Fungal inoculation and methyl jasmonate application induce highly variable terpene accumulation in Norway spruce

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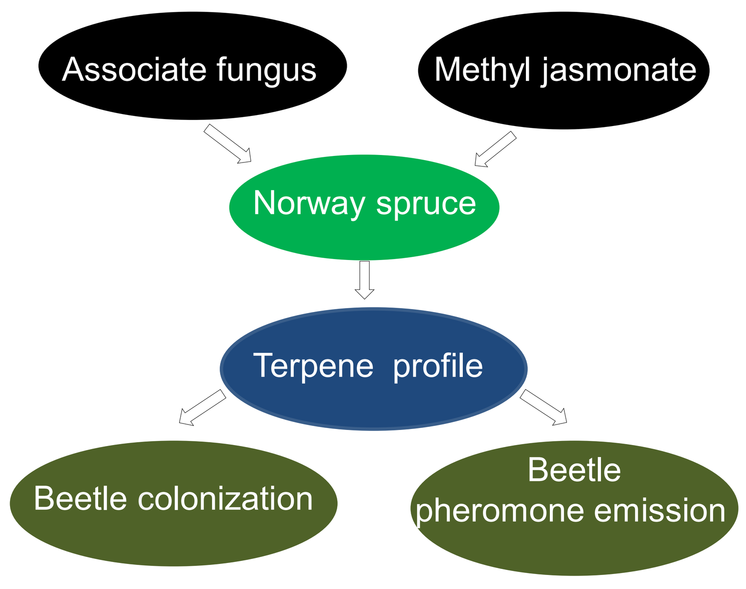
Introduction

Knowledge about tree chemical defense is vital for developing practical methods to maintain healthy forests. **With the aims of characterizing the defensive chemical induction in Norway spruce *Picea abies* and demonstrating its ecological function to spruce bark beetle *Ips typographus*,** we measured the terpenoid content in the bark of mature Norway spruce trees inoculated with *Ceratocystis polonica*, or treated with methyl jasmonate (MeJA), and investigated the colonization and pheromone emission of *I. typographus*.



Experimetal scheme

Pheromone collection

2DGC-MS instrument

Fungal inoculation elicited strong and highly variable terpene accumulation in Norway spruce

The total terpene in the reaction zone were 7- and 95- fold higher 14 days and 35 days after treatment, respectively, than in untreated stem section.

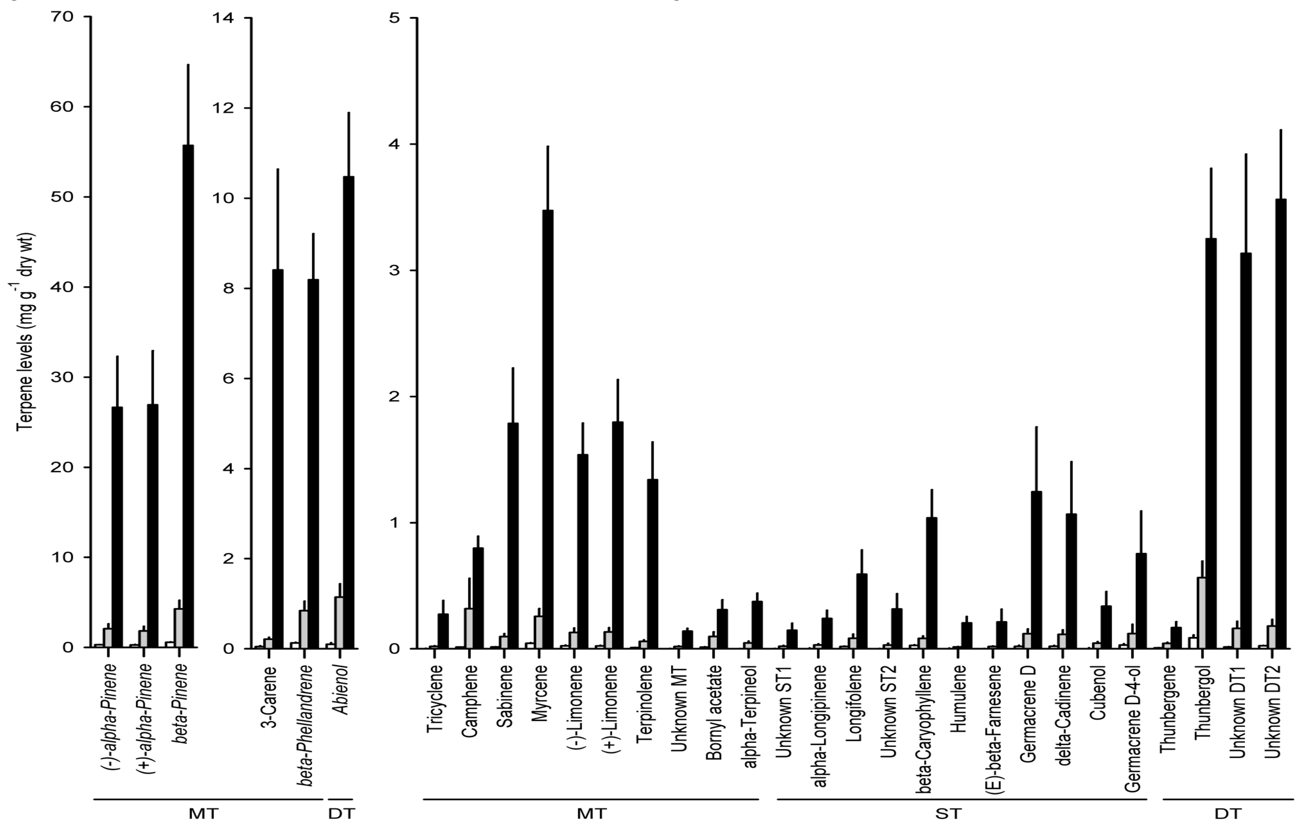


Fig 1. Levels of individual mono- (MT), sesqui- (ST) and diterpenes (DT) in untreated Norway spruce bark (white bars) and in bark close to inoculation sites with *Ceratocystis polonica* 14 days (grey bars) and 35 days (black bars) after inoculation. Data are expressed as means + 1 SE. n = 20 trees (PLoS ONE 6: e26649).

MeJA elicited terpene accumulation and primed the trees to susequent mechanical wounding

MeJA treatment increased terpene level at both sampling times. The levels of (-)-germacrene D, germacrene D-4-ol and thunbergol were significantly higher 14 days than 35 days after treatment. Extensive variation were observed between experimental trees.

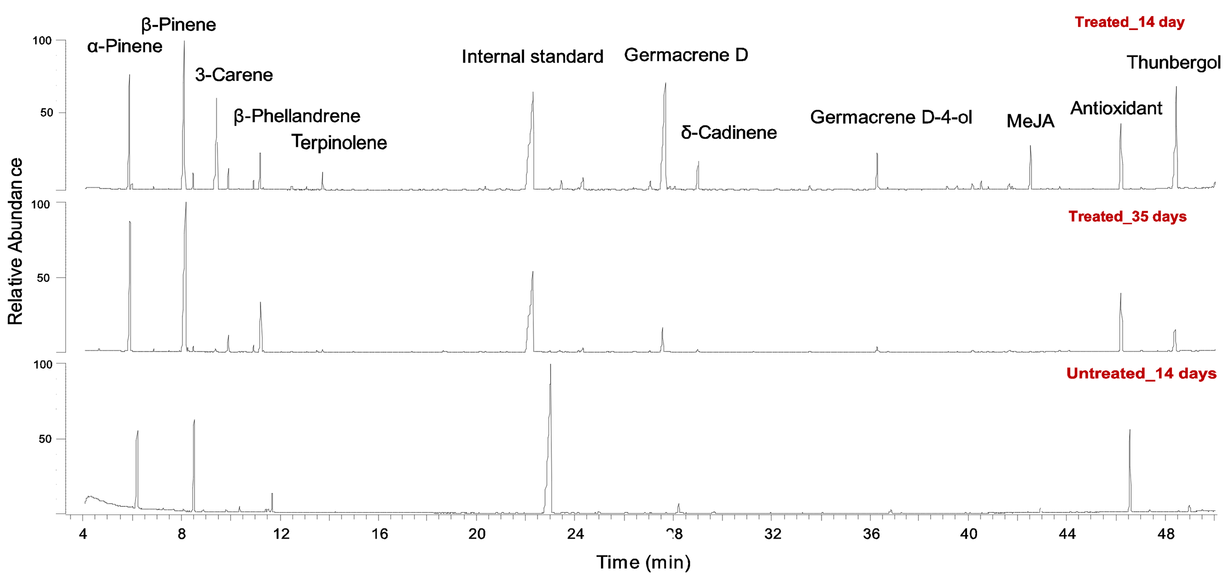


Fig 2. Representative total ion chromatograms from treated and untreated section of MeJA treated trees14 days and 35 days after treatment.

MeJA-treated logs also showed a much stronger response to mechanical wounding than control logs. In wounded phloem all terpene classes were significantly more abundance in MeJA-treated than control logs, but diterpens incresed more than others.

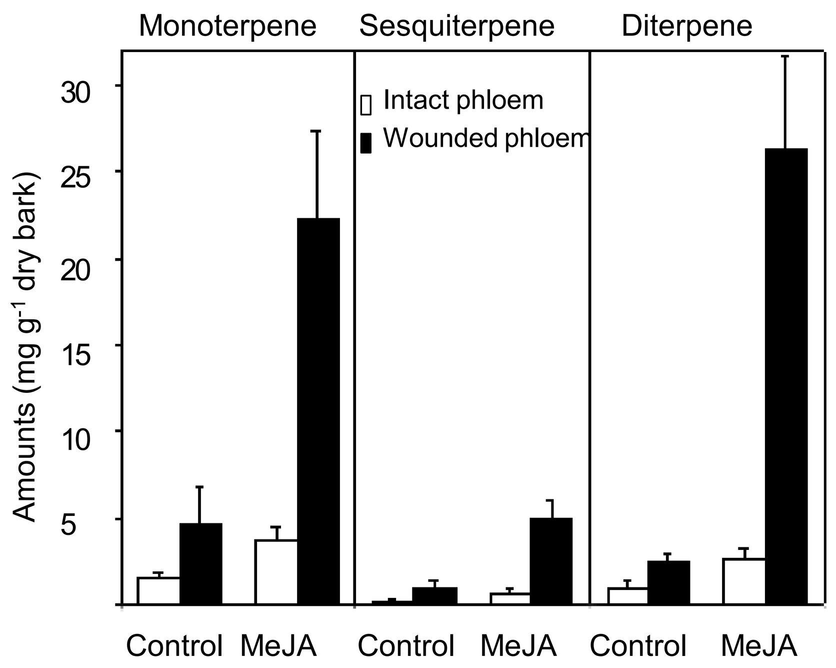


Fig 3. Amounts of terpenes in intact and wounded phloem of control logs and MeJA-treated Norway spruce logs. Wounded phloem was intensively wounded using a push pin 24 h before sampling. n = 3 logs, except for wounded phloem in MeJA-treated logs where n = 5 (Oecologia 167:691–699).

Fungal induced terpene accumulation inhibits beetle colonization in a dose-dependent manner

Beetle attack density and gallery length increased markedly with decreasing terpene levels in the bark. Trees with low terpene levels had 3.5 and high terpene level had 3.5 and 20.3 fold higher attack density, and 2.9 and 37.9 fold longer gallery length than medium and high terpene trees, respectively.

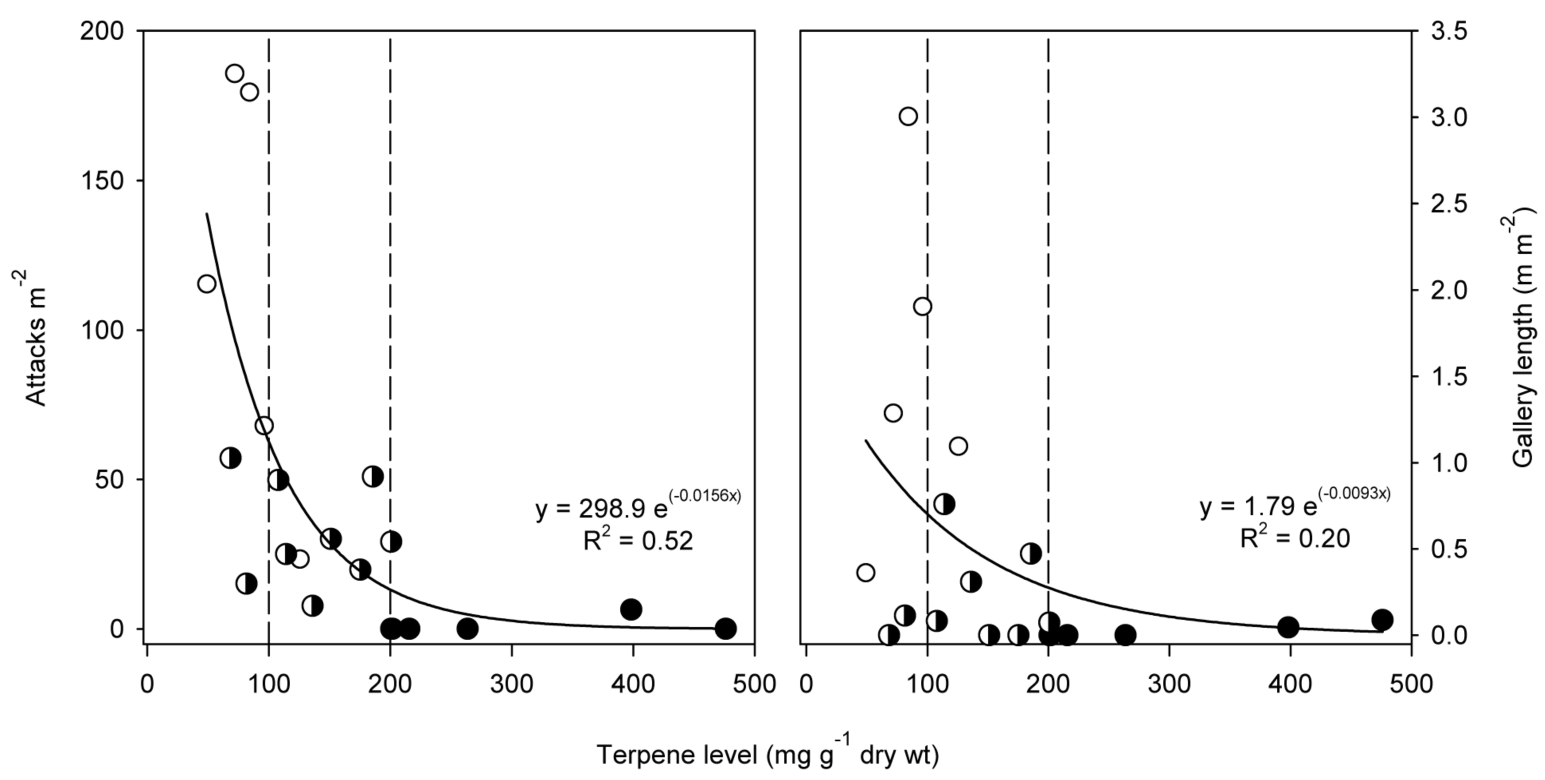
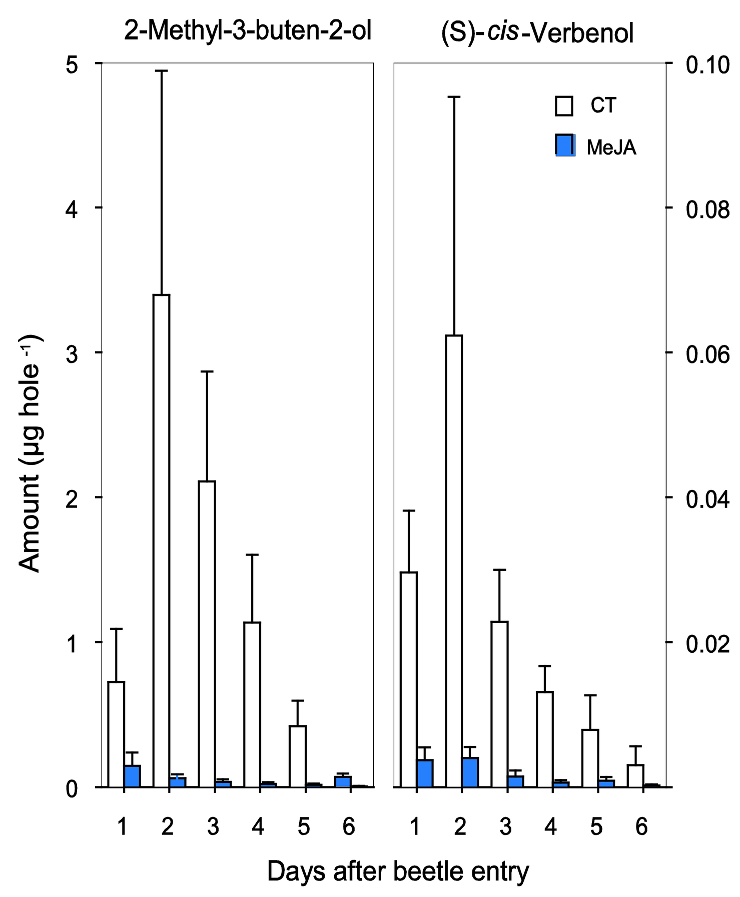


Fig 4. Attack densities and gallery lengths in 20 Norway spruce trees with different induced terpene levels in the bark. Chemical samples were taken from the reaction zone 35 days after *Ceratocystis polonica* inoculation. Dotted lines show two potential thresholds in terpene levels (200 and 100 mg g^{-1} dry wt) with different inhibitive effects on beetle colonization. Trees were classified as resistant (black dots), susceptible (white dots) or intermediate (black and white dots) with respect to beetle colonization success (PLoS ONE 6: e26649).

Host resistance elicited by MeJA reduced emission of aggregation pheromones by *Ips typographus*



Beetles tunneling in control logs produced 35.9-fold more methyl butenol and 13.9-fold more (S)-*cis*-verbenol than beetle tunneling in MeJA-treated logs over the collection time.

Fig 5. Amounts of pheromones emitted from *Ips typographus* entrance holes in control logs (*CT*, n = 7 holes) and methyl jasmonate treated (*MeJA*, n = 13 holes) Norway spruce logs the first 6 days after beetle entry (Oecologia 167:691–699).

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