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Drought increases Norway spruce susceptibility to the Eurasian spruce bark beetle and its associated fungi

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Summary

- Drought affects the complex interactions between Norway spruce, the bark beetle *Ips typographus* and associated microorganisms. We investigated the interplay of tree water status, defense and carbohydrate reserves with the incidence of bark beetle attack and infection of associated fungi in mature spruce trees.
- We installed roofs to induce a 2-yr moderate drought in a managed spruce stand to examine a maximum of 10 roof and 10 control trees for resin flow (RF), predawn twig water potentials, terpene, phenolic and carbohydrate bark concentrations, and bark beetle borings in field bioassays before and after inoculation with *Endoconidiophora polonica* and *Grosmannia penicillata*.
- Drought-stressed trees showed more attacks and significantly longer fungal lesions than controls, but maintained terpene resin defenses at predrought levels. Reduced RF and lower mono- and diterpene, but not phenolic concentrations were linked with increased host selection. Bark beetle attack and fungi stimulated chemical defenses, yet *G. penicillata* reduced phenolic and carbohydrate contents.
- Chemical defenses did not decrease under mild, prolonged drought in our simulated small-scale biotic infestations. However, during natural mass attacks, reductions in carbon fixation under drought, in combination with fungal consumption of carbohydrates, may deplete tree defenses and facilitate colonization by *I. typographus*.

Introduction

Outbreaks of the Eurasian spruce bark beetle, *Ips typographus* (L.) (Coleoptera: Curculionidae: Scolytinae), have reached unprecedented levels and resulted in extensive mortality of Norway spruce (*Picea abies*) (L.) Karst. (Pinales: Pinaceae), which may eventually lead to the regional loss of this economically important tree species in Central Europe (Hlásny *et al.*, 2021; Netherer & Hammerbacher, 2022). Well adapted to cool and wet climatic conditions, *P. abies* is a dominant tree species of montane, nemoral and boreal forest ecosystems (Clear *et al.*, 2015; Kameniar *et al.*, 2023). Owing to its excellent wood quality, spruce has also been planted extensively outside its natural range (Seidl *et al.*, 2011). Heat and drought events have been identified as important drivers of *I. typographus* outbreak dynamics (Seidl *et al.*, 2016; Marini *et al.*, 2017; Krejza *et al.*, 2020). However, at tree level scarce evidence exists on how stress-induced physiological and biochemical changes in mature *P. abies* are linked to drought-induced bark beetle outbreaks (Huang *et al.*, 2020a; Netherer *et al.*, 2021).

During drought, trees reduce stomatal conductance to control water loss from transpiration, resulting in persistently lower xylem sap flow rates, but ultimately also in declining predawn twig water potentials (TWP; Gebhardt *et al.*, 2023; Hesse *et al.*, 2023). Such impacts on the tree water status in turn negatively affect defense traits such as resin pressure and flow (Rissanen *et al.*, 2021). Water saving via stomatal closure reduces carbon fixation and shifts the investment of limited resources away from growth. Instead, moderately drought-stressed trees prioritize storage, respiration and other life-sustaining functions such as biosynthesis of defense metabolites (Martinez-Vilalta, 2014; Huang *et al.*, 2021). Yet, partitioning carbon into defense may become too costly during severe or long-term water stress (McDowell, 2011).

Monoterpene olefins and diterpene resin acids are the predominant components of conifer resin accumulating in concentrations of up to 10% of dry weight in the bark of mature trees (Mageroy *et al*., 2020), thus representing a substantial carbon sink. Terpenes and their derivatives accumulate in specialized anatomical structures such as radial and axial resin ducts in the phloem and xylem (Franceschi *et al*., 2005). The detrimental effects of resin on attacking bark beetles are due to its contact toxicity, feeding inhibition (Everaerts *et al*., 1988; Fang *et al*., 2020) and physical trapping of beetles through polymerization of diterpene resin acids (Celedon & Bohlmann, 2019). The living bark or phloem of mature conifers further includes bands of polyphenolic parenchyma (PP) cells that contain a diverse range of phenolic compounds (Li *et al*., 2012). The major phenolics in *P. abies* include the stilbenes astringin and isorhapontin, the flavonoids taxifolin, catechin and procyanidin B1 (PB1) and an assortment of lignans (Hammerbacher *et al*., 2011, 2019). These are present collectively at a concentration of *c*. 10% of phloem dry weight (Zhao *et al*., 2019), and so also represent a substantial carbon sink for trees. Phenolic compounds act as fungicides and antioxidants, for instance to fend off fungal colonization of pine heartwood (Ekeberg *et al*., 2006) and spruce bark (Hammerbacher *et al*., 2014).

The preformed terpene and phenolic defenses of conifer trees can be boosted in response to herbivore or pathogen attack by the formation of traumatic resin canals and the activation of PP cells (Franceschi *et al*., 2005; Mageroy *et al*., 2020; Mercado *et al*., 2023). However, there is little evidence on how drought stress affects the magnitude of such induced defenses (Holopainen *et al*., 2018). In severely stressed trees with constrained carbon uptake, the reduced carbohydrate reserves may limit the extent of induced resistance and increase the success of biotic attacks (McDowell *et al*., 2008; Krokene *et al*., 2023).

Bark beetle attack of *P. abies* involves the transfer of spores or mycelia of facultative fungal symbionts, mainly of the class Ascomycota, which grow in the phloem and wood (Solheim, 1998; Kirisits, 2004; Linnakoski *et al*., 2012). Among the frequent associates of *I. typographus*, *Endoconidiophora polonica* (Siemaszko) Z.W. de Beer, T.A. Duong & M.J. Wingf. and *Grosmannia penicillata* (Gosmann) Goid. are suggested to play an important role in the life cycle of the beetles by supplying nutrients, defending against insect pathogens and detoxifying terpenoids and phenolics (Krokene, 2015; Wadke *et al*., 2016; Six & Elser, 2019). The presence of these fungi early in the bark beetle colonization process likely amplifies the attraction of conspecifics as *E. polonica* is able to synthesize the aggregation pheromone component 2-methyl-3-buten-2-ol (Zhao *et al*., 2015). Furthermore, *G. penicillata* might signal favorable breeding sites by converting monoterpenes into oxygenated derivates (Kandasamy *et al*., 2023).

The role of bark beetle-associated fungi in tree mortality has long been debated (Netherer & Hammerbacher, 2022). Fungal invasion of tree tissues may accelerate the exhaustion of defenses (Krokene *et al*., 1999; Lieutier *et al*., 2009). Virulent fungal species, or strains, cause larger hypersensitive wound reaction zones on weakened trees (Christiansen *et al*., 1987; Sallé *et al*., 2005; Linnakoski *et al*., 2017). Fungal growth may also induce the jasmonate signaling pathway, stimulating tree defense and leading to an accumulation of terpenes in the lesions (Nagel *et al*., 2022). By contrast, no increase in stilbene and flavonoid content was observed despite the upregulation of genes involved in the synthesis of phenolics, likely due to fungal metabolism (Brignolas *et al*., 1998; Hammerbacher *et al*., 2013).

This study is the first rainfall-exclusion experiment employing a systematic field approach that combines the simulation of attack by *I. typographus* and two important associated fungi in a mature spruce forest stand. Experiments were conducted during a period when bark beetle abundance was low. Since few pioneer beetles were landing on potential host trees, tree defenses were expected to strongly determine tree susceptibility to attacks and host selection by *I. typographus* (Supporting Information Fig. S1). We monitored (Q1) soil water content (SWC), tree water status, resin flow (RF) and tree growth and examined (Q2) if initial bark beetle–host tree interactions were reduced or increased in drought-stressed trees (full list of questions and hypotheses in Table S1). We anticipated (Q3) only minor effects of drought on terpene and phenolic defenses (Holopainen *et al*., 2018), but an increased number of attacks with lower concentrations of terpenoid and phenolic compounds. We expected that (Q4) drought would increase the susceptibility of spruce to bark beetle-associated fungi, and (Q5) the combination of drought, bark beetles and fungi boost chemical defenses. Finally, we hypothesized (Q6) an increase in nonstructural carbohydrates (NSC) in response to mild drought and a depletion of primary and secondary metabolites by associated fungal species.

Materials and Methods

Study site and drought stress monitoring

We conducted the drought experiment in a 90–100-yr-old Norway spruce stand (*Picea abies* L., > 90% of basal area) mixed with European beech (*Fagus sylvatica*, < 10%) and Scots pine (*Pinus sylvestris*, < 10%) in the Rosalia Mountains, Lower Austria (47.691228 N, 16.290998 E; decimal degrees, WGS84; Fig. 1a). The slope was slightly northwest exposed, at 650 m AMSL. Over the past 30 yr, mean annual temperatures recorded at the permanent, local weather station (Heuberg) increased from 6.5°C (1990–1999) to 9.8°C (2011–2020) and annual precipitation decreased from 796 to 736 mm.

The drought treatment, established in October 2018, included 10 rain-out shelters (roofs, 8 × 8 m) of 1.20 m height, each enclosing one tree sealed with pond liner, further designated roof trees (1–10). Ten nonsheltered trees at the site served as controls (K1–K10; Fig. 1a,b). Trees were randomly assigned to either roofs or controls, all of which had a DBH of 40–50 cm, 25–30 m height and healthy crowns. We recorded SWC discontinuously at 0–30 cm soil depth by connecting 30 pairs of waveguides, installed at *c*. 1 m distance from the study trees (one per control, two per roof tree), to a portable time domain reflectometry

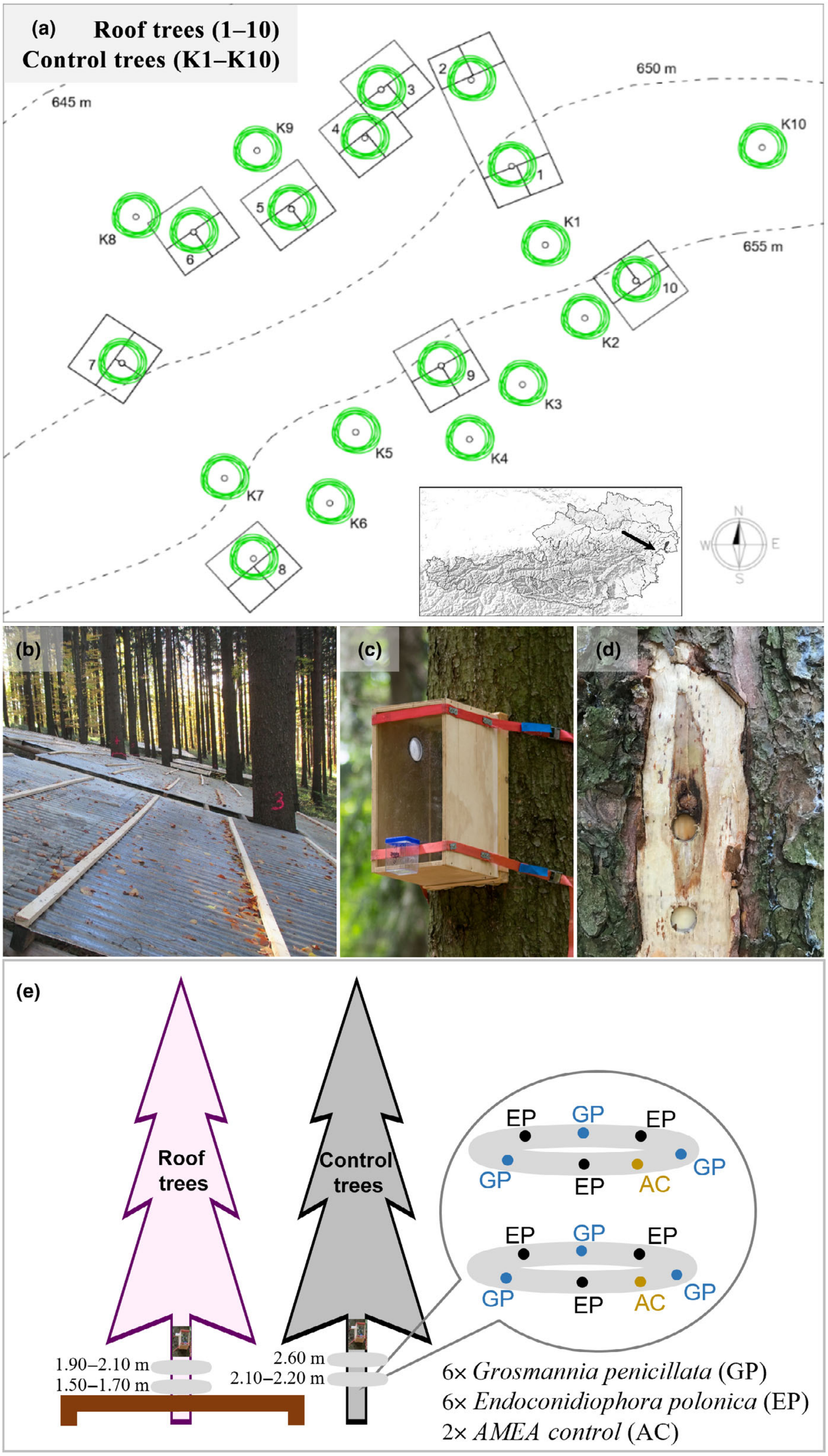

Fig. 1 (a) Location of the study site and view of the experimental set-up in a mature, managed Norway spruce (*Picea abies*) forest including the drought treatment (10 roof trees, 1–10) and 10 nonmanipulated control trees (K1–K10). The roofs measuring 8 × 8 m were established *c.* 1.20 m aboveground. The pictures show (b) the roofs, (c) an attack box mounted on the tree at 5–6 m height on a preattached wooden frame; the frames were used for all bioassays throughout season 1 and changed to a different location on the stem in season 2, and (d) a hypersensitive wound reaction (lesion) caused by inoculation of fungi associated with the bark beetle *Ips typographus*; the lesion was exposed by removing the bark; bark cores were sampled close to and 5 cm below the inoculation hole. (e) Conceptual scheme of the inoculation experiment: each roof and each control tree received 2 × 3 inoculations of *Endoconidiophora polonica* (EP) and *Grosmannia penicillata* (GP) and 2 × 1 inoculations of the agar control (AC) on 6 August 2020. The fungal inoculations were evenly distributed around the trunk in a lower and an upper circle, below the preattached frames for the attack boxes.

(TDR) measurement unit (Trase system 1; Soilmoisture Equipment Corp., Santa Barbara, CA, USA). SWC values at 0–30 and 0–60 cm did not differ at this site (Netherer *et al.*, 2015). We recorded air temperature and rainfall at a weather station set-up in an open area, 300 m from the stand. Twig water potential was measured before sunrise (predawn) using a pressure chamber (Soilmoisture Equipment Corp.) on 21 May, 3 July, 31 July, 28 August 2019 and 20 May, 1 July, 5 August 2020 on twigs taken

Table 1 *Ips typographus* host tree selection bioassays were conducted with beetles in wooden 'attack boxes' mounted on Norway spruce (*Picea abies*) trees from both roof and control treatments.

		2019			2020		
	RD	Roof trees	Control trees	Total	Roof trees	Control trees	Total
Start bottles	−	939	914	1853	328	343	671
Attack boxes	±	285	279	564	471	520	991
Exits	−	311	317	628	283	176	459
Bark	+	29	38	67	176	156	332
Attempted attacks	+	7	8	15	23	17	40
Defended attacks	+	17	31	48	42	37	79
Successful attacks	+	3	7	10	18	10	28
Beetles not recovered	±	79	76	155	249	331	580
Total number		1670	1670	3340	1590	1590	3180

Listed are the number of attacks that were successful (fresh bore holes), defended (repelled by resin) and attempted (bore dust without bore hole), and the total number of beetles found at different locations in the attack box systems or lost during the experiments (not recovered). Column RD indicates response direction, that is whether the presence of beetles at this location signifies attraction: positive attraction (+), unknown (±) or negative attraction (−). All attacks were taken to signify positive (+) attraction.

from the crowns with a shotgun. Mean RF in $mm^3\ h^{-1}$ was calculated from two recordings per tree made at breast height on the north and south sides of the trunks. By collecting resin overnight, we ensured that the flow rates were neither affected by sunlight nor temperature. Minor mechanical wounding near the sampling site did not induce RF (Netherer *et al.*, 2015). Glass tubes (inner Ø = 3 mm, l = 120 mm) were inserted for *c.* 12 h in holes made by a cork borer into the cambium at xylem surface on 21 May, 5 June, 3 July, 31 July, 28 August 2019 and 20 May, 17 June, 1 July, 14 July, 5 August, 19 August and 3 September 2020.

In October 2021, wood cores (Ø = 7 mm) were obtained at breast height and dried at ambient temperature. The transverse surfaces were thereafter shaved 90° to the grain and scanned with a high-resolution flatbed scanner (Epson Perfection V700 Photo). Ring widths (1995–2021) were measured with IMAGEJ software (Schneider *et al.*, 2012) and the mean annual ring widths calculated for both, drought and control treatments.

Attack box bioassays

Host selection by *Ips typographus* (L.), assessed as possible types of initial interactions with the host tree, was monitored every second week from May to September 2019 and 2020 using 'attack boxes' (Netherer *et al.*, 2015). This was achieved by mounting wooden boxes with windows of transparent acrylic glass on preassembled frames on the trees, at a height of 5–6 m. Bark beetles were introduced via start bottles screwed onto the boxes and collected in exit jars as they left the boxes (Fig. 1c). Temperature conditions were similar inside and outside the boxes (Fig. S2). We carried out a total of 18 bioassays on the following consecutive days: 20–21 May, 4–5 June, 18–19 June, 2–3 July, 8–9 July, 30–31 July, 5–6 August, 20–21 August, 27–28 August 2019 and 19–20 May, 2–3 June, 16–17 June, 30 June–1 July, 14–15 July, 28–29 July, 04–05 August, 18–19 Aug and 2–3 September 2020 (Fig. S3a,b). On the first day, the same number of bark beetles (maximum 20), not sexed and freshly emerged from logs in the laboratory, were added to each of the boxes at noon. About 24 h later, the beetles found in the start bottles (displaying low attraction to trees), attack boxes, on the bark, and in the exit jars, where they had landed when orienting toward the natural light (denoting tree avoidance and flight readiness) were counted. The number of beetles that could not be recovered was also recorded (Table 1). Successful attacks (fresh boreholes with bore dust), defended attacks (repelled by resin) and attempted attacks (bore dust without clear bore holes pointing to examination/tasting of the bark) were recorded to assess host selection.

Tree responses to inoculation of associated fungi

We examined induced tree defense after inoculation of *Endoconidiophora polonica* (Siemaszko) and *Grosmannia penicillata* (Gosmann) by measuring the hypersensitive wound reaction (Fig. 1d) and accumulation of terpene and phenolic compounds. Local isolates of *E. polonica* (strain LF/Va/3/3) (EP) and *G. penicillata* (strain LF/Ia/9/1) (GP) were obtained in July 2020 from desiccated sapwood of a *P. abies* log attacked by *I. typographus*. The strains were identified based on morphological characteristics and grown for 13 d on ash leaf malt extract agar (Kirisits *et al.*, 2013) in plastic Petri dishes (Ø = 5.2 cm). For inoculation, we punched out pieces of fungal culture with a cork borer (Ø = 5 mm) with sterile agar serving as a control. Following Netherer *et al.* (2016), each study tree received 2 × 3 inoculations of both EP and GP and 2 × 1 inoculations of agar controls (AC) on 6 August 2020. Inoculations were distributed evenly around the trunk along a lower circle at 160 cm (R) or 210 cm (K) and an upper circle at 210 cm (R) or 260 cm (K) height (Fig. 1e). Two and four weeks postinoculation (2 wpi, 18 August; 4 wpi, 2 September), we sketched the outlines of one EP and one GP lesion per tree on transparent plastic foils after the bark was removed (Fig. 1d). On 16 September (6 wpi), the remaining four EP, four GP and two AC inoculation sites were checked. We measured the area (mm^2) and length (mm) of lesions from the sketches using the software DATINF® MEASURE v.2.1d (DatInf GmbH, Tübingen, Germany).

Bark cores (Ø = 1 cm) were sampled from all trees, shock-frozen in liquid nitrogen and stored at −80°C. The samples were taken from unaffected tissue at the trunk base (at least 50 cm below fungi inoculation) on 19 May, 30 June and 2 September 2020 and from lesions next to and 5 cm below EP and GP inoculation holes on 18 August (2 wpi) and 2 September (4 wpi) 2020 (Fig. 1d). Chemical analyses of bark samples, including periderm and secondary phloem, were performed for 14 of the 20 study trees. We minimized the number of samples per tree and date as multiple sampling may have influenced subsequent results by a wounding effect. We divided bark disks to allow different analyses to be carried out on the same samples. However, for some samples, biomass was not sufficient to do all analyses so that the number of data points are not equal across the different parameters and sampling dates (Table S2).

Monoterpenes and diterpene resin acids (MT and DT)

Portions (100 mg) of frozen bark ground in liquid nitrogen were extracted for 24 h in 1 ml of tert-butyl-methyl ether containing 50.0 μg ml^{-1} 1,9-decadiene (Merck) and 47.3 μg ml^{-1} dichlorodehydroabietic acid (CanSyn Chem Group, Toronto, ON, Canada) as internal standards. The extract was removed from the plant material, made alkaline with 0.3 ml of 0.1 M $(NH_4)_2CO_3$, pH 8.0 and dehydrated using Na_2SO_4. To 0.4 ml of the dehydrated extract, 50 μl of N-trimethylsulfonium hydroxide (Macherey-Nagel GmbH & Co. KG, Düren, Germany) was added for methylation of DT, while the rest of the extract was used for MT analysis. Samples were analyzed using *gas chromatography* (*GC*)-*flame ionization detection* (*FID*) and GC-mass spectrometry according to Schmidt *et al.* (2011). We identified compounds by using identical standards or by comparing their retention times and mass spectra to those of reference spectra in the Wiley and National Institute of Standards and Technology libraries. We quantified compounds using the peak areas obtained from the FID relative to the internal standards (1,9-decadiene for MT and dichlorodehydroabietic acid for DT) and standardized to sample fresh weight.

Phenolic compounds

Phenolic compounds were extracted according to Huang *et al.* (2020b) with minor modifications. Approximately 30 mg of freeze-dried and ground bark was extracted with 1 ml methanol containing 20 μg of apigenin-7-glucoside (Carl Roth GmbH, Germany) as internal standard. The extracts were vortexed for 10 min and centrifuged for 10 min at 13 000 ***g***. The supernatant was collected and the remaining pellet re-extracted with 0.5 ml of methanol. Both supernatants were combined and analyzed with a high-performance liquid chromatography (HPLC)-mass spectrometry (MS) (HPLC, Agilent, Santa Clara, CA, USA; MS, Sciex, Darmstadt, Germany). Phenolic compounds were separated on a Zorbax Eclipse XDB-C18 column (4.6 × 50 mm, 1.8 μm; Agilent) using mobile phase 0.05% (v/v) formic acid (phase A) and acetonitrile (phase B) at a flow rate 1.1 ml min^{-1} (profile Methods S1). All compounds were identified with standards and peak areas quantified by calculating response factors of internal standards (Huang *et al.*, 2019). Linearity of quantification was confirmed by analyzing a gradient of catechin.

Soluble sugars and starch

Soluble sugars (Sol S, sum of glucose, sucrose and fructose) and starch were extracted following Landhausser *et al.* (2018). Ground bark and the internal standard were dried at 60°C. Briefly, *c.* 10 mg of soluble sugars were extracted with 1.5 ml of 80% ethanol, vortexed for 1 min, incubated at 90°C for 10 min and centrifuged at 13 000 ***g*** for 1 min. The supernatant was collected and the pellet re-extracted twice following the same procedures. Supernatants were combined, diluted and analyzed with HPLC coupled to a pulsed amperometric detection (HPLC-PAD) according to Raessler *et al.* (2020). From the remaining pellet, starch was digested with 1.0 ml of α-amylase, vortexed for 1 min, incubated at 85°C for 30 min and centrifuged at 13 000 g for 1 min. An aliquot of supernatants was collected, digested with amyloglucosidase (Sigma-Aldrich) and incubated at 55°C for 30 min (Landhausser *et al.*, 2018). The glucose hydrolysate was collected and measured by HPLC-PAD. We used a multiplication factor of 0.9 to calculate starch as glucose equivalents.

Statistics

We used repeated measures ANOVA, including Mauchly tests for sphericity and adjustment by the Greenhouse–Geisser correction to test for date-related differences in mean TWP, RF, SWC and tree ring widths (Q1) and differences in lesion length and area caused by EP and GP (Q4). Data normality and homogeneity of variances were tested by Shapiro–Wilk and Levene's tests. Independent sample *t*-tests or ANOVA were used to examine differences in mean TWP, RF, tree ring widths, MT, DT, phenolic and carbohydrate contents, and lesion size between treatments (roof/control trees; EP/GP/AC; Q1, Q3–Q6); in case of heteroscedasticity, we applied Welch and Games–Howell *post hoc* tests. Primary and secondary metabolite concentrations before and after the inoculation of fungi were compared by paired sample *t*-tests (Q5, Q6). We ran *chi-squared* statistics with the null hypotheses that in both seasons roof and control trees were equally attacked and that treatments did not differ regarding the proportions of successful and defended attacks (Q2). The strength and direction of the relationships between variables (e.g. number of attacks and TWP or RF; lesion size and MT or DT contents) were determined by Pearson correlation (two-tailed); for non-normally distributed data, we used Spearman's rank correlation. We used multiple linear regression analyses to test for the relative contribution of RF and TWP to host selection (defended and successful attacks, attack attempts) (Q2) and single linear regressions to assess the influence of terpene and phenolic levels on attacks (Q3). Natural log transformation of terpene data served to meet the assumption of normal distribution. Multicollinearity of variables was excluded at a variance inflation factor between 1 and 10. A high goodness-of-fit of the regression models was indicated by an $R^2 \geq 0.26$ (Cohen, 1988). All statistical analyses were performed in IBM SPSS STATISTICS 27 (IBM Corp., Armonk, NY, USA).

Results

Roofs increased drought stress on trees and reduced wood growth, but not resin flow (Q1)

Soil water content varied significantly with the date ($F(4.00, 68.04) = 62.42$, $P < 0.001$, partial $\eta^2 = 0.79$) and was significantly lower under the roofs (9.0–11.7%) than near the control trees (11.4–25.4%; Fig. 2a; Table S3a). The mean SWC on control plots was low from July 2019 to May 2020 since the period from April to September was much warmer and drier in 2019 than in 2020, with a mean temperature of 15.2°C and total precipitation of 479.5 mm (Table S3b). Mean predawn TWP were significantly lower in roof trees (−0.54 to −0.85 MPa) in comparison with controls (−0.35 to −0.62 MPa) on all dates except 21 May 2019 (Fig. 2b, Table S3a). TWP also varied with the date ($F(3.76, 67.72) = 50.41$, $P < 0.001$, partial $\eta^2 = 0.74$) and was lowest for roof trees between 31 July 2019 and 20 May 2020 (minimum of −1.13 MPa, tree 2). In July and August 2020, TWP increased in both treatments, likely due to rainfall (Fig. 2b; Table S3a). TWP correlated with precipitation ($r = 0.61$, $P = 0.148$, $n = 7$) and SWC under the roofs ($r = 0.80$, $P = 0.030$, $n = 7$; Table S4). Drought stress was severe enough to affect wood growth. Tree ring widths showed significant annual variations ($F(6.75, 125.5) = 26.68$, $P < 0.001$, partial $\eta^2 = 0.61$) with a significant decrease in the width values of 2020 and 2021, in comparison with 1995 ($P < 0.001$) and with most other dates, except for 2018 and 2016. Only in 2020 and 2021, tree rings were smaller for trees of the drought treatment compared with control individuals (2020: $t(18) = 3.97$, $P < 0.001$; 2021: $t(18) = 4.18$, $P < 0.001$; Fig. 3). RF varied significantly over time ($F(11, 198) = 14.36$, $P < 0.001$, partial $\eta^2 = 0.44$), with peaks in July and August. The flow increased strongly with air temperature (T Mean: $r = 0.95$, $P < 0.001$, $n = 12$), but was not influenced by SWC (Fig. 2c; Tables S3a, S4).

Prolonged drought increased the number of bark beetles–host tree interactions (Q2)

The beetles' behavior varied across drought treatments, seasons and years (Table 1; Fig. S3a–d). Considering the total number of beetles in all trials, in 2020 the number of beetles found in the start bottles was higher with 55.5% compared with 21.1% in 2019, but lower for those landing in the exits, denoting tree avoidance/lower flight readiness (2020: 14.4%; 2019: 18.8%). The number of beetles inside the attack boxes or sitting on the bark increased from 2019 to 2020, respectively, from 16.9 to 31.2% and from 2.0 to 10.4%. From 2019 to 2020, all attacks increased: successful from 0.3 to 0.9%, defended from 1.4 to 2.5%, and attempted from 0.5 to 1.3%. The ratio of attacked roof and control trees differed significantly between years (2020: 56.1%, 2019: 34.4% roof trees; $\chi^2 = 7.021$, df = 1, $P < 0.05$).

Temperature conditions during bioassays and tree RF rates strongly correlated with bark beetle behavior (season 2; Table S5). The number of beetles landing in the exits increased with mean air temperatures ($r = 0.81$, $P < 0.001$, $n = 18$) and RF ($r = 0.65$, $P < 0.05$, $n = 12$). Surprisingly, the direction of the correlations (positive or negative) between TWP or RF and the number of beetle attacks was the same for attempted, defended and successful ones, so we combined them. RF of roof trees was strongly negatively correlated with the number of successful and defended (total) attacks (RF Max: $r = -0.91$; Table 2) and of beetles on the bark (RF Mean: $r = -0.91$, $P < 0.001$, $n = 12$; Table S5). While the number of attacks on trees with larger negative TWP was lower in year 1 (2019) than in year 2, regardless of treatment ($r = 0.53$, $P < 0.05$, $n = 20$; not shown), the trend was reversed for roof trees (larger negative TWP values led to increased host selection) in 2020 ($P > 0.05$, Table 2). Multiple regression models showed a significant negative relationship between RF Max and total (successful, defended and attempted) attacks on all trees ($R^2 = 0.31$, $F(2, 17) = 3.86$; $P < 0.05$; Fig. S4a) with an even stronger relationship for roof trees (Fig. 4a). However, the influence of TWP was not significant in these models (both treatments: $T = 1.06$; $P = 0.304$; roof trees: $T = 0.92$; $P = 0.391$; Figs 4b, S4b).

Interplay of drought, terpene and phenolic content, and bark beetle host selection (Q3)

Of the total MT, α-pinene accounted for 52.2% (26–81%), β-pinene 38.1% (6–64%), limonene 7.6% (5–11%), camphene 0.9% (0.02–5%), myrcene 0.8% (0.02–2%) and Δ-3-carene 0.4% (0.01–2%) in May 2020. Of the total DT, levopimaric acid accounted for 34.2% (23–47%), dehydroabietic acid 18.3% (6–34%), neoabietic acid 15.4% (8–24%), isopimaric acid 13.6% (9–27%), abietic acid 11.7% (8–22%) and sandaracopimaric acid 6.8% (3–15%). In absence of fungi, total bark concentrations of MT and DT as well as of individual terpene compounds did not differ among drought treatments or dates (May/September 2020; Fig. 5a–h; Table S6a). However, total phenolics differed significantly between treatments (Table S6b), mainly due to a pronounced increase in the stilbene isorhapontin in roof trees in June ($t(12) = 3.49$, $P = 0.004$) and September ($t(13) = 2.82$, $P < 0.05$; Fig. 6a,c). The flavonoids taxifolin and catechin did not differ between treatments (Fig. 6d,e), only the proanthocyanidin B1 (PB1) content increased in September ($t(13) = 2.19$, $P < 0.05$; Fig. 6f).

Terpene levels clearly influenced host selection by *I. typographus*. The number of successful and defended attacks (in total for each tree over the 2020 season) was higher when the MT content (recorded in May) was lower (Fig. 4c), whereby limonene significantly contributed to this outcome (Fig. 4d). Attack numbers also increased at lower total resin acid contents (Fig. 4e), with dehydroabietic acid (Fig. 4f) most strongly influencing host selection. Similar strong negative relationships were found between the total number of attacks and terpene concentrations in September (MT: $R^2 = 0.57$, $F(1, 12) = 15.57$; $P < 0.01$; Fig. S4c; DT: $R^2 = 0.31$, $F(1, 12) = 5.35$; $P < 0.05$; Fig. S4d). Defended attacks were less frequent at higher contents of alpha-pinene ($r = -0.55$, $P < 0.05$, $n = 14$), beta-pinene ($r = -0.63$, $P < 0.05$, $n = 14$), limonene ($r = -0.53$, $P < 0.05$, $n = 14$) and neoabietic acid ($r = -0.57$, $P < 0.05$, $n = 14$; Table S7).

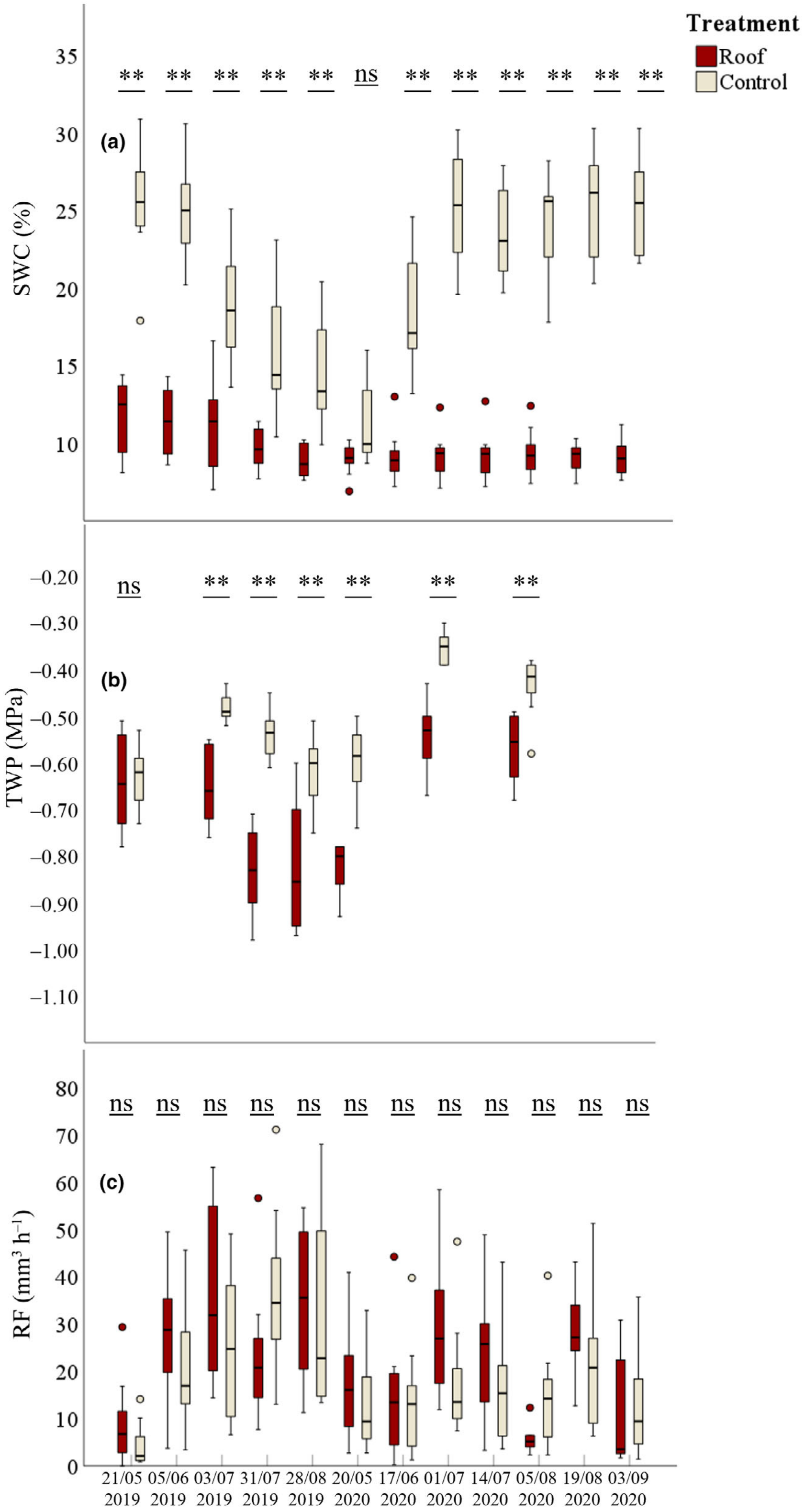

Fig. 2 Boxplots for hydrological and physiological characteristics of 20 Norway spruce (*Picea abies*) study trees by date and drought treatment (roof or control trees). (a) Volumetric soil water content (SWC, %), (b) predawn twig water potential (TWP, MPa), (c) resin flow (RF) recorded within 12 h overnight. All boxplots show median, 25 and 75 percentiles with 1.5× interquartile ranges and outliers. Significant differences are denoted by asterisks above the boxplots ($n = 20$, independent samples t-test with ns, not significant; *, $P < 0.05$; **, $P < 0.001$).

Host selection was weakly correlated with the trees' phenolic contents (Table S7). The number of defended attacks decreased in May and was correlated with increased levels of catechin ($r = -0.46$, $P = 0.098$, $n = 14$) and PB1 ($r = -0.39$, $P = 0.173$, $n = 14$).

Drought-decreased resistance to bark beetle-associated fungi (Q4)

The trees' hypersensitive wound reaction depended on the presence and species of the bark beetle-associated fungus inoculated.

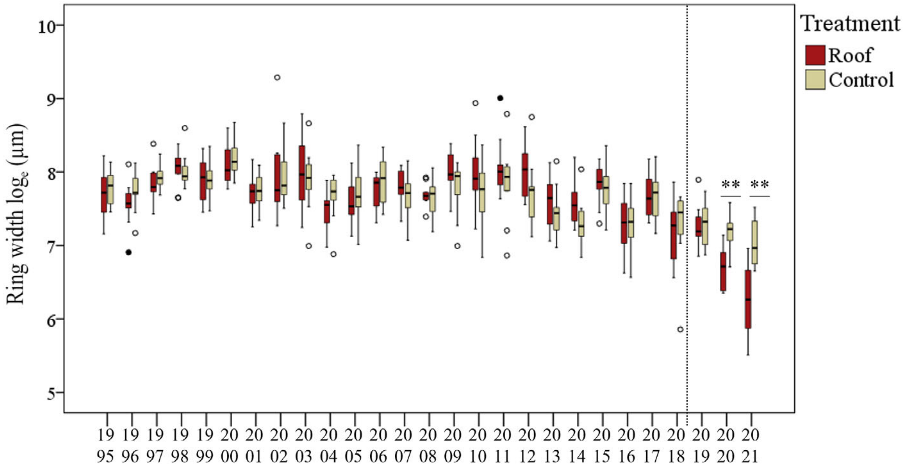

Fig. 3 Boxplots for annual ring width ($\log_e$ µm) of Norway spruce (*Picea abies*) study trees for the time span from 1995 to 2021 determined after cross-dating. The roof ($n = 10$) and control ($n = 10$) treatments were started with the setup of roofs in October 2018, marked by the dashed line. All boxplots show median, 25 and 75 percentiles with 1.5× interquartile ranges and outliers. Significant differences are denoted by asterisks above the boxplots (independent samples *t*-test, *, $P < 0.05$; **, $P < 0.001$).

Table 2 Pearson or Spearman-Rho correlations[1] (*r*) of host selection by *Ips typographus* recorded throughout May to September 2020 with physiological and defense parameters of *Picea abies* study trees.

		Parameters				
Indications of host selection		TWP Mean[2]	RF Mean[3]	RF Max[4]	Rel MT[5]	Rel DT[6]
Roof and control trees						
Total attacks (defended+successful)	*r*	−1.19	−0.29	**−0.49***	**0.67***	0.41
	P	0.420	0.211	**0.030**	**0.009**	0.150
	N	20	20	20	14	14
Total attacks + attempts	*r*	−0.23	−0.42	**−0.52***	**0.64***	0.48
	P	0.332	0.066	**0.020**	**0.015**	0.082
	N	20	20	20	14	14
Roof trees						
Total attacks (defended + successful)	*r*	−0.26	**−0.81****	**−0.91****	**0.81***	0.06
	P	0.474	**0.004**	**<0.001**	**0.050**	0.913
	N	10	10	10	6	6
Total attacks + attempts	*r*	−0.15	**−0.88****	**−0.87****	**0.99****	0.46
	P	0.686	**0.001**	**<0.001**	**<0.001**	0.354
	N	10	10	10	6	6
Control trees						
Total attacks (defended + successful)	*r*	0.17	0.17	−0.02	0.36	0.58
	P	0.631	0.646	0.963	0.387	0.137
	N	10	10	10	8	8
Total attacks + attempts	*r*	0.15	0.08	−0.03	0.22	0.42
	P	0.678	0.818	0.927	0.601	0.307
	N	10	10	10	8	8

[1] Nonparametric tests were employed if data were not normally distributed; high positive and negative correlations are marked by more intense orange and blue colors, respectively. Significant correlations are indicated by bold numbers (*, $P \leq 0.05$; **, $P \leq 0.01$).

[2] Mean predawn twig water potential (TWP).

[3] Mean resin flow rate (RF Mean).

[4] Maximum resin flow rates (RF Max).

[5] Relative increase in monoterpene concentrations (Rel MT).

[6] Relative increase in diterpene concentrations (Rel DT) close to *Grosmannia penicillata* (GP) inoculation holes compared with noninoculated bark tissue 4 wk postinoculation.

Mean lesion areas recorded at 6 wpi (16 September 2020) in both treatments were with significance the smallest for pure AC (327.31 mm^2), intermediate for the fungus EP (737.65 mm^2) and significantly larger for GP (2451.21 mm^2; Table 3). In response to GP, lesions grew in size mainly until 4 wpi, while lesions caused by EP expanded until 6 wpi (Fig. S5a–d). Roof trees formed longer and larger lesions in response to both fungi, although the differences were only significant for GP, 6 wpi (Table 3). Lesion length in response to GP increased with more negative TWP in 2019 ($r = -0.53$) and 2020 ($r = -0.52$), but was not correlated with RF (Table 4). Trees with larger lesions had a higher number of successful bark beetle attacks (Lesion Area, $r = 0.47$; Table 4).

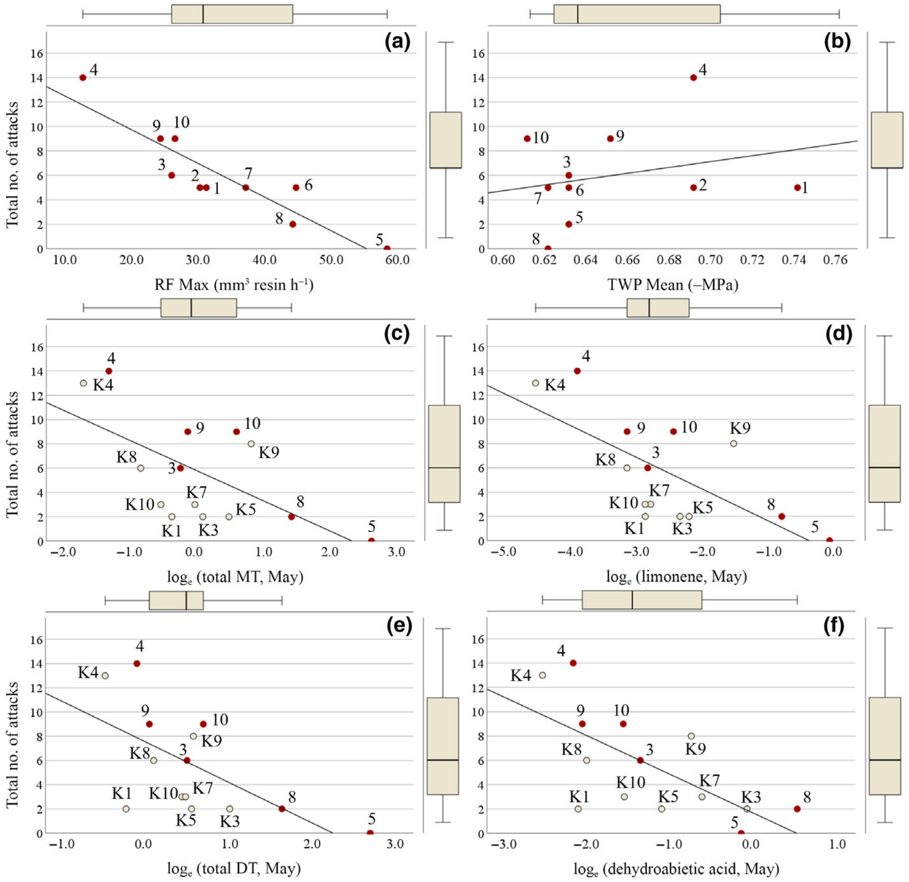

Fig. 4 (a, b) Linear regression plots for the Norway spruce (*Picea abies*) study trees of the roof treatment (red, $n = 10$) with various parameters plotted against the total number of attacks (successful and defended) by *Ips typographus* summed for all bioassays conducted in 2020 per tree. Linear regression plots for seasonal (a) maximum resin flow (RF Max; $T = -6.052$; $P < 0.001$) and (b) mean twig water potential (TWP Mean × (−1); $T = -0.92$; $P = 0.391$; overall model fit: $R^2 = 0.85$, $F(2, 7) = 19.85$; $P = 0.001$). (c–f) Linear regression plots for six roof (red) and eight control (K, white) trees ($n = 14$) with attacks plotted against the natural logarithms of (c) total concentration of monoterpenes (MT) ($R^2 = 0.39$, $F(1, 12) = 7.57$; $P = 0.017$), (d) limonene ($R^2 = 0.47$, $F(1, 12) = 10.69$; $P = 0.007$), (e) total concentration of diterpenes (DT) ($R^2 = 0.38$, $F(1, 12) = 7.85$; $P = 0.018$) and (f) dehydroabietic acid ($R^2 = 0.45$, $F(1, 12) = 9.70$; $P = 0.009$). Boxplots on the right and above each panel show the distributions of dependent and response variables. All boxplots show median, 25 and 75 percentiles with 1.5× interquartile ranges.

Bark beetles, their fungal associates and drought induced the accumulation of terpenes, but not of phenolics (Q5)

Monoterpenes and diterpene resin acids were strongly induced in lesions caused by EP and GP at 2 and 4 wpi in comparison with noninoculated tissues (Fig. 5a–h; Table S6a). The relationship between lesion area and MT content in response to EP was positive and significant ($r = 0.60$; Table 4). Regarding GP, lesion length was significantly and positively correlated with induced levels of DT ($r = 0.62$) but weakly negatively correlated with MT. Drought had no effects on EP- and GP-induced production of MT, except for camphene, which increased significantly in roof trees in August (GP) ($t(12) = -2.38$, $P < 0.05$) and September (EP) ($t(12) = 3.58$, $P < 0.05$). By contrast, the induction of total and most individual DT by EP and GP was stronger under drought at 2 and 4 wpi (Fig. 5e–h; Table S6a).

As for phenolics, drought amplified the induction of isorhapontin by EP, although this was only significant at 2 wpi, 5 cm below inoculation holes ($t(6) = 3.48$, $P < 0.05$). The contents of the flavonoids catechin and PB1 slightly increased, while taxifolin decreased under drought (Fig. 6a–f; Table S6b). After inoculation with GP, however, phenolic contents were not affected by drought (Table S6b).

Host selection was positively correlated with the relative increase in monoterpenes (Rel MT) and the relative increase in diterpene resin acids (Rel DT) in lesions close to GP inoculation holes, compared with the concentrations in tissues without fungus (Table 2). Particularly in roof trees, there was a strong correlation between attempted attacks and the relative increase in MT content ($r = 0.99$).

Associated fungi reduced phenolic and carbohydrate content but drought showed weak effects (Q6)

GP but not EP significantly reduced the concentration of total phenolics, in comparison with uninfected tissues in June (close to inoculation: $t(6) = 18.11$, $P < 0.001$; 5 cm below: $t(8) = 5.08$, $P < 0.001$) and in September (close to inoculation: $t(5) = 3.19$, $P < 0.05$). Consequently, the phenolic content in the GP-induced lesions was significantly lower on all dates and at all sites except for cores sampled 5 cm below inoculation holes in September (Fig. 7a; Table S8a). Soluble sugars (Sol S) and starch were significantly lower in lesions caused by EP and GP, resulting in a strong decrease in NSC compared with June concentrations (close to inoculation: EP $t(10) = 5.01$, $P < 0.001$; GP $t(9) = 10.23$, $P < 0.001$; 5 cm below: EP $t(5) = 11.07$, $P < 0.001$; GP $t(10) = 11.95$, $P < 0.001$). NSC content was (significantly) lower in GP-induced lesions on all dates (Fig. 7b; Table S8a).

Carbohydrate contents varied only slightly with drought treatment. Drought led to increased starch concentrations, irrespectively of the intensity of bark beetle attack and fungal inoculations. This increase was significant for locations close to GP inoculations in September (4 wpi) ($t(8) = 3.21$, $P < 0.05$; Table S8b). Consequently, NSC levels tended to increase under drought, with the exception of EP tissue sampled in August, 2

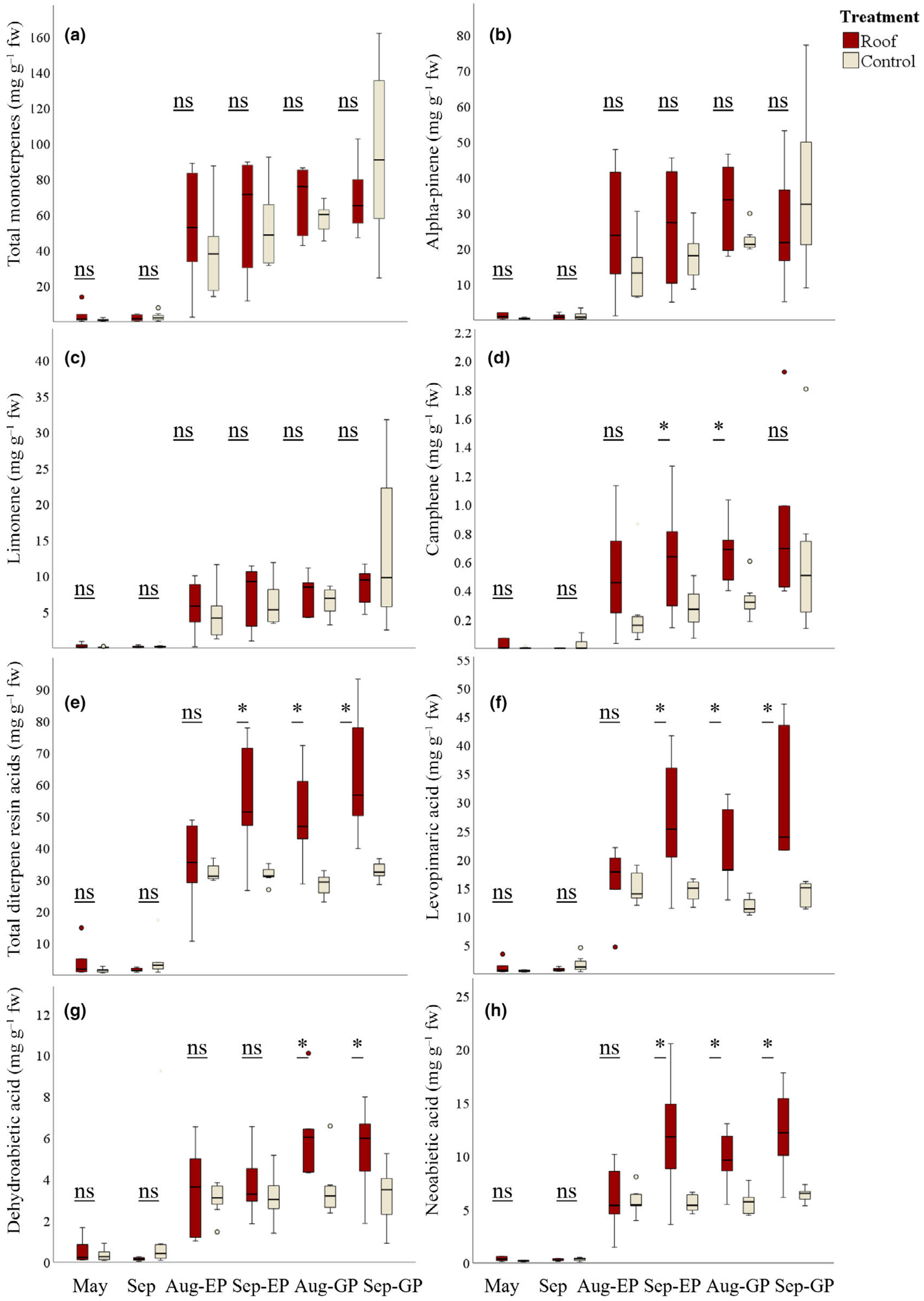

Fig. 5 Boxplots for concentrations of (a) total monoterpenes and the individual compounds (b) alpha-pinene, (c) limonene, (d) camphene as well as of (e) total diterpene resin acids and the individual compounds (f) levopimaric acid, (g) dehydroabietic acid and (h) neoabietic acid in mg g^{-1} fresh weight of bark (periderm and phloem) sampled from six roof and eight control Norway spruce (*Picea abies*) trees. Contents were determined from bark tissue without fungal infection in May and September 2020, while amounts induced by *Endoconidiophora polonica* (EP) and *Grosmannia penicillata* (GP) were determined from cores taken from hypersensitive wound reaction zones close to inoculation holes in August and September 2020. All boxplots show median, 25 and 75 percentiles with 1.5× interquartile ranges and outliers. Significant differences are denoted by asterisks above the boxplots (independent samples *t*-test with ns, not significant; *, $P < 0.05$; **, $P < 0.001$).

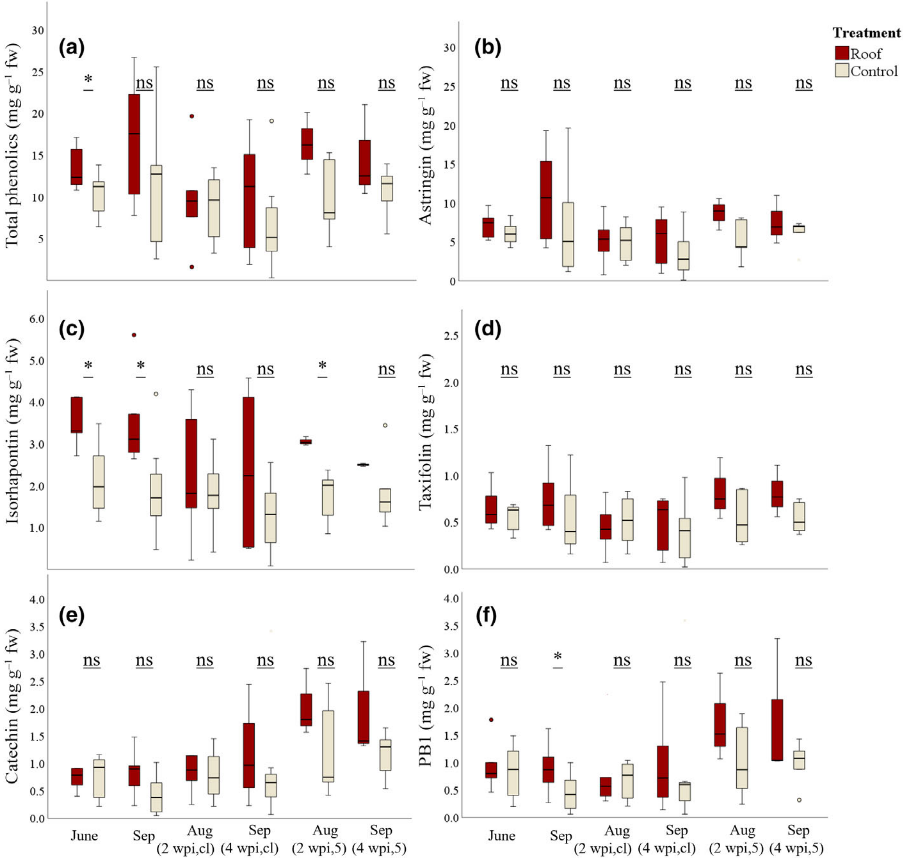

Fig. 6 Boxplots for concentrations of (a) total phenolics and individual compounds: the stilbenes (b) astringin and (c) isorhapontin, and the flavonoids (d) taxifolin, (e) catechin and (f) PB1, procyanidin B1 in mg g^{-1} fresh weight of bark (periderm and phloem) sampled from roof and control Norway spruce (*Picea abies*) trees. Contents were determined from bark tissue without fungal infection in June and September 2020, while induced concentrations were determined from hypersensitive wound reaction zones close (cl) and 5 cm below (5) the inoculation holes of *Endoconidiophora polonica* in August (2 wpi, weeks postinoculation) and September 2020 (4 wpi). Number of samples for roof/control trees = 6/8 (June), 7/8 (September), 6/7 (August, September, cl) and 3/5 (August, September, 5). All boxplots show median, 25 and 75 percentiles with 1.5× interquartile ranges and outliers. Significant differences are denoted by asterisks above the boxplots (independent samples *t*-test with ns, not significant; *, $P < 0.05$; **, $P < 0.001$).

wpi, which contained less Sol S, in particular fructose ($t(10) = -3.11$, $P < 0.05$). Sol S concentrations were equally high close to EP and GP, for both treatments, in September (Table S8a).

Discussion

Periods of drought and warmer temperatures have a negative impact on tree resistance to biotic disturbances and can promote the development of certain herbivorous insects such as bark beetles (McNichol *et al*., 2022). To understand how environmental stress increases tree susceptibility to the initial attacks of bark beetles and potentially leads to population build-up and mass outbreaks, it is necessary to consider the complex relationships between host trees, bark beetles and their associated microbes (Netherer *et al*., 2021; Niinemets *et al*., 2021). Here, we observed increased susceptibility of mildly drought-stressed, mature *P. abies* trees to the attack of *I. typographus* and infection by beetle-associated fungi. However, these trees could still induce terpene and phenolic defenses. Bark beetles more often attacked trees with lower rates of terpene resin exudation and lower contents of monoterpenes and diterpene resin acids. Phenolic compounds played a less important role in host selection and were even reduced in the presence of fungi, especially *G. penicillata*. Fungal infection also reduced the levels of starch and soluble sugars. We argue that the combined effect of continuous attacks by pioneer beetles, fungal invasion and drought eventually increase tree susceptibility to bark beetle invasion and promote mass attacks of entire forest stands.

Spruce resistance to bark beetles decreases during prolonged drought

The roof treatment strongly reduced SWC in the main rooting layer and clearly affected the water status of roof trees. Minimum predawn TWPs of −0.98 to −1.13 MPa recorded in the second study season correspond with values reported for *P. abies* in other drought manipulation experiments (Lu *et al*., 1995) or subjected to naturally occurring water deficits (Rothe & Roloff, 2002). These values indicate that our treatment caused a mild but prolonged drought, compared with the known thresholds of −3.3 to −3.5 MPa inducing 50% loss of hydraulic conductivity (Cochard, 1992; Mayr *et al*., 2002). Previously, Netherer *et al*. (2015) recorded TWP of −2.80 to −4.15 MPa in spruce trees enclosed by larger roofs and therefore under lower water availability. Mean TWP of all trees decreased during 2019, but then increased again, likely due to nearly 80% of average annual rainfall occurring from April to September 2020.

Despite the mild drought conditions, there was a significant decline in annual wood increment in 2020 and 2021, indicating that the roof trees invested less resources into growth. However, RF rates of the roof trees were not significantly affected by the drought treatment, yet, in accordance with earlier findings, were highly variable and strongly correlated with temperature differences between sampling dates (Netherer *et al*., 2015). These observations confirm the assumption that trees maintain defensive traits under mild to moderate stress (Herms & Mattson, 1992; Lombardero *et al*., 2000). Only severe or extended drought

Table 3 Length and area of hypersensitive wound reaction zones (lesions) established by *Picea abies* study trees, recorded 2, 4 and 6 wk postinoculation (wpi) of *Endoconidiophora polonica* (EP) and *Grosmannia penicillata* (GP).

				Lesion length (mm)[1]		Lesion area (mm^2)[1]	
Date/wpi	Treatments[2]	Groups[2]	N	Mean	95% CI	Mean	95% CI
18 August 2020 2 wpi	R + K	**EP**	20	**45.66^a**	**(34.13, 57.20)**	**446.34^a**	**(307.36, 585.33)**
		GP	20	**147.05^b**	**(130.24, 163.86)**	**1949.63^b**	**(1612.60, 2286.66)**
	EP	R	10	50.50^a	(31.13, 69.86)	483.22^a	(255.39, 711.05)
		K	10	40.83^a	(24.87, 56.78)	409.46^a	(204.96, 613.97)
	GP	R	10	154.98^a	(125.67, 184.29)	2087.22^a	(1494.52, 2679.92)
		K	10	139.12^a	(117.59, 160.64)	1812.05^a	(1379.13, 2244.97)
02 September 2020 4 wpi	R + K	**EP**	20	**51.21^a**	**(41.20, 61.23)**	**491.67^a**	**(377.88, 605.46)**
		GP	20	**209.58^b**	**(166.46, 252.69)**	**2940.70^b**	**(2084.09, 3797.30)**
	EP	R	10	51.63^a	(36.51, 66.75)	504.73^a	(323.78, 685.68)
		K	10	50.79^a	(34.49, 67.09)	478.61^a	(302.74, 654.47)
	GP	R	10	225.34^a	(142.60, 308.07)	3374.01^a	(1625.92, 5122.09)
		K	10	193.82^a	(148.64, 239.00)	2507.39^a	(1915.97, 3098.80)
16 September 2020 6 wpi	R + K	**EP**	80	**69.13^a**	**(61.75, 76.51)**	**737.65^a**	**(630.54, 844.76)**
		GP	80	**187.58^b**	**(166.55, 208.62)**	**2451.21^b**	**(2134.78, 2767.64)**
		AC	40	**34.47^c**	**(28.27, 40.66)**	**327.31^c**	**(246.95, 407.67)**
	EP	R	40	71.51^a	(59.75, 83.27)	776.78^a	(589.82, 963.73)
		K	40	66.75^a	(57.35, 76.14)	698.52^a	(585.72, 811.31)
	GP	**R**	40	**217.31^a**	**(181.16, 253.47)**	**2839.41^a**	**(2296.13, 3382.70)**
		K	40	**157.86^b**	**(138.93, 176.78)**	**2063.01^b**	**(1759.49, 2366.52)**

[1]Mean values for lesion lengths and areas according to [2]treatments and compared groups (R, roof trees; K, control trees; EP; GP; AC, agar control) are shown with 95% credible interval (CI). Significant differences between groups (ANOVA, $P \leq 0.05$) are indicated in bold and different superscript letters (a, b, c).

eventually leads to decreases in resin exudation in spruce and pine trees (Gaylord *et al.*, 2013; Netherer *et al.*, 2015). Nonetheless, our bioassays show that drought increased *I. typographus*–host tree interactions. During the first study year host selection by bark beetles was low, yet increased in the second year in both treatment groups, most notably for the roof trees (3, 4, 9 and 10) that had previously been spared from attack. Roof trees with comparably lower water potential and reduced RF capacity were more often attacked, but it did not matter whether trees immediately reacted to beetle attacks with resin exudation or not. Strong RF capacities alone reduced attacks and attack attempts. Such relationships were neither found for the control trees nor for trees of both treatments in the first year; suggesting that trees become more susceptible to bark beetle attack (or more attractive to beetles) during prolonged drought and so increasingly depend on defense traits for resistance.

Terpene and phenolic compounds are only slightly affected by drought, but these metabolites are important in bark beetle host selection

The effectiveness of oleoresin defenses against bark beetles increases with the concentration of mono- and diterpenes (Celedon & Bohlmann, 2019). Warming has a positive effect on the volatility of terpenes, while drought tends to reduce emission rates; however, less is known about stress-induced changes in tissue concentrations (Holopainen *et al.*, 2018) and changes in bark permeability with moisture content (Rosner & Morris, 2022). Although our drought treatment group did not differ significantly from the controls in RF and terpene content in the bark tissue, these parameters attained the highest values in some of the least stressed trees (5, 6 and 8), and the lowest values in one of the most stressed trees (4) (Figs 4, S4). In fact, a more severe drought might amplify this trend, by acting in conjunction with additional biotic stress factors. In accordance, Kolb *et al.* (2019) demonstrated that severely water-limited mature *Pinus ponderosa* trees were less able to mobilize resin defenses against bark beetle attack than control trees. By contrast, monoterpene contents increased in twigs of drought-stressed potted *Pinus edulis*, especially under hot conditions (Trowbridge *et al.*, 2021), as well as in stressed *Pinus halepensis*, *Pinus sylvestris* and *P. abies* seedlings and saplings (Kainulainen *et al.*, 1992; Turtola *et al.*, 2003; Kelsey *et al.*, 2014). These observations point to an ontogenetic influence on drought-induced changes in terpene defense.

In the present study, the overall effect of drought on phenolic compounds was weak, although roof trees responded to drought with increased bark concentrations of the stilbene isorhapontin and of the flavonoid PB1. The specialized phloem cells producing phenolics are known to be alive even in 100-yr-old trees (Li *et al.*, 2012), the approximate age of spruce in this study. Phenolics protect trees from a variety of biotic and abiotic factors, including oxidative and drought stress (Rosemann *et al.*, 1991; Song *et al.*, 2016), or herbivore attack (Singh *et al.*, 2021). Certain phenolic compounds such as isorhapontin were originally suggested as indicators of *P. abies* susceptibility to bark beetles and their associated fungi, or proposed as resistance markers, such as (+)-catechin (Brignolas *et al.*, 1995, 1998). Direct evidence for their protective effect was found when an artificial diet enriched with catechin and taxifolin reduced tunneling activity and weight gain of *I. typographus* adults (Faccoli &

Table 4 Pearson or Spearman-Rho correlations[1] (*r*) of physiological and defense parameters of *Picea abies* study trees recorded in 2019 or 2020 and host selection by *Ips typographus* (May to September 2020) with lesion length and lesion area in response to inoculation of *Endoconidiophora polonica* (EP) and *Grosmannia penicillata* (GP).

Parameters		Lesion length	Lesion area
TWP mean 2019	*r*	**−0.53***	**0.51***
	P	**0.015**	**0.022**
	N	**20**	**20**
TWP mean 2020	*r*	**−0.52***	**−0.46***
	P	**0.020**	**0.041**
	N	**20**	**20**
RF mean 2020	*r*	0.01	−0.03
	P	0.971	0.890
	N	20	20
Defended attacks	*r*	−0.25	−0.28
	P	0.280	0.233
	N	20	20
Successful attacks	*r*	0.43	**0.47***
	P	0.060	**0.036**
	N	20	**20**
MT (4 wpi, EP)[2]	*r*	0.41	**0.60***
	P	0.143	**0.023**
	N	14	**14**
DT (4 wpi, EP)[3]	*r*	0.30	0.35
	P	0.303	0.215
	N	14	14
MT (4 wpi, GP)[2]	*r*	−0.28	−0.09
	P	0.326	0.759
	N	14	14
DT (4 wpi, GP)[3]	*r*	**0.62***	0.28
	P	**0.019**	0.334
	N	**14**	14

[1] Nonparametric tests were employed if data were not normally distributed; high positive and negative correlations are marked by more intense orange and blue colors, respectively.
Significant correlations are indicated by bold numbers (*, $P \leq 0.05$; **, $P \leq 0.01$); total concentrations of [2]monoterpenes (MT) and [3]diterpenes (DT) in bark and phloem measured 4 wk postinoculation (4 wpi) of EP and GP.

Schlyter, 2007; Hammerbacher *et al.*, 2019). Here, increased catechin and PB1 contents in May were actually associated with fewer entrance holes from May to September, which might be due to feeding inhibition by flavonoids. However, host selection in our field bioassays was less dependent on concentrations of phenolics than on terpenes.

Apart from drought effects, our bioassays clearly demonstrate the importance of resin monoterpenes and diterpenes in the selection process by pioneer *I. typographus*. Attack frequency decreased with increasing levels of all major and minor compounds detected. This negative relationship was particularly significant for limonene and dehydroabietic acid contents in May. Resin traits likely affect successful colonization of *P. abies*, even at high beetle infestation rates. During a mass attack of *I. typographus* in Sweden, the phloem of surviving *P. abies* trees contained significantly greater amounts of limonene and 1-8-cineole than the bark of killed trees (Schiebe *et al.*, 2012). Similarly, increased limonene and Δ-3-carene concentrations enhanced the resistance of *P. contorta* var. *latifolia* to mountain pine beetle attack (Erbilgin *et al.*, 2017). In another study with *P. abies*, bark beetle infestation was prevented by high levels of monoterpenes induced after inoculation of *E. polonica* (Zhao *et al.*, 2011).

Drought increases susceptibility of Norway spruce to infection by bark beetle-associated fungi

The infection of bark beetle-associated fungi in phloem tissue is facilitated by beetle transmission, which we here simulated by artificial inoculation. These fungi typically cause necrotic lesions indicating the defense reaction of trees (Solheim, 1998; Franceschi *et al.*, 2000; Lieutier *et al.*, 2009). The fungal species used, *G. penicillata* (GP) and *E. polonica* (EP), are potential tree pathogens, but differ in their infection strategies. EP, which mainly grows into the sapwood, resulting in blue staining and occlusion of water-conducting cells, caused smaller and slower hypersensitive wound responses in our study trees than GP (Krokene & Solheim, 1997). Among the common fungal associates of *I. typographus*, GP can invade the secondary phloem most extensively (Zhao *et al.*, 2019). Significantly longer and larger lesions were formed in roof than in control trees following infection by GP (but not EP), suggesting that drought-stressed trees were less capable of restraining GP growth. The size of the GP lesions increased with the severity of the drought that affected the trees, both in the current and in previous years. The negative effects of past/prolonged drought on tree resistance to bark beetle-associated fungi were previously demonstrated in inoculation experiments with *G. europhioides* in a 20-yr-old *P. abies* stand (Öhrn *et al.*, 2021) and *G. clavigera* on 60-yr-old *Pinus contorta* × *banksiana* hybrids (Arango-Velez *et al.*, 2014).

Drought and bark beetle attack amplify the accumulation of terpenes induced by associated fungi

The study trees showed strong local defense reactions to fungal infection within 2–4 weeks after inoculation, which corresponds to the period normally required for spruce to resist to attacks of *I. typographus* and associated fungi (Zhao *et al.*, 2010, 2011). Compared with uninfected tree tissues, the relative increase in terpenes was pronounced in trees of both treatments after infection by both fungal species. EP and GP triggered a similar accumulation of monoterpenes and diterpene resin acids in the necrotic areas, yet the content of monoterpenes was positively correlated with lesion size for EP only (Viiri *et al.*, 2001). We found a particularly high accumulation of camphene, myrcene and Δ-3-carene compared with the resin of uninfected tissue of the same trees, as well as for limonene (26- to 57-fold) that can effectively inhibit fungal growth (Zeneli *et al.*, 2006; Novak *et al.*, 2014). Drought did not additionally increase the accumulation of monoterpenes, with the exception of camphene. By contrast, diterpene resin acids were more strongly induced in roof than in control trees as well as in trees with larger GP-induced lesions. Diterpenes are known to be particularly important defense mechanisms against blue-stain and other pathogenic

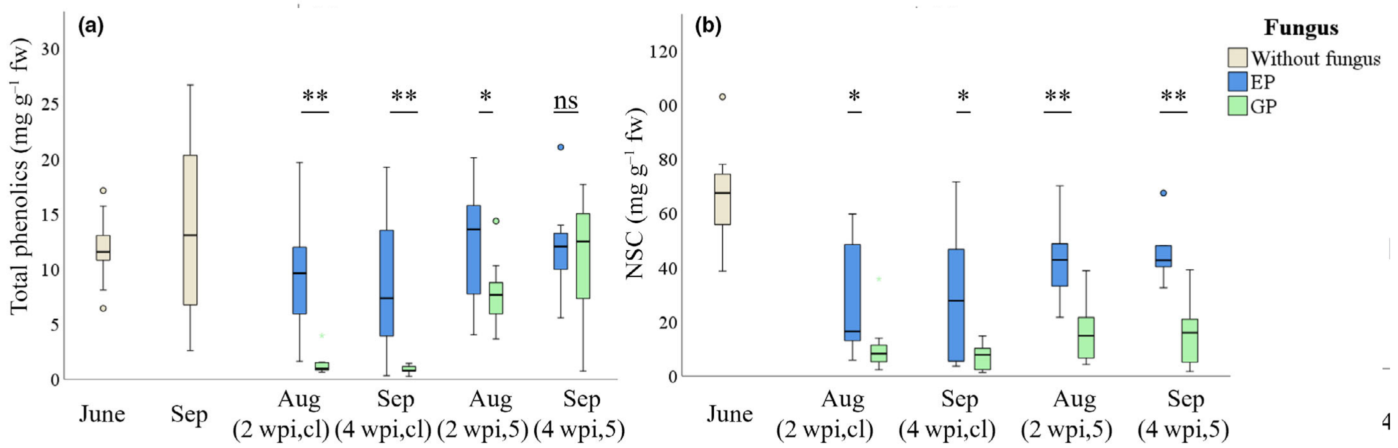

Fig. 7 Boxplots for concentrations of (a) total phenolics and (b) nonstructural carbohydrates (NSC) in mg g^{-1} fresh weight of phloem sampled from Norway spruce (*Picea abies*) study trees. Contents were determined for bark without fungal infection in June and September 2020, while induced concentrations were measured in August (2 wpi, weeks postinoculation) and September 2020 (4 wpi) for cores sampled from the lesions close (cl) and 5 cm below (5 cm) inoculation holes of *Endoconidiophora polonica* (EP) and *Grosmannia penicillata* (GP). The number of samples *N* for total phenolics and NSC in vital tissue = 14 and 11 (June), 16 (September); for EP/GP = 13/7 and 12/11 (August, cl), 13/6 and 12/10 (September, cl), 8/9 and 6/12 (August, 5), 8/10 and 6/12 (September, 5). All boxplots show median, 25 and 75 percentiles with 1.5× interquartile ranges and outliers. Significant differences are denoted by asterisks above the boxplots (independent samples *t*-test with ns, not significant; *, $P < 0.05$; **, $P < 0.001$).

fungi such as *Heterobasidion* spp. (Axelsson *et al*., 2020; Jyske *et al*., 2020).

In our bioassays, the successful, defended and attempted attacks of bark beetles triggered additional defensive compound increases, especially in monoterpenes. *Picea abies* apparently invests in terpene defenses as an immediate reaction to minor or even unsuccessful attacks (Christiansen *et al*., 2007). Such an upregulation of inducible defenses, triggered by single or combined abiotic and biotic stress elicitors, may prepare trees for further attacks (Mageroy *et al*., 2020; Nagel *et al*., 2022). However, it is likely that an increased terpene production under drought, bark beetle attack and/or fungal infestations may divert resources away from tree growth and maintenance processes (Huang *et al*., 2019). This is supported by the smaller annual rings of roof trees in the second and third year after the onset of drought (Fig. 3). Yet, as carbon costs of terpene and phenolic defenses are high, prolonged drought could promote a progressive reduction in defenses (McDowell *et al*., 2008; Huang *et al*., 2020a).

Associated fungi contribute to a faster depletion of primary and secondary metabolites

Phenolic compounds, such as stilbenes and flavonoids, have long been known to act as plant defenses because of their toxicity and deterrence to herbivores and pathogens (Yu *et al*., 2005; Ullah *et al*., 2017). At the same time, these metabolites can be tolerated by the fungal flora associated with *I. typographus* (Evensen *et al*., 2000) because of their ability to metabolize or even use them as carbon sources (Hammerbacher *et al*., 2013; Wadke *et al*., 2016). We showed that GP has a higher capacity than EP to degrade phenolics (Zhao *et al*., 2019) and that the tissues' soluble sugar and starch reserves were more efficiently consumed near the GP inoculation sites. The utilization of spruce carbohydrate reserves by bark beetle fungi has been previously reported (Lahr & Krokene, 2013) and may significantly hamper the production of new defenses, thus enhancing the probability of successful attack by the beetles and their associated fungi. On the tree side, we found no evidence for a depletion of primary metabolites that might limit resources for tree defenses under the drought conditions. NSC concentrations tended to be higher in the phloem of roof than of control trees, a drought-induced physiological response frequently observed that can fuel the costly production of secondary metabolites and drought resilience/resistance mechanisms (Martinez-Vilalta, 2014; Tomasella *et al*., 2019; Huang *et al*., 2021).

Conclusions

We addressed multilateral relationships between *P. abies* and its main biotic antagonists *I. typographus* and its associated fungi in a unique and complex field experiment to shed light on the effects of mild-to-moderate prolonged drought on tree susceptibility in the early phase when pioneer beetles locate potential host trees in the absence of aggregation pheromone signaling by conspecifics. We demonstrated that the increased flow of terpene-containing resin in *P. abies* and increased concentrations of its monoterpene and diterpene constituents are closely related to a reduced *I. typographus* host selection in the decisive period when pioneer beetles land on potential hosts. Despite the weak influence of drought on RF and terpene concentrations, these traits were less strongly expressed in the trees of the drought treatment that were under greater stress. Drought stress also decreased *P. abies* resistance to the infection of bark beetle fungal mutualists. Together, fungi and beetle attack increased terpene accumulation and depleted sugar and starch reserves. Such reductions in carbohydrate supply may be exacerbated by the effects of more severe droughts that are predicted to occur under future climate change scenarios (Hartmann *et al*., 2022), leading to increased susceptibility to bark beetle attack and mass outbreaks.

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Competing interests

None declared.

Author contributions

SN planned and designed the research, performed experiments, analyzed and interpreted the data, and wrote the manuscript. EM, HH, JG, MRP and SR, contributed to conceptualization and methodology. AB, AS, LL, JH, SR and TS conducted field and/or laboratory work. All authors reviewed the manuscript.

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its Supporting Information.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Sequence of critical periods in the population dynamics of *Ips typographus* over space and time.

Fig. S2 Mean hourly temperatures recorded inside and outside (Air) the attack boxes.

Fig. S3 Host selection of *Ips typographus* in the attack box bioassays.

Fig. S4 (Multiple) linear regression plots with total number of *Ips typographus* attacks and attack attempts and RF Max, TWP Mean, total MTs and total DTs in September.

Fig. S5 Boxplots for mean lesion length and area established by *Picea abies* study trees in response to (a) *Grosmannia penicillata* (GP) and (b) *Endoconidiophora polonica* (EP).

Methods S1 Analysis of phenolic compounds, profile.

Table S1 Validation of the main study questions (Q1–Q6) and hypotheses (H1a–H6b).

Table S2 Number of *Picea abies* bark cores analyzed per date and compound group.

Table S3 (a) Mean values for predawn twig water potential (Mean TWP), resin flow (Mean RF), and volumetric soil water content (Mean SWC) of *Picea abies* study trees. (b) Precipitation sum (P Sum) and minimum, mean and maximum temperature (T Min, T Mean, T Max).

Table S4 Pearson correlations (*r*) of *Picea abies* physiological characteristics, weather and soil hydrological parameters recorded during experimental season 2 (2020).

Table S5 Correlations of *Ips typographus* behavior, *Picea abies* resin flow and the climate parameters temperature and precipitation.

Table S6 (a) *Picea abies* study tree bark contents of total monoterpenes and diterpene resin acids and individual compounds in mg g^{-1} fresh weight. (b) Bark contents of total phenolics and individual compounds in mg g^{-1} fresh weight.

Table S7 Pearson correlations (*r*) of *Ips typographus* attacks defended by resin flow and concentrations of total and individual monoterpenes (MT), diterpene resin acids (DT) and phenolic compounds in *Picea abies* study trees.

Table S8 (a) *Picea abies* study tree bark contents of total phenolics, Sol S, soluble sugars, starch, and NSC, nonstructural carbohydrates. (b) Phloem contents of glucose, sucrose, fructose and starch.