Tree-ring volatile terpenes show potential to indicate fungal infection in asymptomatic mature Norway spruce trees in the Alps

Laura C. Vezzola[1*], Marco Michelozzi[2], Luca Calamai[3], Paolo Gonthier[4], Luana Giordano[4,5], Paolo Cherubini[6] and Manuela Pelfini[1]

[1]University of Milano, Department of Earth Sciences "A. Desio", Via Mangiagalli 34, Milano (MI), 20133, Italy.

[2]Institute of Biosciences and Bioresources, National Research Council of Italy, Via Madonna del Piano 10, Sesto Fiorentino (FI), I-50019, Italy.

[3]University of Firenze, Department of Agrifood Production and Environmental Sciences, Piazzale delle Cascine 18, Firenze (FI), I-50144, Italy.

[4]University of Torino, Department of Agricultural, Forest and Food Sciences (DISAFA), Largo Paolo Braccini 2, Grugliasco (TO), I-10095, Italy.

[5]University of Torino, Centre of Competence for the Innovation in the Agro-Environmental Field (AGROINNOVA), Largo Paolo Braccini 2, Grugliasco (TO), I-10095, Italy.

[6]Swiss Federal Research Institute WSL, Zürcherstrasse 111, Birmensdorf, CH-8903, Switzerland.

*Corresponding author: Tel: +39 0250315514; Fax: +39 02503111; Email: laura.vezzola@studenti.unimi.it

Volatile terpenes (VT) content in tree-ring resin, in response to natural infection by *Heterobasidion* spp. in asymptomatic mature Norway spruce (*Picea abies*) trees was investigated. Twenty-three randomly selected mature trees were sampled in a stand in the western Italian alps by extracting cores using an increment borer. Based on fungal isolations from cores and molecular typing using taxon-specific competitive-priming (TSCP)-PCR, 12 out of the 23 trees were identified as infected by *Heterobasidion parviporum*. Tree-ring growth

patterns and VT content in tree rings were determined. Analysis of VT content was performed by means of gas chromatography mass spectrometry on a subset of trees. Results show slightly but not significantly lower tree-ring width in infected compared to non-infected trees in the past two decades. Total concentrations of sesquiterpenes and relative proportions of α-pinene, β-pinene and longifolene were significantly greater in infected trees; while relative proportions of camphene, 3-carene, ρ-cymene, sesquiterpene 15.90 and α-farnesene were significantly lower. This is the first study showing that VTs in tree-ring resin may indicate infection of trees by a fungal forest pathogen, even when trees are mostly asymptomatic.

Introduction

Inducible volatile terpenes (VTs) are abundantly produced and released by different plant organs following abiotic stresses (e.g., Loreto and Schnitzler, 2010; Leonelli et al., 2014) and biotic attacks, including those by insects and pathogens (e.g., Holopainen, 2004; Jansen et al., 2011).

In conifers, VTs are produced and stored in several plant structures, including constitutive resin ducts (CRDs), i.e., species-specific wood anatomical characteristics, and traumatic resin ducts (TRDs). Resin is toxic for most pathogens due to its composition and physical properties (Phillips and Croteau, 1999). In fact, resin contains monoterpenes, diterpenes and sesquiterpenes and some, especially when produced and released abundantly, are known to be insecticidal, antimicrobial and fungicidal (Schuck, 1982; Michelozzi, 1999; Trapp and Croteau, 2001). Conifer resin is produced in bark, phloem and xylem by constitutive and inducible secretory structures, releasing primary and secondary resin, respectively.

In Norway spruce [*Picea abies* (L.) Karst], resin accumulates both in CRDs and in TRDs, which appear within the developing xylem after mechanical wounding, in stem xylem. The formation of TRDs associated with enhanced production of VTs is part of a complex mechanism of plant defence that is activated to induce the successful tree reaction to the attack of pathogens and mechanical damage (Franceschi et al., 2000; Nagy et al., 2000; Fäldt et al., 2003; Krokene et al., 2008; Gärtner and Heinrich, 2009; Danielsson et al., 2011; Brauning et al., 2016). TRDs considerably enhance the oleoresin content of

Norway spruce, considering that they are larger, and thus their volume is much higher, than CRDs. TRDs usually develop in high number in the proximity of the injury caused by mechanical wounding or pathogens, and their number decreases as the distance from the wound increases (Schmidt et al., 2011). TRDs are commonly used for dating events which injure the cambium in geomorphology (e.g., Stoffel, 2008; Butler et al., 2010; Garavaglia and Pelfini, 2011), but their frequency and distribution within tree rings are poorly investigated. In some tree species, most of the resin ducts seem to develop in the latewood (Reid and Watson, 1966), but their distribution is highly variable within the same tree, due to environmental and climatic conditions (Wimmer et al., 1999).

Norway spruce is susceptible to heart rots caused by some fungi included in the *Heterobasidion annosum sensu lato* (*s.l.*) species complex, namely *H. annosum* (Fr.) Bref. and *H. parviporum* Niemelä & Korhonen (Garbelotto and Gonthier, 2013). While the former species is more generalist being able to attack several coniferous tree species, the latter displays a preference for Norway spruce. Regardless of which one of the two species is involved, the disease is mostly asymptomatic in mature trees. In fact, the progressive development of the decay in the heartwood rarely results in the appearance of external symptoms (Garbelotto and Gonthier, 2013). Heart rots caused by *Heterobasidion* spp. are among the most destructive and widespread diseases of Norway spruce in Europe, including the Alpine region (Asiegbu et al., 2005; Gonthier et al., 2012; Giordano et al., 2015). Infection occurs through airborne spores (primary infections) colonising freshly exposed wood surfaces (stumps or wounds in the stem or roots). Subsequently, the fungus can infect uninjured trees by vegetative growth of mycelium through root contacts or grafts (secondary infections) (Garbelotto and Gonthier, 2013).

The production of spores by *Heterobasidion* spp. is more abundant when air temperature are above 5°C (Gonthier et al., 2005). For this reason, climate warming may prolong the time interval favourable for sporulation and infection during the year for *Heterobasidion* spp, The altitude at which pathogens can be found may also be shifted to higher elevations (La Porta et al., 2008).

Defensive strategies and VT production are usually studied under controlled experimental conditions obtained from controlled crosses, and that are artificially inoculated with the pathogen (e.g., Cellini et

al., 2014; Piesik et al., 2015) or in which the pathogen attack is mimicked by treatment with methyljasmonate (e.g., Arnerup et al., 2013). In particular, experiments conducted on Norway spruce revealed that the oleoresin of trees affected by *Heterobasidion* spp. was different to that of non-affected trees in terms of amounts of (+)-α-pinene, (+)-sabinene, (-)-sabinene, δ-3-carene, (-)-limonene and γ-terpinene (Zamponi et al., 2007). However, we are not aware of any studies conducted on the oleoresin content of mature trees infected by *Heterobasidion* spp. in forest stands. Moreover, little is known about VT production in asymptomatic trees. A better understanding of this topic may be crucial for developing strategies allowing the set-up of useful markers enabling the early diagnosis of tree diseases, that could prevent losses in forest productivity, and to assess which factors can influence the climatic signal recorded in tree rings at high altitude (Leonelli et al., 2012).

The main aim of this research was to detect possible differences in VT content in tree-ring resin in response to natural infection by *Heterobasidion* spp. in asymptomatic mature Norway spruce trees. Tree-ring growth was also analysed in infected and non-infected trees in order to investigate if any difference in growth patterns could be attributed to the presence of the pathogen.

Methods

Study site and sampling design

The study site is located in the Western Italian Alps at about 1450 m a.s.l. close to the area called Ermitage (45°47’46.11’’N; 6°58’56.39’’E), in the municipality of Courmayeur (Aosta Valley Region), where *Heterobasidion* spp. were previously detected in a mature mixed Norway spruce-European larch (*Larix decidua* Mill.) forest stand. About 55% of trees were estimated to be infected (Gonthier et al., 2012). The stand, with a standing volume of 227 m^3 ha^{-1} and a density of 410 trees ha^{-1}, was thinned in 1995. This area and adjacent valleys, i.e., Val Veny and Val Ferret, have been well studied in order to better understand the impact of the climatic and related environmental changes on vegetation (for a review see Bollati et al., 2015).

In an attempt to compare a similar number of infected and putatively non-infected trees, 23 randomly selected trees were sampled at the end of June 2015 by extracting four wood cores at 90° from one another at the base of stems (20 cm above the ground) using a Pressler's increment borer (for details about sampling techniques see, e.g., Pelfini et al., 2007). The minimum and mean distance among sampled trees was 25 m and 80 m, respectively. The diameter at breast height (DBH) of sampled trees ranged between 68 cm and 145 cm (mean 99 cm). Cores were transported to the laboratory in plastic straws and stored at 5°C before subsequent analyses. Two cores were used for isolation and pathogen detection, one for the dendrochronological analyses and one for VT analyses in tree rings (Fig. 1).

Pathogen detection and identification at species level

Cores were sprayed with a benomyl solution (0.010 g benomyl, 500 μL methanol, 1 L distilled water) and incubated for about 10 days at room temperature (25°C ± 2°C) in a moist chamber as described by Gonthier et al. (2003). After incubation cores were inspected under a dissecting microscope (x20 magnification) in order to check for the presence of emerging colonies of the conidial stage of *Heterobasidion* spp.

Fungal isolations were made by transferring infected wood or fungal hyphae onto 6-cm Petri dishes containing a PCNB-based selective medium for *Heterobasidion* spp. (Kuhlman and Hendrix, 1962). All isolates were subsequently subcultured and stored at 5°C on MEA (malt extract agar: 20 g glucose, 20 g malt extract, 2 g peptone, 20 g agar, 1 L distilled water).

DNA from fungal isolates was extracted by a hyphal tipping method (Schweigkofler et al., 2004), modified as follows: fungal mycelium was collected with the tip of a micropipette and suspended in 100 μL of distilled water, frozen on dry ice for 3 minutes, thawed at 75°C, vortexed for 1 minute, and finally centrifuged for 5 minutes at 19,000 g. Freezing and thawing were repeated three times, with the last thaw extended to 15 minutes. Samples were then centrifuged for 5 minutes at 19,000 g and the supernatant was used as template for polymerase chain reactions (PCRs). Identification of *Heterobasidion* isolates at the species level was carried out by a taxon-specific competitive-priming

(TSCP)-PCR (Garbelotto et al., 1996) combined with a PCR-mediated detection of species-specific DNA insertions in the ML5-ML6 DNA region of the mitochondrial large ribosomal RNA (mt LrRNA) gene as described by Gonthier et al. (2001).

Dendrochronological analysis

The cores were prepared for tree-ring dating and ring-width measurements following standard methods (Stokes and Smiley, 1968), usually applied in dendrochronological studies conducted in mountain environments and in the nearest geographical areas (e.g., Pelfini et al., 2007; Garavaglia et al., 2010). Tree-ring widths were measured to the nearest 0.01 mm using the LINTAB system with the TSAPWin software (Frank Rinn, Heidelberg, Germany), and the obtained series were visually and statistically cross-dated using the COFECHA software (Grissino-Mayer, 2001) in order to find and correct any dating error in the dataset. Two main ring-width mean chronologies were built: one, named “pathogen”, using the trees found to be infected by *Heterobasidion* spp., and one, named “no pathogen”, using trees putatively non-infected by the pathogen.

To analyse tree-ring growth trends in the two groups of trees, the raw ring-width series were standardized using the software Arstan (Holmes, 1992) and a residual chronology for each category was prepared applying a negative exponential curve.

VT analysis in tree rings

Five trees infected and five trees putatively non-infected by *Heterobasidion* spp. were selected for the analyses of VTs. Selection was mainly based on the overall conditions of the cores: priority was given to the cores with no broken tree rings, at least in the terminal part of the core, and characterised by easily identifiable tree rings. The last five tree rings of each core (corresponding to the years from 2010 to 2014) were split from each other using a scalpel, for a total of 50 samples (Fig. 1).

VT relative content was determined by means of gas chromatography mass spectrometry. For this procedure, about 25 mg of cortical and xylem tissues were placed into a sterilised vial, and 200 µL of

153 pentane with tridecane as internal standard was added to each vial, after which the vials were put in a
154 Soltec ultrasound machine Sonica 2200 S3 at the temperature of 30°C for 60 minutes. The vials were left
155 in a Gerhardt Thermoshake THO 5 for 24 hours, and the extracts were then filtered with 0.45 µm PTFE
156 syringe filters and injected (3 µL) in the GC-MS system. An Agilent 7820 GC-chromatograph equipped
157 with a 5977A MSD mass spectrometer with EI ionisation operating at 70 eV was used for analysis. A
158 chromatographic column J&W Innovax 50 m, 0.20 mm, ID 0.4 µm DF was used. The GC injection
159 temperature was 250°C, splitless mode, and the oven was programmed at 40°C for 1 minute, followed
160 by a ramp of 5°C/minute to 200°C, and of 10°C/minute to 260°C. This high temperature was held for 5
161 minutes. Mass spectra were acquired within the 29-350 M/Z interval with an Agilent 5977 MSD
162 spectrometer at three scans s^{-1}. VT identification was done on the basis of both peak matching with
163 library spectral database (NIST 08) and kovats indeces as retrived in literature for the identified
164 compounds.
165 Total absolute amounts (total concentrations) of monoterpenes (total MTs) and sesquiterpenes (total
166 SQTs) were expressed as milligrams of terpenes per grams of fresh tree tissue and they were analysed
167 by non-parametric Mann-Whitney U Test, in order to test differences between the two groups
168 “pathogen” and “no pathogen”.
169 The relative amount (proportions or percentages) of each monoterpene was expressed as a percentage
170 of total monoterpenes (monoterpene profiles), while the relative amount of each sesquiterpene was
171 expressed as a percentage of the sum of mono- and sesquiterpenes (terpene profiles). The average and
172 standard error (SE) of the percentage were calculated for each compound and compared between
173 “pathogen” and “no pathogen” trees.
174 In order to analyse variations in total concentrations of terpenes of Norway spruce tree rings between
175 different sampling years we performed the statistical Friedman Test. Friedman test results
176 (Supplementary material: tables S1 and S2) showed no significant variations in total MTs, SQTs, MTs +
177 SQTs and the relative content of terpenes between different sampling years,; based on these results,
178 mean value of total MTs, SQTs , MTs + SQTs and relative content of terpenes were calculated within

179 treatment from 2010 to 2014. Mean values were not normally distributed (Kolmogorov-Smirnov one-
180 sample test) and were analysed using the Mann-Whitney U Test for comparison among disease
181 treatments of the plants. A 0.05 threshold was used as cut-off value for all analyses. Statistical analyses
182 were carried out using SPSS (statistical package for social science, SPSS software, v.22.0, SPSS Inc.,
183 Chicago, USA).
184
185 **Results**
186 *Pathogen detection and identification at species level*
187 Out of the 23 sampled trees, 12 were infected by *Heterobasidion* spp. (52%) while the remaining 11
188 samples were putatively non-infected by the pathogen. None of the cores analysed displayed visible
189 symptoms of wood decay. Based on the molecular diagnostic assay, all infected trees were colonized by
190 *H. parviporum*.
191
192 *Dendrochronological analysis*
193 The tree-ring width mean chronologies covered the period 1902-2015 for “pathogen” trees and 1901-
194 2015 for “no pathogen” trees. Median age was similar for the two series, i.e., 65 years for “pathogen”
195 trees and 64 years for “no pathogen” trees. The two mean chronologies showed similar growth trends,
196 especially after 1970 when more than five trees contributed to the chronology (Fig. 2, continuous line).
197 “Pathogen” trees were characterised by slightly, but not significantly, lower tree-ring width in the last 15
198 years compared to “no pathogen” trees. The two residual chronologies show similar growth patterns
199 along the entire considered time interval, with the more recent relative peaks of positive growth in 1998
200 (“pathogen” trees) and 2000 (“no pathogen” trees) (Fig. 3).
201
202 *VT analysis in tree rings*
203 *Changes in total concentrations*

204 Mann-Whitney U test results showed that mean values of SQTs were significantly different ($\chi^2 = 5.8$; $P < 0.05$) between "pathogen" and "no pathogen" trees, while mean values of MTs ($\chi^2 = 0.9$; $P = 0.35$) and MTs plus SQTs ($\chi^2 = 0.8$; $P = 0.34$) did not show significant differences between the two groups (Fig. 4).

207

208 *Changes in the relative content of terpenes (terpene profiles)*

209 The Mann-Whitney U test showed significant differences in the relative content of 8 terpenes between
210 tree rings of "pathogen" and "no pathogen" trees. As regards the monoterpenes, α-pinene ($\chi^2 = 4.8$; $P < 0.05$) and β-pinene ($\chi^2 = 5.8$; $P < 0.05$) were significantly higher in "pathogen" trees compared to "no pathogen" trees, while camphene ($\chi^2 = 6.8$; $P < 0.01$), 3-carene ($\chi^2 = 6.8$; $P < 0.01$), and ρ-cymene ($\chi^2 = 6.81$ $P < 0.05$) were significantly higher in "no pathogen" compared to "pathogen" trees. The monoterpenes sabinene ($\chi^2 = 1.8$; $P = 0.18$), myrcene ($\chi^2 = 1.7$; $P = 0.17$), limonene ($\chi^2 = 0.3$; $P = 0.60$), β-phellandrene ($\chi^2 = 0.1$ $P = 0.75$), cineole ($\chi^2 = 2.6$; $P = 0.11$) and γ-terpinene ($\chi^2 = 0.9$; $P < 0.35$) did not show statistically significant differences between the two groups.

217 Among the analysed sesquiterpenes, sesquiterpene 15.90 ($\chi^2 = 3.9$; $P < 0.05$) and α-farnesene ($\chi^2 = 3.9$; $P = 0.05$) showed higher proportions in "no pathogen" compared to "pathogen" trees, while higher relative contents of longifolene were observed in infected compared to non-infected samples ($\chi^2 = 5.7$; $P < 0.05$). α-Humulene ($\chi^2 = 0.3$; $P = 0.6$) and β-caryophyllene ($\chi^2 = 1.8$; $P = 0.18$) did not show significant differences between the analysed categories (Fig. 5).

222

223 **Discussion**

224 This study represents the first attempt to detect possible differences in mono- and sesquiterpene
225 content in annual tree rings of adult asymptomatic Norway spruce trees in response to natural infection
226 by a fungal pathogen, i.e., *Heterobasidion* spp.

227 All *Heterobasidion* infected trees were colonized by *H. parviporum* and none by *H. annosum*, thus
228 confirming that the overwhelming majority of Norway spruce decays in the area are caused by the
229 former species, as previously documented (Gonthier et al., 2003). Although the dates of infection of

trees remain unknown, which may complicate the interpretation of the results of this work, all lines of evidence suggest infection occurred relatively recently, possibly in the last 15 years. First, none of the cores analysed displayed visible symptoms of decay, pointing to a recent upward colonization of the fungus from the point of infection in the roots. Second, the infection courts for primary infections by means of airborne spores, i.e. stumps, have been most likely created during thinning performed in 1995. Third, and incidentally, the mean ring-width chronology of trees infected by *H. parviporum* showed lower values starting from the late 1990s compared with non-infected trees, and this may suggest infection of trees occurred at that time. In fact, growth reduction in conifers is common during infection by fungi, e.g. *Heterobasidion parviporum* (Gori et al., 2013). This pattern was also observed by Cherubini et al. (2002) on *Pinus mugo* Turra trees killed by *H. annosum* and *Armillaria* sp. Although these authors found a more remarkable difference in ring-width between infected and non-infected trees than we did in this study, it should be noted that pine trees compared to Norway spruce trees are more susceptible to root rot and mortality rather than heart rot (Garbelotto and Gonthier, 2013), and this may explain the higher levels of growth reduction in pines than in Norway spruce trees (Mallett and Volney, 1999).

The progressive reduction in tree-ring width can affect the climatic signal recorded in tree rings, thus negatively influencing dendroclimatic reconstructions (Trotter et al., 2002). Our results, even if limited to only a small number of trees, support previous investigations conducted on conifers, revealing that Norway spruce infected by *Heterobasidion* spp. shows lower tree-ring width compared to non-infected trees (Cherubini et al., 2002).

Total concentrations of both monoterpenes and sesquiterpenes were lower in trees infected by *H. parviporum* compared to putatively non-infected ones and, for sesquiterpenes, the difference between "pathogen" and "no pathogen" trees appeared to be significant. Both mono- and sesquiterpenes have an important role in counteracting pathogen infection in Norway spruce trees. However, the Friedman Test did not show any significant difference in the terpene content between different years (Supplementary material), suggesting that this method does not allow the identification of any difference in terpene content following pathogen infection at the yearly resolution.

256 The relative content (percentage) of the monoterpenes α-pinene and β-pinene and of the sesquiterpene
257 longifolene are significantly higher in infected compared to non-infected trees. In particular, the
258 monoterpenes α-pinene and β-pinene are known for their role in conifer defence strategies in stems
259 and roots (Huber et al., 2005). These results are in agreement with research performed by Zamponi et
260 al. (2007) on branches of Norway spruce trees experimentally inoculated with *H. parviporum*. In that
261 study, α-pinene and β-pinene were significantly different between infected and non-infected trees,
262 which is also in agreement with our study. However, there were some differences between our study
263 and the results obtained by Zamponi et al. (2007), i.e., we did not detect a significant increase in the
264 relative content of 3-carene and myrcene following *Heterobasidion* attack. These differences could be
265 due to the tissues colonized by the pathogens in the two studies, i.e. heartwood vs sapwood,
266 respectively. In fact, while branches, hence sapwood, was inoculated with *Heterobasidion* spp. by
267 Zamponi et al. (2007), it is likely that our adult Norway spruces were colonized by *H. parviporum* in the
268 heartwood as it occurs as a general rule (Garbelotto and Gonthier, 2013).
269 The relative content of the monoterpenes camphene, 3-carene and ρ-cymene and of the sesquiterpenes
270 sesquiterpene 15.90 and α-farnesene was significantly lower in infected compared to non-infected
271 trees. This can be a consequence of the defence mechanism activated by the tree following infection:
272 the plant reduces the production of the biologically less active compounds and increases the synthesis
273 of the more toxic terpenes (Michelozzi, 1999). When the infection begins, Norway spruce trees start
274 increasing the level of several terpenes in order to contrast the pathogen attack but if the defence
275 mechanism is not successful (Luchi et al., 2005), then the tree reduces the production of the terpenes
276 that are less effective for restricting the pathogen, because their production has a relevant cost for the
277 tree itself (e.g., Ghimire et al., 2016).
278
279 **Conclusions**
280 In summary, this study reveals that both dendrochronological and VT analyses may indicate fungal
281 infection in adult trees. In particular, the tree-ring mean chronology showed lower values in infected

compared to non-infected trees in the more recent years and the relative content of some terpenes, i.e., α-pinene, β-pinene and longifolene showed significantly higher values in infected compared to non-infected trees. This is the first study suggesting that VT composition in tree rings may be an indicator of fungal disease and this is particularly important in the case of Norway spruce, where external symptoms of infection, for example by *H. parviporum,* are usually poor. A future study considering different geographical regions and trees from diverse genetic lineages, as well as a larger sample size, should be carried out to identify which markers can be used for the identification of diseased trees.

Acknowledgements

The authors thank the Editor Dr. Gary Kerr and the two anonymous reviewers for insightful comments that considerably helped improving this manuscript. Special thanks to Gabriele Cencetti (IBBR-CNR of Firenze and ARCA Laboratory, CNR of Firenze) for technical assistance with GC-MS analyses and to the Regione Autonoma Valle d'Aosta for sampling permission in the study area.

Conflict of interest statement

None declared.

References

Arnerup, J., Nemesio-Gorriz, M., Lundén, K., Asiegbu, F.O., Stenlid, J. and Elfstrand, M. 2013 The primary module in Norway spruce defence signalling against *H. annosum* s.l. seems to be jasmonate-mediated signalling without antagonism of salicylate-mediated signalling. *Planta* **237**, 1037-1045

Asiegbu, F.O., Adomas, A. and Stenlid, J. 2005 Conifer root and butt rot caused by *Heterobasidion annosum* (Fr.) Bref. s.l.. *Mol Plant Pathol* **6**, 395-409

307 Bollati, I., Leonelli, G., Vezzola, L. and Pelfini, M. 2015 The role of ecological value in geomorphosite
308 assessment for the Debris-Covered Miage Glacier (Western Italian Alps) based on a review of 2.5
309 centuries of scientific study. *Geoheritage* **7**, 119-135
310
311 Brauning, A., De Ridder, M., Zafirov, N., Garcia-Gonzales, I., Dimitrov, D.P. and Gartner, H. 2016 Tree-
312 ring features: indicators of extreme event impacts. *IAWA J* **37**, 206-231
313
314 Butler, D.R., Sawyer, C.F. and Maas, J.A. 2010 Tree-ring dating of snow avalanches in Glacier National
315 Park, Montana, USA. In *Tree rings and natural hazards*. *Advances in global change research*, vol. 41. M.
316 Stoffel, M. Bollschweiler, D. Butler and B. Luckman (eds). Springer, Dordrecht, pp 35-46
317
318 Cellini, A., Biondi, E., Buriani, G., Farneti, B., Rodrigues-Estrada, M.T., Braschi, I., Savioli, S., Blasioli, S.,
319 Rocchi, L., Biasioli, F., Costa, G. and Spinelli, F. 2014 Characterization of volatile organic compounds
320 emitted by kiwifruit plants infected with *Pseudomonas syringae* pv. *actinidae* and their effects on host
321 defences. *Trees – Struct Funct* **30**, 795-806
322
323 Cherubini, P., Fontana, G., Rigling, D., Dobbertin, M., Brang, P. and Innes, J.L. 2002 Tree-life history prior
324 to death: two fungal root pathogens affect tree-ring growth differently. *J Ecol* **90**, 839-850
325
326 Danielsson, M., Lundén, K., Elfstrand, M., Hu, J., Zhao, J., Zhao, T., Arnerup, J., Ihrmark, K., Swedjemark,
327 G., Borg-Karlson, A.K. and Stenlid, J. 2011 Chemical and transcriptional responses of Norway spruce
328 genotypes with different susceptibility to *Heterobasidion* spp. infection. *BMC Plant Biol* **11**: 154
329
330 Fäldt, J., Martin, D., Miller, B., Rawat, S. and Bohlmann, J. 2003 Traumatic resin defence in Norway
331 spruce (*Picea abies*): methyl jasmonate-induced terpene synthase gene expression, and cDNA cloning
332 and functional characterization of (+)-3-carene synthase. *Plant Mol Biol* **51**, 119-133

Franceschi, V.R., Krokene, P., Krekling, T. and Christiansen, E. 2000 Phloem parenchyma cells are involved in local and distant defence responses to fungal inoculation or bark-beetle attack in Norway spruce (*Pinaceae*). *Am J Bot* **87**, 314-326

Gärtner, H., Heinrich I. 2009 The formation of traumatic rows of resin ducts in *Larix decidua* Mill. and *Picea abies* (L.) Karst. as a result of wounding experiments in the dormant season. *IAWA J* **30**, 199-215

Garavaglia, V., Pelfini, M. and Motta, E. 2010 Glacier stream activity in the proglacial area of debris covered glacier in Aosta Valley, Italy: an application of dendroglaciology. *Geogr Fis Din Quat* **33**, 15-24

Garavaglia, V. and Pelfini, M. 2011 The role of border areas for dendrochronological investigations on catastrophic snow avalanches: a case study from the Italian Alps. *Catena* **87**, 209-215

Garbelotto, M., Ratcliff, A., Bruns, T.D., Cobb, F.W. and Otrosina, W. 1996 Use of taxon-specific competitive-priming PCR to study host specificity, hybridization, and intergroup gene flow in intersterility groups of *Heterobasidion annosum. Phytopathology* **86**, 543-551

Garbelotto, M. and Gonthier, P. 2013 Biology, epidemiology, and control of *Heterobasidion* species worldwide. *Annu Rev Phytopathol* **51**, 39-59

Ghimire, R.P., Kivimäenpää M., Blomqvist M., Holopainen T., Lyytikäinen-Saarenmaa, P., Holopainen J.K. 2016 Effect of bark beetle (*Ips typographus* L.) attack on bark VOC emissions of Norway spruce (*Picea abies* Karst.) trees. *Atmos Environ* **126**, 145-152

Giordano, L., Sillo, F., Guglielmo, F. and Gonthier, P. 2015 Comparing visual inspection of trees and molecular analysis of internal wood tissues for the diagnosis of wood decay fungi. *Forestry* **88**, 465-470

Gonthier, P., Garbelotto, M., Varese, G.C. and Nicolotti, G. 2001 Relative abundance and potential dispersal range of intersterility groups of *Heterobasidion annosum* in pure and mixed forests. *Can J Botany* **79**, 1057-1065

Gonthier, P., Garbelotto, M. and Nicolotti, G. 2003 Swiss stone pine trees and spruce stumps represent an important habitat for *Heterobasidion* spp. in subalpine forests. *Forest Pathol* **33**, 191-203

Gonthier, P., Garbelotto, M.M. and Nicolotti, G. 2005 Seasonal patterns of spore deposition of *Heterobasidion* species in four forests of the western Alps. *Phytopathology* **95**, 759-767

Gonthier, P., Brun, F., Lione, G. and Nicolotti, G. 2012 Modelling the incidence of *Heterobasidion annosum* butt rots and related economic losses in alpine mixed naturally regenerated forests of northern Italy. *Forest Pathol* **42**, 57-68

Gori, Y., Cherubini, P., Camin, F. and La Porta, N. 2013 Fungal root pathogen (*Heterobasidion parviporum*) increases drought stress in Norway spruce stand at low elevation in the Alps. *Eur J Forest Res* **132**, 607-619

Grissino-Mayer, H.D. 2001 Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. *Tree-Ring Res* **57**, 205-221

Holmes, R.L. 1992 *Dendrochronology program library user's manual*. Laboratory of Tree-Ring Research, University of Arizona, Tucson, Arizona, USA.

Holopainen, J.K. 2004 Multiple functions of inducible plant volatiles. *Trends Plant Sci* **9**, 529-533

Huber, D.P.W., Philippe, R.N., Madilao, L.L., Sturrock, R.N. and Bohlmann, J. 2005 Changes in anatomy and terpene chemistry in roots of Douglas-fir seedlings following treatment with methyl jasmonate. *Tree Physiol* **25**, 1075-1083

Jansen, R.M.C., Wildt, J., Kappers, I.F., Bouwmeester, H.J., Hofstee, J.W. and van Henten, R.J. 2011 Detection of diseased plants by analysis of Volatile Organic Compounds emission. *Annu Rev Phytopathol* **49**, 157-174

Krokene, P., Nagy, N.E. and Krekling, T. 2008 Traumatic resin ducts and polyphenolic parenchyma cells in conifers. In *Induced plant resistance to herbivory* A. Schaller (ed.). Springer, Netherlands, pp. 147-169

Kuhlman, E.G. and Hendrix, F.F. Jr. 1962 A selective medium for the isolation of *Fomes annosus*. *Phytopathology* **52**, 1310-1312

La Porta, N., Capretti, P., Thomsen, I.M., Kasanen, R., Hietala, A.M. and Von Weissenberg, K. 2008. Forest pathogens with higher damage potential due to climate change in Europe. *Can J Plant Pathol* **30**, 177-195

Leonelli, G., Battipaglia, G., Siegwolf, R., Saurer, M., Morra Di Cella, U., Cherubini, P. and Pelfini, M. 2012 Climatic isotope signals in tree rings masked by air pollution: A case study conducted along the Mont Blanc Tunnel access road (Western Alps, Italy). *Atmos Environ* **61**, 169-179

409 Leonelli, G., Pelfini, M., Panseri, S., Battipaglia, G., Vezzola, L. and Giorgi, A. 2014 Tree-ring stable
410 isotopes, growth disturbances and needles volatile organic compounds as environmental stress
411 indicators at the debris covered Miage Glacier (Monte Bianco Massif, European Alps). *Geogr Fis Din*
412 *Quat* **37**, 101-111
413
414 Loreto, F. and Schnitzler, J.P. 2010 Abiotic stresses and induced BVOCs. *Trends Plant Sci* **15**, 154-166
415
416 Luchi, N., Ma, R., Capretti, P. and Bonello, P. 2005 Systemic induction of traumatic resin ducts and resin
417 flow in Austrian pine by wounding and inoculation with *Sphaeropsis sapinea* and *Diplodia scrobiculata*.
418 *Planta* **221**, 75-84
419
420 Mallett, K.I. and Volney, W.J.A. 1999 The effect of *Armillaria* root disease on lodgepole pine tree growth.
421 *Can J Forest Res* **29**, 252-259
422
423 Michelozzi, M. 1999 Defensive roles of terpenoid mixtures in conifers. *Acta Bot Gallica* **146**, 73-84
424
425 Nagy, N.E., Franceschi, V.R., Solheim, H., Krekling, T. and Christiansen, E. 2000 Wound induced traumatic
426 resin duct development in stems of Norway spruce (*Pinaceae*): anatomy and cytochemical traits. *Am J*
427 *Bot* **87**, 302-313
428
429 Pelfini, M., Santilli, M., Leonelli, G., Bozzoni, M. 2007 Investigating surface movements of debris-covered
430 Miage glacier, Western Italian Alps, using dendroglaciological analysis. *J Glaciol* **53**, 141-152
431
432 Phillips, M.A. and Croteau, R.B. 1999 Resin-based defences in conifers. *Trends Plant Sci* **4**, 184-190
433

Piesik, D., Miler, N., Lenańczyk, G., Bocianowski, J. and Buszewski, B. 2015 *Botrytis cinerea* infection in three cultivars of chrysanthemum in “Alchimist” and its mutants: volatile induction of pathogen-infected plants. *Sci Hortic-Amsterdam* **193**, 127-135

Reid, R.W. and Watson, J.A. 1966 Sizes, distribution, and numbers of vertical resin ducts in lodgepole pine. *Can J Botany* **44**, 519-525

Schmidt, A., Nagel, R., Kreklimg, T., Christiansen, E., Gershenzon, J. and Krokene, P. 2011 Induction of isoprenyl diphosphate synthases, plant hormones and defence signalling genes correlates with traumatic resin duct formation in Norway spruce (*Picea abies*). *Plant Mol Biol* **77**, 577-590

Schuck, H.J. 1982 Monoterpenes and resistance of conifers to fungi. In *Resistance to diseases and pests in forest trees*. H.M. Heybroeck, B.R. Stephan, K. von Weissenberg (eds.). Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands, pp. 169-175.

Schweigkofler, W., O’Donnell, K. and Garbelotto, M. 2004 Detection and quantification of airborne conidia of *Fusarium circinatum*, the causal agent of pine pitch canker, from two California sites by using a real-time PCR approach combined with a simple spore trapping method. *Appl Environ Microb* **70**, 3512-3520

Stoffel, M. 2008 Dating past geomorphic processes with tangential rows of traumatic resin ducts. *Dendrochronologia* **26**, 53-60

Stokes, M.A. and Smiley, T.L. 1968 *An introduction to tree-ring dating*. University of Chicago Press, Chicago, USA, 73 pp.

460 Trapp, S. and Croteau, R. 2001 Defensive resin biosynthesis in conifers. *Annu Rev Plant Physio* **52**, 689-
461 724
462
463 Trotter, III R.T., Cobb, N.S. and Whitham, T.G. 2002 Herbivory, plant resistance, and climate in the tree
464 ring record: interactions distort climatic reconstructions. *PNAS* **99**, 10197-10202
465
466 Wimmer, R., Grabner, M., Strumia, G. and Sheppard, P.R. 1999 Significance of vertical resin ducts in the
467 tree rings of spuce. In *Tree Ring Analysis. Biological, methodological and environmental aspects*. R.
468 Wimmer and R. Vetter (eds.). CABI Publishing, Oxon, United Kingdom, pp.107-118
469
470 Zamponi, L., Michelozzi, M. and Capretti, P. 2007 Terpene response of *Picea abies* and *Abies alba* to
471 infection with *Heterobasidion* s.l. *Forest Pathol* **37**, 243-250
472
473
474
475
476
477
478
479
480
481
482
483
484
485

Figure captions

Figure 1. Experimental design of the analyses.

Figure 2. Ring-width mean chronologies for “pathogen” and “no pathogen” trees. Discontinuous lines characterize the curve built with less than five trees.

Figure 3. The two residual chronologies “pathogen” and “no pathogen”. Discontinuous lines characterize the curve built with less than five trees.

Figure 4. Mean (+ SE) values of total monoterpenes (MTs), sesquiterpenes (SQTs) and mono + sesquiterpenes (MTs + SQTs) concentrations detected in tree rings of “pathogen” and “no pathogen” trees. Values of columns with different letters differ significantly ($P < 0.01$).

Figure 5. Average percentage of terpenes in “pathogen” and “no pathogen” tree rings. Statistical difference was determined by Mann-Whitney test. Error bars indicate SE. Values of columns with different letters differ significantly (the values of statistical significance are reported in the text).