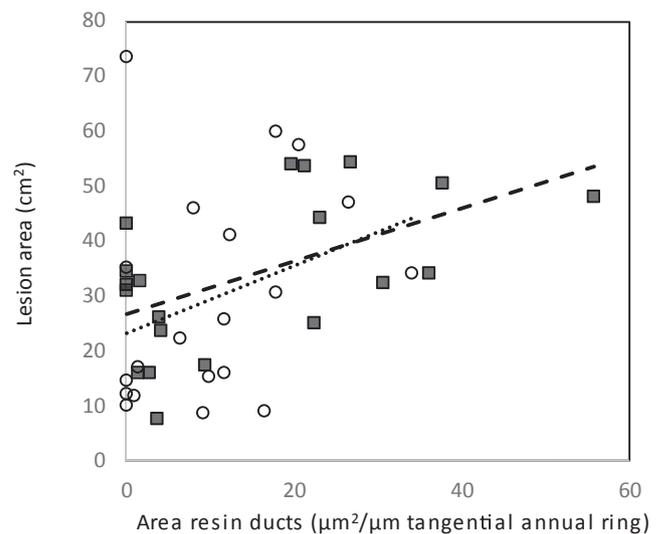


Fig. 8. Correlation between necrotic lesion area in Norway spruce following inoculation with *Grosmannia europhoides* and number and area of traumatic resin ducts in Swedish provenances (○, ..., $r^2 = 0.10$, $p = 0.18$) and East European (■, —, $r^2 = 0.31$, $p = 0.01$) provenances. Data are from trees in five blocks at site Lugnet, $n_{\text{total}} = 40$.

Kolb, 2010) the likelihood that individual trees will survive bark beetle outbreaks has been found to correlate with their capacity to produce traumatic resin ducts. In whitebark pine *Pinus albicaulis*, individuals with large resin ducts relative to sapwood growth had lower mortality, which indicates a trade-off between growth and defense (Kichas et al., 2020). To predict tree growth responses and resistance in a climate change-context it is important to further disentangle how the interplay between tree genetics and environmental factors forms phenotypic responses (Vazquez-Gonzalez et al., 2020). While traditional tree breeding with both Swedish and East European provenances has focused on rapid growth and high wood quality, there might be reasons to also breed for tree resistance to biotic attacks.

4.3. Seasonal differences in tree defense capacity

Both provenance classes displayed seasonal differences in defense capacity. Trees at Skärnsnäs and Norberg provided support for our third hypothesis, stating that lesions would be larger due to a lower tree defense capacity during the period with most active growth, which is June. At Lugnet, the largest lesions were observed in May, and Swedish provenances in particular produced larger lesions in May than in June. This may be explained by the contributing effect of May being dryer, and hence more stressful for the trees, than June at Lugnet. Comparable studies of seasonal defense capacity of Norway spruce to bluestain fungi are sparse, but the few available studies show similar results. Horntvedt (1988), studying 25-year-old trees, and Krokene et al. (2012), studying 2- and 8-year old trees, found tree defense capacity to be lowest in mid-June. Low tree defenses in June, coinciding with the peak of sister brood swarming by the spruce bark beetle (Öhrn et al., 2014), indicate that sister broods may be important for the build-up of univoltine spruce bark beetle populations at higher latitudes and altitudes (Davidková and Doležal, 2017; Wermelinger and Seifert, 1999).

Trees growing on dry soils at Skärnsnäs had lower defense capacity than trees growing on moister soils. It is, however, worth noting that in our study the trees growing on moister soils were also generally smaller. Moreover, the observed difference was not significant when expressed as lesion area per diameter. Lower resistance due to water stress has been observed in earlier studies (Matthews et al., 2018; Netherer et al., 2016; 2015). However, the relationship between tree resistance and drought stress is complex and non-linear, since there is also evidence that mild drought may enhance tree resistance (Christiansen and Glosli, 1996; Zhao et al., 2011a; 2011b).

5. Conclusion

In this field study we found evidence for a threshold in the sensitivity of Norway spruce to drought stress, where trees receiving less than about 100 mm precipitation in the two months preceding fungal infection had lower defense capacity than other trees. The relationship between accumulated precipitation and defense capacity was consistent across three study sites in southern Sweden, all being affected by a severe drought stress event the year before. The observed differences in spruce defense capacity between sites and throughout the growing season were likely caused by site-specific combinations of water- and NSC-limitations, influencing the trees' ability to produce resin-based defenses during their recovery phase following a severe drought. However, because this study included data on traumatic resin ducts from one site only, additional analysis is needed to further elucidate the relationship between resin-based defenses and drought stress. While 2018 was the first example of a drought-induced spruce bark beetle outbreak in southern Sweden, warm and dry summers are projected to become more frequent in this region in response to climate change. An increased awareness about provenance-specific predisposing factors in combination with triggering weather and climate events is therefore needed to identify timely countermeasures to limit the size and consequences of spruce bark beetle outbreaks.

Author statement

All authors contributed to the conceptualization and writing of the manuscript. PÖ carried out the field work, and the data analysis. Financial support was provided by FORMAS project 2019-00097 granted to AMJ.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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