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Chemical similarity between introduced and native populations of Scots pine can facilitate transcontinental expansion of mountain pine beetle in North America

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Abstract Introduced forest tree species are frequently attacked by insects in their new range; however, it has been seldom investigated whether the presence of such tree species increases the risk of range expansion of native insect herbivores in the introduced range. European Scots pine has been introduced to North America including within a portion of the range of the mountain pine beetle (MPB). We investigated Scots pine suitability to MPB as a host in the introduced range of the pine populations. We compared chemotypic similarity of foliage between introduced and native Scots pine populations, and then determined the suitability of introduced populations to MPB. Suitability was assessed based on whether beetles produce aggregation pheromone components and complete development in Scots pine bolts. We also assessed whether or not suitability was affected by the host chemotypes. Introduced and native pine populations had the same sesquiterpene chemotypes and shared one of the two monoterpene chemotypes. All introduced populations were suitable for MPB but the suitability varied slightly with host chemotype. This is the first report of chemotypic variations of Scots pine populations

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outside its native range. Chemotypic similarity between the introduced and native pine populations, ability of beetles to produce pheromones and to complete its life cycle on bolts from all chemotypes, and preferential colonization of Scots pine over native pine species by MPB in field suggest that introduced Scots pine populations could facilitate transcontinental expansion of MPB in North America.

Keywords Bark beetles · *Dendroctonus* · Invasion ecology · *Pinus sylvestris* · Terpenes

Introduction

Biological invasions of novel habitats by insect herbivores have been frequently reported during the last decades (Walther et al. 2009; Brockerhoff and Liebhold 2017). Despite the frequent attacks of introduced plant species by insects in the plant's new range, few studies in forestry have investigated whether introduced tree species could increase the risk of range expansion of native insect herbivores in the introduced range (Fraser and Lawton 1994; Lindelöw and Björkman 2001; Branco et al. 2015).

Scots pine (*Pinus sylvestris* L.) is a Eurasian forest tree species that has been widely planted in North America, including parts of the range of the mountain pine beetle (MPB, *Dendroctonus ponderosae* Hopkins, Col: Curculionidae). Although its suitability to MPB as a host has been demonstrated in the field (Furniss and Schenk 1969; McCambridge 1975; Fries 2016; Rosenberger et al. 2017); in all cases, Scots pine trees were studied either in only a single location (disregarding the variations within the species) or using small sample sizes of trees. Thus conclusions cannot be reasonably extrapolated to other populations in North America or to the populations in Eurasia. Particularly, it is unknown whether Scots pine populations in North America show similar chemotypic variation (phenotypes with distinct chemical profiles) as reported in Europe. Furthermore, given that host phytochemicals can strongly influence MPB-host interactions (reviewed by Erbilgin 2019), it is unknown whether phytochemical variation among Scots pine populations could influence their suitability to MPB. Thus, a clear understanding of chemical similarity between native and introduced Scots pine

populations and variation in suitability to MPB among introduced pine populations could be used to extrapolate a potential suitability of Scots pine as a host by MPB across North America as well as to determine the possible transcontinental expansion of MPB to regions where Scots pine is present.

Scots pine has a very large geographical range in Eurasia from the UK, Norway, Finland, Sweden, and Russia (Eastern Siberia to the Sea of Okhostsk) to Spain and Turkey. In this range, Scots pine populations show two chemotypes based on the amounts of two monoterpenes, 3-carene and α-pinene (Sjödin et al. 2000; Manninen et al. 2002; Semiz et al. 2007; Thoss et al. 2007; Kännaste et al. 2013). The α-pinene chemotype occurs more commonly in the southern part of the range; whereas, the 3-carene chemotype is more prevalent in northern than in southern populations (Muona et al. 1986). α-Pinene and 3-carene are major monoterpenes of both foliage and vascular (phloem) tissues (Manninen et al. 2002).

Scots pine is also widely planted in southern Canada and the United States (USA). It is largely used as a Christmas tree in the USA or to control wind and water erosion and to a large extent in ornamental plantings. In the USA, Tobolski and Hanover (1971) sampled Scots pine seedlings growing in a common garden environment in Michigan and reported that α-pinene and 3-carene were the most abundant monoterpenes among 108 populations that originated mainly from northern Europe. Furthermore, Rosenberger et al. (2017) sampled five mature Scots pine trees from Minnesota (USA) and reported that α-pinene and 3-carene were the most abundant monoterpenes. To our knowledge, no other studies have reported the chemistry of mature Scots pine populations in North America, especially at the continental level.

Mountain pine beetle has a large host range in western North America, colonizing many native pine species, primarily lodgepole pine (*Pinus contorta*) (Wood 1982). It is considered to be regionally invasive due to its recent host (*Pinus banksiana*) and geographical range expansions in western North America (Cudmore et al. 2010; Cullingham et al. 2011; Raffa et al. 2017). The MPB-host interaction is well characterized (Safranyik et al. 2010). Briefly, beetles colonize and reproduce within the phloem (living subcortical vascular tissue of trees located between the outer bark and the outermost layer of xylem) of their host pines and must overcome tree defenses via

aggregation elicited by beetle pheromones and host phytochemicals. After successful entry into the phloem, beetles mate, excavate egg galleries, and oviposit. The larvae hatch within several days; they feed and develop under the bark, and emerge as adults in summer of the subsequent year. These beetles have a 1-year life cycle in western Canada.

Host phytochemicals, mainly monoterpenes, can influence MPB interactions with host trees. First, phytochemicals are closely linked to MPB pheromone production (reviewed by Blomquist et al. 2010). For example, the female aggregation pheromone *trans*-verbenol is a bicyclic monoterpenoid alcohol and is likely produced via hydroxylation of α-pinene present in host phloem (Gries et al. 1990; Chiu et al. 2018). The male aggregation pheromone *exo*-brevicomin is synthesized de novo by the beetle from its precursor fatty acids in the host tissues. Verbenone is an anti-aggregation pheromone produced by beetles (Byers et al. 1984), by auto-oxidation of α-pinene via the intermediary compounds *cis*- and *trans*-verbenol (Hunt et al. 1989; Hunt and Borden 1990), and by degradation of host material by microorganisms associated with bark beetles (Leufvén et al. 1984). Second, host volatiles (mainly monoterpenes) emitted during host colonization by bark beetles can influence beetle aggregation on hosts and can synergize or inhibit beetle attraction to its pheromones (Borden et al. 2008; Erbilgin et al. 2014, 2017). Finally, the toxic host phytochemicals, such as monoterpenes, present in pine resin can lead to unsuccessful host colonization, and thus failed beetle reproduction by MPB (Raffa and Berryman 1983; Erbilgin et al. 2017).

Historical hosts of MPB show considerable chemotypic variation. For example, lodgepole pine monoterpenes show three common chemotypes defined by different concentrations of the most abundant three monoterpenes, β-phellandrene, α-pinene, and β-pinene (Forrest 1980). These chemotypes are further subdivided into subtypes on the basis of the presence or absence of 3-carene and of limonene. Such chemotypic variation in lodgepole pine monoterpenes can influence MPB biology, including egg gallery excavation, fecundity, survivorship, fitness, and pheromone production (reviewed by Erbilgin 2019).

We hypothesize that Scots pine trees in the introduced range may facilitate expansion of MPB across the North American continent through "phytochemical stepping stone" hypothesis proposed by Erbilgin (2019). Thus we tested whether: (1) introduced Scots pine populations are chemotypically similar to the European populations, and (2) suitability of introduced Scots pine populations to MPB depends on the chemotypes of the populations. For the chemotypic investigations, we sampled foliage of different Scots pine populations from Canada, Sweden, and Finland, and compared the introduced and Eurasian populations. Due to climatic similarity between Scandinavia and Canada, we focused on these regions (Bentz et al. 2019). Even though MPB feeds and breeds in the phloem, we sampled foliage, because we could sample a large number of trees, particularly from Europe. Suitability of introduced Scots pine populations to MPB was assessed with cult bolts. We focused on two biological criteria: whether beetles can produce pheromone components and complete development in the bolts from introduced range at levels similar to those in known MPB hosts. We also determined whether chemotypes based on phloem monoterpene profiles of Scots pine populations in Canada affect beetle pheromone production and biological development.

Materials and methods

Foliage sampling

We collected the foliage of 240 Scots pine trees in Canada, Sweden, and Finland (Electronic Supplementary Material (ESM)-Table 1): We sampled 12 trees in two sites in Nova Scotia, 28 trees in five sites in New Brunswick, 48 trees in five sites in Ontario, 47 trees in one site in Alberta (Canada), and 30 and 75 trees in Sweden and Finland, respectively. Trees from Finland represented varieties of provenances from western Russia (85%) and some local varieties from Finland (15%). Trees from Sweden were all from northern European sources. From each tree, we collected 15 current-year needles from the top third of the tree crown. The diameter at 1.4 m height of trees was 20 ± 3.8 cm. Collections were completed in all locations from July to early August in 2017. After sampling, needles were either stored in the liquid N or dry ice in the field and transferred within a few hours to the respective storage facilities in each sampling location, and then stored at -40 °C until shipment to

the University of Alberta, where samples were stored at -40 °C.

Phloem sampling

To evaluate pheromone production and reproduction by MPB in the Scots pine bolts, we selected 24 trees from three locations in Canada: four trees from Alberta, ten trees from New Brunswick, and ten trees from Nova Scotia (ESM-Table 1). These trees were subsets of trees sampled for foliage above. We cut a single bolt (35 cm long, 25 ± 4.3 cm diam. at 1.4 m height) from each tree. In the field, both ends of the bolts were covered with paraffin wax to reduce moisture loss. Bolts from Nova Scotia and New Brunswick were wrapped up in tarps, put into nylon duffle bags, and then shipped to the University of Alberta. We used tarps and bags to prevent insects from attacking bolts during transport. Bolts were delivered within 2–3 days of the shipment. After receiving the bolts, we stored them at 4 °C for a maximum of 3 days until used in the beetle pheromone and reproduction experiments as descried below. After introduction of live beetles (see details below), from each bolt we sampled one 2 cm × 2 cm piece of phloem on the side of the bolt opposite to the beetle entrance holes. These samples were stored at − 40 °C.

Chemical analysis of foliage and phloem

Hexane-extractable mono- and sesquiterpene compounds were identified and quantified (Klutsch et al. 2016). Briefly, we extracted 100 mg of ground tissue (foliage or phloem) twice with 0.5 ml of hexane containing 0.004% pentadecane (internal standard). Samples were vortexed for 30 s, sonicated for 10 min, and centrifuged at 16,100 rcf at 2 °C for 15 min for each extraction. Afterwards we pooled the two extracts. We injected the extract (1 μl) with a 10:1 split ratio into a gas chromatograph/mass spectrometer (GC/MS, Agilent 7890A/5975C, Agilent Tech., Santa Clara, CA, USA) equipped with a HP-CHIRAL-20β column (I.D. 0.25 mm, length 30 m) (Agilent Tech.) with helium carrier gas flow at 0.9 ml/min, and a temperature of 50 °C for 5 min, increased to 75 °C by 40 °C/min and held at 3 min, increased to 100 °C by 1.5 °C/min and held for 0.5 min, and then to 250 °C by 15 °C/min and held for 0.2 min. Compounds were

quantified using standard curves from a series of four dilutions prepared from analytical standards of pulegone, α-terpinene, γ-terpinene, α-terpineol, geranyl acetate, α-humulene (Sigma-Aldrich, St. Louis, MO, USA), (−)-caryophyllene oxide (Acros Organics, NJ, USA), (−) and (+)-α-pinene, (−) and (+)-β-pinene, (−) and (+)-limonene, (−)-camphene, myrcene, (+)-3-carene, cymene, naphthalene, 4-allylanisole, borneol, p-cymene (Fluka, Sigma-Aldrich, Buchs, CHE), (+)-camphene, *cis*-ocimene, bornyl acetate, terpinolene, β-caryophyllene (SAFC Supply Solutions, St. Louis, MO, USA), and β-phellandrene (Erbilgin Lab). Chemical purity of all these chemicals was greater than 97%. For identified sesquiterpenes for which we did not have standards, we calculated concentrations from the nearest eluting sesquiterpene standard compound.

Pheromone collection

To obtain live MPB, we cut three naturally infested lodgepole pine trees located near Hinton, Alberta (Lat: 53.42448 N, Long: 117.51428 W) in late May of 2017. We obtained several bolts from each tree and placed them in rearing containers to allow beetles to complete their development. Beetles emerging from these containers were stored at 4 °C for 2–3 days and used in the following experiment. We introduced two pairs of beetles (2–3 days old) into opposite sides of each bolt and collected pheromone components (Erbilgin et al. 2014). Briefly, for each introduction point, we drilled a single hole (0.5 cm in diam.) through the outer bark at 5 cm from the bolt end and secured a single female over the hole with one half of a gelatin capsule. Once the female initiated excavation of an egg gallery and its abdomen was no longer visible in the entrance hole, we placed a male beetle in the same capsule. If the female rejected the first male introduced, within 24 h we replaced the rejected male with a new one. We continuously collected pheromones emitted from holes for 4 h, using the following method.

Briefly, a small Teflon funnel (2.5 cm in diam. of mouth) was placed above each beetle entrance hole and a charcoal filter (Honeywell, Southborough, MA, USA) was placed at the gap between the bark and the mouth of the funnel (Pureswaran and Sullivan 2012). Each funnel was attached to a vacuum pump (Cole-Parmer Canada Inc., Montreal, QC, CAN) with a

Teflon tube, and an adsorbent cartridge [Porapak Q (OD, 6 mm; length, 110 mm; adsorbent: front layer, 150 mg; back up layer, 75 mg; separated by glass wool), SKC Inc., Eighty Four, PA, USA] was inserted in the tube between the pump and the funnel. Pheromone components emitted from individual beetle entrance holes were trapped in the adsorbent cartridges for 4 h. The flow rate (100 ml/min) of the pumps was kept constant during pheromone collection. Additional pheromone collections were made from the same entrance holes using the same collection method but a new adsorbent cartridge at 12, 24, 36, 60, 84, and 108 h after female beetle introduction. After each collection, the adsorbent cartridges were capped and stored at − 40 °C before extraction. Bolts were kept at room temperature (23–25 °C, 55% RH) during pheromone collection.

Pheromone chemical analysis

We extracted pheromones trapped in the adsorbent cartridges with 1 ml of dichloromethane (internal standard 0.0025% heptyl acetate) (Sigma-Aldrich) using established methods (Erbilgin et al. 2014). Sample extract (1 µl) was injected in splitless mode (the entire extract was loaded on the column) into a GC/MS equipped with a HP-Chiral-20βcolumn (Agilent Tech.) with helium carrier gas flow at 1.2 ml/min, and a temperature of 50 °C for 2 min, increased to 90 °C by 45 °C/min and held at 2 min, increased to 155 °C by 6 °C/min and held for 1 min, and then to 230 °C by 25 °C/min and held for 3 min. The MS was set to SCAN and SIM mode with the following ion mass and time program: ion masses 72, 85 100, and 114 starting at 3.5 min, ion mass 43 starting at 16.5 min, ion mass 170 starting at 18.0 min, and ion masses 107 and 109 starting at 21.7 min. Pheromone components were quantified using standard curves from a series of four dilutions prepared from analytical standards of *cis-/trans*-verbenol (ion masses: 107, 109), *exo*-brevicomin (ion masses: 72, 85, 114), frontalin (ion masses: 72, 85, 100), and verbenone (ion masses: 107, 109) (Contech Enterprise Inc. BC, CAN). Chemical purity of these compounds was greater than 85%.

Assessing MPB reproduction

To evaluate how Scots pine chemotypes affected beetle colonization and brood quality, after pheromone collection, we placed inoculated bolts in individual rearing containers for 10–12 weeks at room temperature to allow the offspring of the inoculated beetles to complete their development and emerge during the next 3 months. We randomly selected one of the two beetle galleries under the bark and exposed it by removing the outer bark. We measured egg gallery length and counted the number of larval galleries, pupal chambers, and emergence holes per egg gallery. We also calculated the incidence of bolts with at least one larva, pupal chamber, and emergence hole.

Statistical analysis

All statistical analyses were conducted using the R software environment version 3.4.0 (R Core Team 2017). When necessary, data were log-transformed to meet assumptions of parametric statistics based on a normal distribution. We used raw data in the construction of figures and tables. The P values were significant at α = 0.05 for all statistical comparisons below.

We analyzed foliar monoterpenes and sesquiterpenes as concentrations [ng/mg dry weight (DW) of plant tissue] for individual compounds. We calculated total monoterpenes and sesquiterpenes by summing the concentrations of each individual compound of the respective chemical class. Whether country or province had significant effects on total monoterpenes and sesquiterpenes was tested using separate general linear models in R (packages "lme4" version 1.1-13 (Bates et al. 2015), and "lmerTest" version 2.0-36 (Kuznetsova et al. 2017). Following a significant omnibus test, we conducted post hoc multiple comparisons between Canada and Europe, or among four Canadian provinces using Tukey HSD tests.

We separately determined monoterpene chemotypes for European and Canadian samples using methods adapted from Taft et al. (2015) by first calculating the proportion of each individual monoterpene in each sample. We used the resulting monoterpene profiles with functions available in the R package "fpc" version 2.1-11 (Hennig 2018) to cluster samples using a partitioning-around-mediods technique, with the number of clusters estimated by optimum average silhouette width. For a given cluster, we centered the matrix of individual monoterpene proportions and scaled to determine the mean relative proportion of

each monoterpene. We labeled clusters based on the compound with the greatest relative proportion within a cluster and/or showed the greatest difference between clusters. We determined characteristics defining each cluster by comparing the 2.5% and 97.5% quantiles between the two clusters for a given region. We characterized sesquiterpene chemotypes using the same methodology.

We analyzed monoterpenes in bolts as concentrations (ng/mg DW) for individual compounds. We calculated total monoterpenes by summing the concentrations of individual compounds. Whether the three Canadian provinces had any effects on total phloem monoterpenes was tested using general linear mixed models as computed using functions available in the R packages "lme4" version 1.1-13 and "lmerTest" version 2.0-36. This model included province as a fixed factor and individual tree as a random factor, which was necessary to account for potential within-tree variation as some of the bolts used originated from the same tree. We followed significant models by multiple comparisons among provinces using Tukey HSD tests. We tested profiles of individual phloem monoterpenes (as concentrations and proportions) for significant inter-province variation using permutational multivariate analysis of variance (PerMANOVA). We used phloem monoterpenes to characterize chemotypes using the methods described for foliar monoterpenes.

We calculated the pheromones emitted from bolts inoculated with MPB as concentration (µg/ml) for each of the six sampling periods. We averaged concentrations of each pheromone across sampling period to determine potential differences between chemotypes based on phloem monoterpenes using general linear mixed models using the R packages mentioned for phloem monoterpenes. Models included mean concentration of each pheromone as a response variable, chemotype as a fixed factor, and bolt identity nested within tree identity as a random factor. This random factor was necessary to account for potential within-bolt variation as pheromones were collected from two holes where beetles were inoculated per bolt. We followed significant models by multiple comparisons using Tukey HSD tests.

We evaluated several factors to assess MPB productivity in bolts: egg gallery length (mm), the number of larvae, pupal chambers, and number of beetle emergence holes per egg gallery per bolt, the incidence of bolts with at least one larva, pupal chamber, and emergence hole. We tested inter-chemotype differences in gallery parameters/measurements for statistical significance using general linear mixed models, with chemotype as a fixed factor and tree as a random factor. Following a significant model, we made multiple comparisons using Tukey HSD tests. Similarly, we investigated the incidence of bolts with at least one larva for significant inter-chemotype differences using a mixed effects logistic regression model. This model used chemotype as a fixed factor and tree as a random effect. We followed significant models by multiple comparisons using Tukey HSD tests on the proportion of bolts for a given province. We tested inter-chemotype differences in the number of bolts with any evidence of pupation and emergence using a Chi squared test.

Results

Foliar monoterpenes

We detected 16 monoterpenes in the foliage of Scots pines from Canada, Finland, and Sweden (ESM-Table 2). The sampling location influenced the total monoterpene concentration ($F_{2,237} = 31.26$, $P < 0.001$). Total monoterpenes were most concentrated in foliage samples from Sweden, which had concentrations 87% and 71% greater than those samples from Finland and Canada, respectively (Fig. 1a). Total concentrations of foliar monoterpenes also varied among Canadian provinces ($F_{3,131} = 24.34$, $P < 0.001$). Concentrations from trees from Alberta were 104%, 60%, and 69% greater than those of trees from New Brunswick, Nova Scotia, and Ontario, respectively (Fig. 1b).

Scots pine foliage from Canada and Europe exhibited a partial overlap in the chemotypes characterizing each region (Table 1). For the Canadian populations, k-medoids clustering partitioned the composition profiles of individual foliar monoterpenes into two clusters that best explained the variation in these data. The first cluster was characterized by relatively high levels of (−)-limonene, whereas high levels of (+)-α-pinene alone characterized the second cluster (Fig. 2a). The European trees were also partitioned into two clusters, with the first cluster being characterized by relatively high levels of 3-carene and the
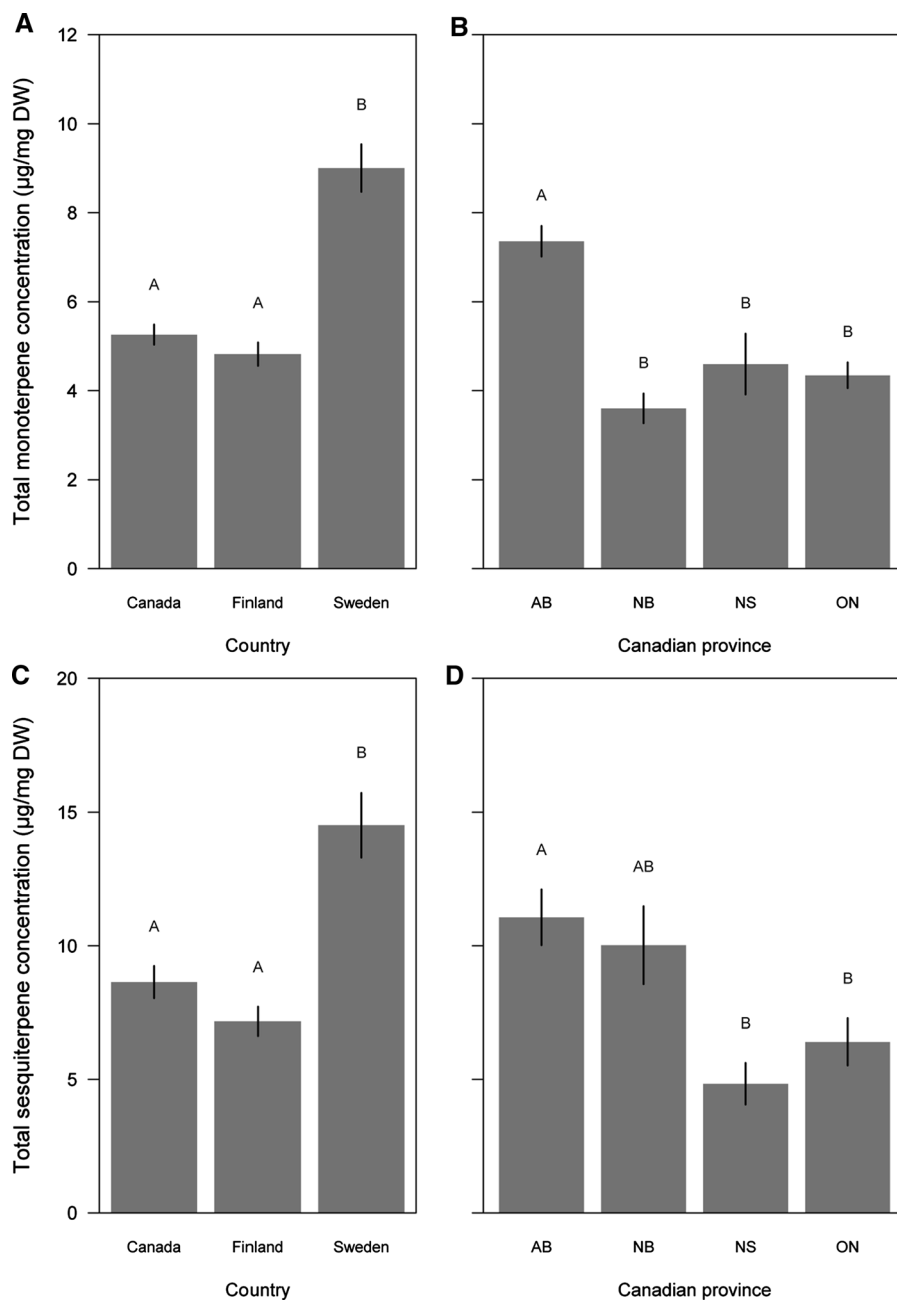
Fig. 1 Mean ($\pm$ SE) concentrations (μg/mg DW) of total monoterpenes (**a, b**) and sesquiterpenes (**c, d**) in *Pinus sylvestris* foliar samples from Finland, Sweden, and four Canadian provinces, Alberta (AB), New Brunswick (NB), Nova Scotia (NS), and Ontario (ON). Bars with different letters represent significantly different means (Tukey HSD tests). Please note that the unit on this figure is (μg/mg DW) whereas the unit reported on ESM-Tables 2 and 3 is (ng/mg DW)

second by high levels of (+)-α-pinene (Fig. 2a). For the first cluster, (+)-α-pinene had the highest relative mean proportion of all monoterpenes, but the cluster was characterized based on 3-carene as this compound allowed differentiation with the second cluster

(Fig. 2a). The (+)-α-pinene clusters represented nearly half of all samples from each region: 57% of Canadian and 50% of European samples. The proportion of samples representing each chemotype varied among Canadian provinces, with the (+)-α-pinene

Table 1 The proportions of foliar monoterpenes and sesquiterpenes separating *Pinus sylvestris* chemotypes between Canada and Europe (Sweden and Finland combined)

Location	Chemotypes	Characteristic proportions
Monoterpenes		
Canada	(−)-Limonene	(+)-α-Pinene < 0.34
	(+)-α-Pinene	(+)-α-Pinene > 0.34
Europe	3-Carene	3-Carene > 0.10
	(+)-α-Pinene	3-Carene < 0.10
Sesquiterpenes		
Canada	Germacrene-D-4-ol	Germacrene-D < 0.31
	Germacrene-D	Germacrene-D > 0.31
Europe	Germacrene-D-4-ol	Germacrnene-D-4-ol > 0.21
	Germacrene-D	Germacrene-D-4-ol < 0.21

Fig. 2 The relative proportions of individual monoterpenes (**a**) and sesquiterpenes (**b**) in *Pinus sylvestris* foliar samples from Canada and Europe. Samples from each location were partitioned into two chemotypes, each characterized by different dominant monoterpenes or sesquiterpenes (black bars)

chemotype being most abundant in all provinces except New Brunswick (Table 2).

Foliar sesquiterpenes

Fifteen sesquiterpenes were detected in foliage samples from Canada, Finland, and Sweden (ESM-Table 2). Percent concentration of sesquiterpenes significantly differed among the countries ($F_{2,227.7} = 14.65$, $P < 0.001$), with samples from Sweden having concentrations 68 and 102 greater than those of samples from Canada and Finland, respectively (Fig. 1c). Total sesquiterpene concentrations also significantly varied among Canadian provinces ($F_{3,98.7} = 5.60$, $P < 0.001$) (Fig. 1d). Foliage from Alberta had the highest sesquiterpene concentrations that were 129% and 73% greater than those from Nova Scotia and Ontario, respectively, but similar to those from New Brunswick.

Foliage from Canadian and European pine populations had similar chemotypes. The proportions of sesquiterpenes characterizing these chemotypes in each region are shown in Table 1. Clustering partitioned the composition of profiles of individual sesquiterpenes into two chemotype groups for each geographical region. The first group represented trees with relatively high proportions of germacrene-d, whereas trees in the second group had high levels of germacrene-d-4-ol (Fig. 2b). The proportion of samples representing each chemotype differed between regions. The germacrene-d-4-ol chemotype

represented 70% of samples from Canada and 30% of samples from Europe. A complementary proportion was observed in each region for the germacrene-d chemotype. The proportion of each chemotype varied among Canadian provinces. The germacrene-d-4-ol chemotype was the most abundant in all provinces except Ontario, where the chemotypes were equally represented (Table 2).
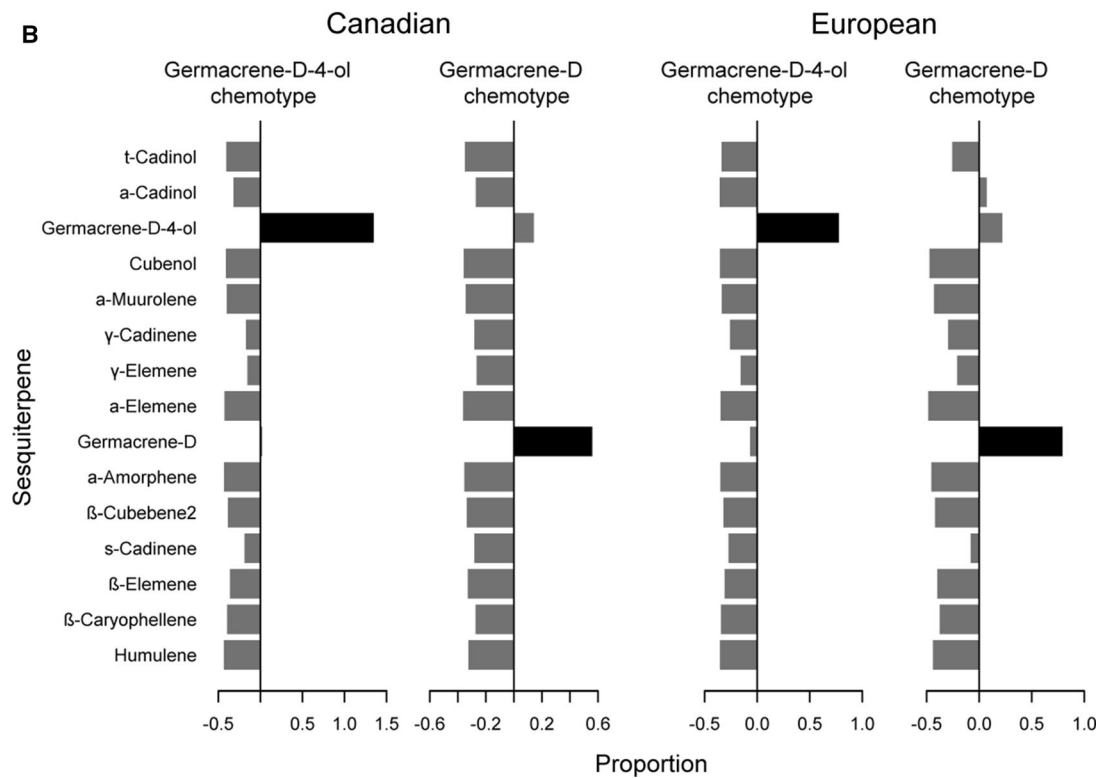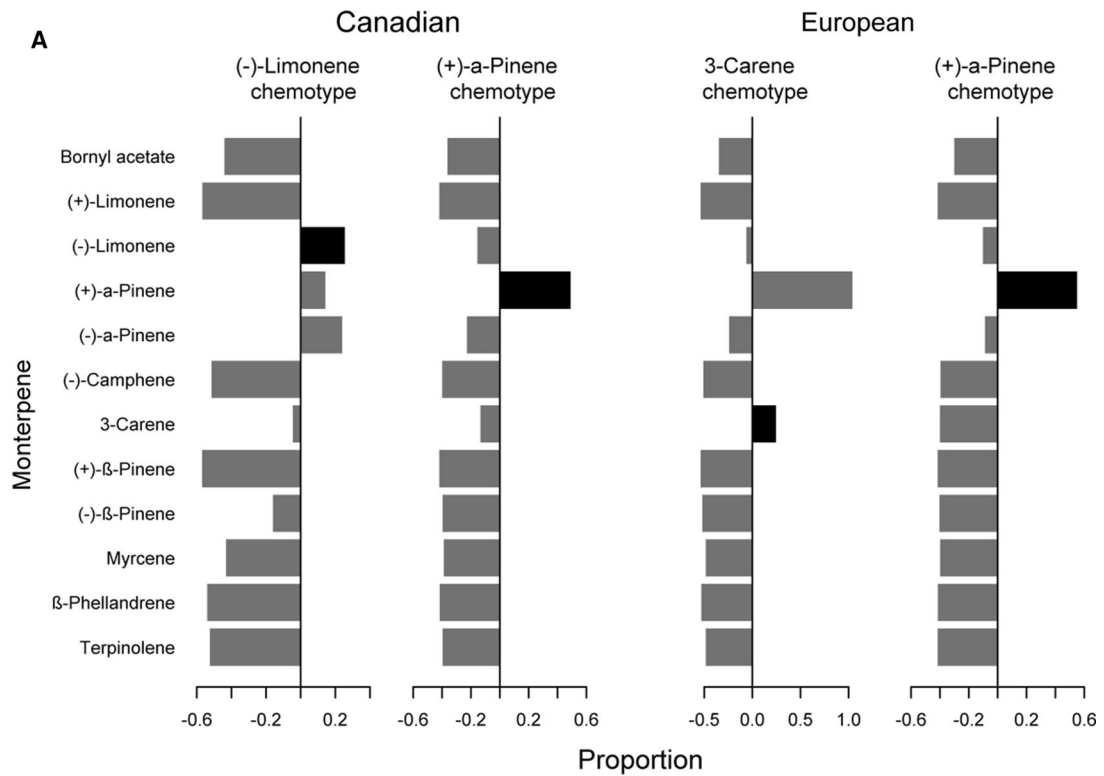
Phloem monoterpenes

Overall, we detected 10 monoterpenes from the phloem of bolts in Alberta, New Brunswick, and Nova Scotia (ESM-Table 3). The sampling locations did not influence total monoterpenes. Similarly, province-of-origin did not affect the profiles of individual monoterpenes in bolts, as profiles calculated from both concentrations and proportions of individual monoterpenes did not differ among provinces.

Using the composition profiles of individual monoterpenes of 24 bolts, *k*-medoids clustering partitioned the profiles into two clusters that best explained the variation in the data (Fig. 3). Cluster one was characterized by relatively high levels of 3-carene, with approximate defining proportion of > 0.35 3-carene and < 0.38 (+)-α-pinene. Cluster two was characterized by relatively high proportion of (+)-α-pinene, with approximate defining proportions of > 0.53 (+)-α-pinene and < 0.29 3-carene. Among the provinces, the 3-carene and (+)-α-pinene chemotypes represented 30% and 70% of bolts respectively. This overall difference in the relative abundance of chemotypes was also observed with slight variation for each province (Table 3).

Pheromone production

We detected all four main pheromones of MPB including *cis-/trans*-verbenol, *exo*-brevicomin, frontalin, and verbenone from bolts inoculated with beetles (Fig. 4). The emission patterns of all pheromones
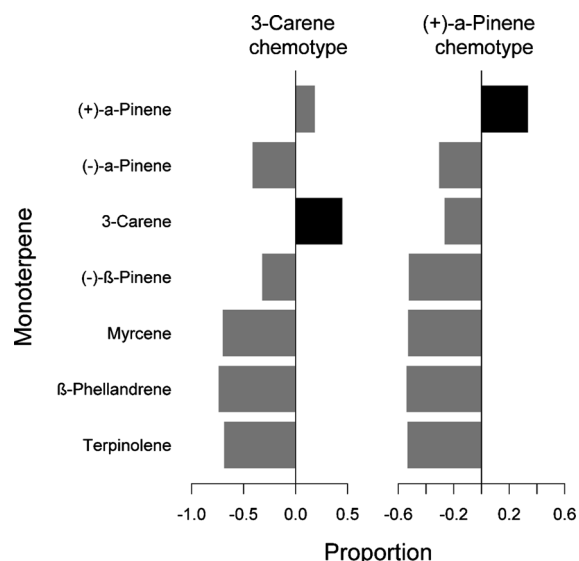
Fig. 3 The relative proportions of individual monoterpenes in *Pinus sylvestris* phloem samples pooled from three Canadian provinces. Samples were partitioned into two chemotypes, each characterized by different dominant monoterpenes (black bars)

except *exo*-brevicomin over the six sampling periods varied between the 3-carene and (+)-α-pinene chemotypes previously determined from bolts, with no consistent emission peak. However, the emission of *cis-/trans*-verbenol tended to decrease over time, frontalin generally increased over time, and verbenone

remain fairly stable over time for the (+)-α-pinene chemotype and decreased for the 3-carene chemotype. For *exo*-brevicomin, peak emission was detected during the 24 h sampling period for each chemotype, though the emission of this pheromone at peak emission was lower for the (+)-α-pinene chemotype. The average concentration of pheromone components detected over all sampling periods was similar between the 3-carene and (+)-α-pinene chemotypes (*cis-/trans*-verbenol: $F_{1,35.9} = 0.726$, $P = 0.4$; *exo*-brevicomin: $F_{1,35.9} = 3.45$, $P = 0.07$; verbenone: $F_{1,20.3}, = 1.26$, $P = 0.275$; frontalin: $F_{1,20.3} = 2.78$, $P = 0.11$) (Fig. 5).

MPB reproduction

Beetles successfully produced brood in bolts originating from trees from Alberta, New Brunswick, and Nova Scotia (Table 4). The average length of beetle egg galleries per bolt did not differ between the 3-carene and (+)-α-pinene chemotypes ($F_{1,35.9} = 1.75$, $P = 0.201$) (Fig. 6). Likewise the proportion of bolts containing any larvae was not different between the two chemotype [$\chi^2(1) = 2.87$, $P = 0.099$]. The number of bolts with evidence of pupation/emergence for all bolts with evidence of larvae was 5 (of a total of 6 bolts) for the 3-carene chemotype and 9 (of 9 bolts) for the (+)-α-pinene

Chemotypes	Proportion of each chemotype in each province (total number of samples)			
	Alberta (47)	New Brunswick (28)	Nova Scotia (12)	Ontario (48)
Monoterpenes				
(−)-Limonene	0.34	0.68	0.25	0.42
(+)-α-Pinene	0.66	0.32	0.75	0.58
Sesquiterpenes				
Germacrene-D-4-ol	0.85	0.82	0.67	0.50
Germacrene-D	0.15	0.18	0.33	0.50

Table 2 The proportion of chemotypes of foliar samples of *Pinus sylvestris* from four Canadian provinces

Table 3 The proportion of *Pinus sylvestris* bolts from three Canadian provinces representing the 3-carene and (+)-α-pinene chemotypes

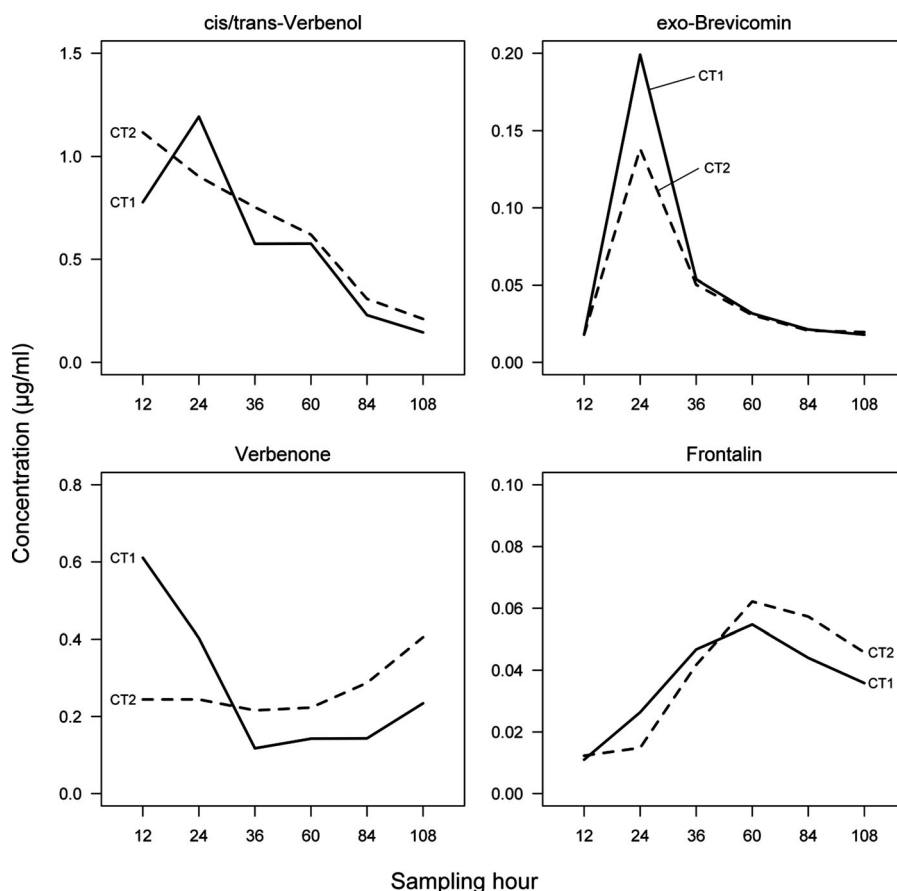| Chemotypes	Proportion of bolts in each chemotype in each province (Total number of bolts)		
	Alberta (7)	New Brunswick (10)	Nova Scotia (10)
3-Carene	0.29	0.20	0.40
(+)-α-Pinene	0.71	0.80	0.60

Fig. 4 Average concentrations (μg/ml) of four *Dendroctonus ponderosae* pheromone components emitted at six sampling periods from *Pinus sylvestris* bolts inoculated with live beetles. Sample collection began at time 12 h (12 h after females were introduced) and ended 108 h later (volatiles collected over 4 h periods in collection tubes replaced at 12, 24, 36, 60, 84, and 108 h). Bolts were cut from trees in Canada and represented two monoterpene chemotypes: 3-carene (CT1; solid) and (+)-α-pinene (CT2; dashed). Concentrations represent amounts (μg) of individual chemicals per 1 ml of extract

chemotype. However, these numbers of bolts did not differ between chemotypes [$\chi^2(1) = 2.75$, $P = 0.097$].

We further investigated if limonene influences beetle performance and pheromone production. Out of 24 trees sampled, only two bolts from New Brunswick had limonene, thus we compared beetle performance on bolts with (n = 8) or without (n = 2) limonene from this province alone. Female beetles excavated egg galleries in both types of bolts, however only in the bolts with limonene, beetle larvae failed to develop (Table 5). Furthermore, the presence of limonene was associated with the reduced production of all three beetle pheromones including *cis/trans*-verbenol (36% of that of bolts without limonene), verbenone (51%), and frontalin (66%) (ESM-Table 4). *exo*-Brevicomin concentration did not change between the two bolt types.

Discussion

Introduced and European Scots pine populations shared one of the two monoterpene chemotypes depending on the proportions of (+)-α-pinene, (−)-limonene, and 3-carene in foliage. The introduced populations had the (+)-α-pinene and (−)-limonene chemotypes while the European populations had the (+)-α-pinene and 3-carene chemotypes. Overall, these results are in partial agreement with the reported Scots pine chemotypes in Eurasia. Furthermore, both introduced and European populations shared the same
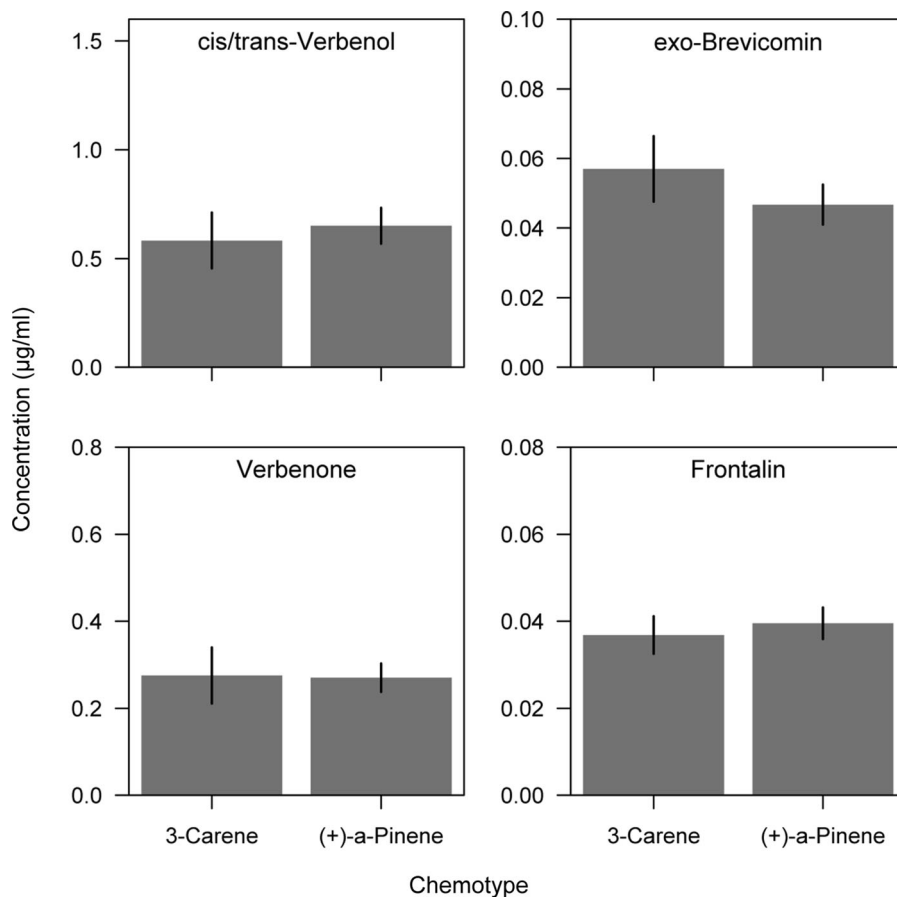
Fig. 5 Mean (± SE) concentrations (μg/ml) of four *Dendroctonus ponderosae* pheromone components emitted from *Pinus sylvestris* bolts inoculated with live females followed by males. Bolts were cut from trees in Canada and represented two monoterpene chemotypes: 3-carene and (+)-α-pinene. Concentrations represent amounts (μg) of individual chemicals per 1 ml of extract

Table 4 Metrics of mountain pine beetle (*Dendroctonus ponderosae*) brood development in artificially inoculated bolts of *Pinus sylvestris* trees from three Canadian provinces

Canadian provinces	Mountain pine beetle brood development (per maternal gallery)		
	Mean number of larvae	Mean number of pupal chambers	Mean number of emergence holes
Alberta	64.00 ± 19.79	57.20 ± 18.07	38.25 ± 15.07
New Brunswick	29.50 ± 10.50	27.50 ± 9.50	12.50 ± 6.50
Nova Scotia	75.78 ± 10.13	62.81 ± 8.66	29.63 ± 4.74

Values are mean ± SE

sesquiterpene chemotypes, germacrene-d and germacrene-d-4-ol. This is the first report of chemotypic variations of Scots pine populations outside its native range. Additionally, bolts from the introduced populations were characterized in either (+)-α-pinene or 3-carene chemotype and both chemotypes were equally suitable for beetle pheromone production and reproduction. This is the first demonstration of MPB pheromone production on Scots pine, providing a mechanistic explanation for the colonization of both
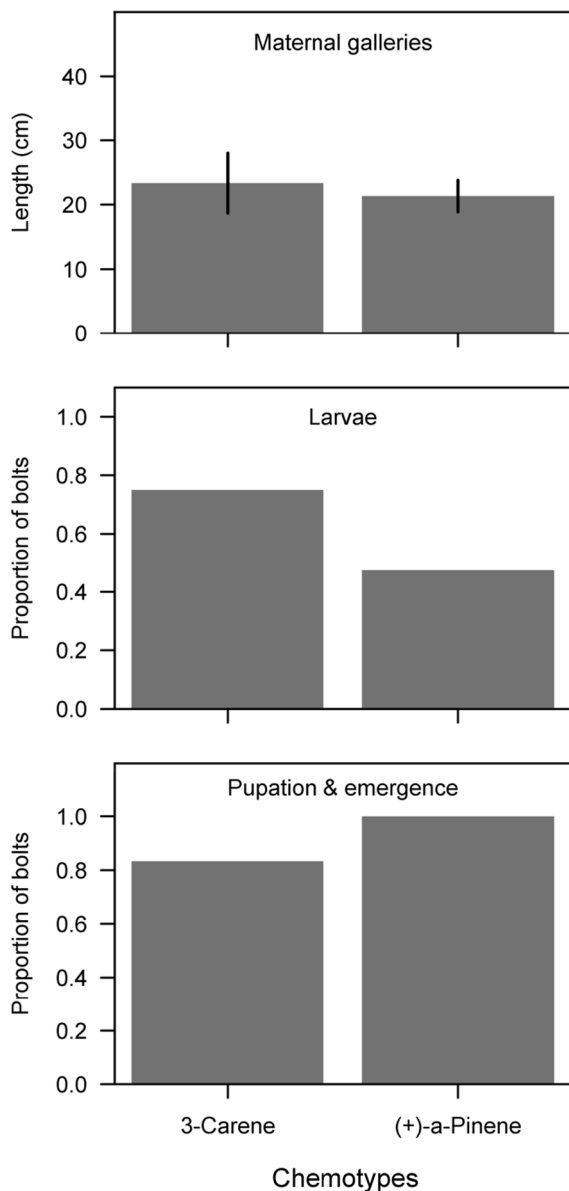
Fig. 6 *Dendroctonus ponderosae* brood performance in bolts of *Pinus sylvestris* that represented two monoterpene chemotypes: 3-carene and (+)-α-pinene. The top panel shows mean (± SE) length (cm) of beetle egg galleries, the center panel shows the proportion of bolts of each chemotype with evidence of larval activity, and the bottom panel shows the proportion of bolts with larvae that had evidence of pupation and emergence

mature trees (Furniss and Schenk 1969; Fries 2016) and cut bolts (Rosenberger et al. 2017) in the field by MPB in North America.

Scots pine is a suitable host for MPB, as indicated here by four lines of evidence. First, both introduced and European populations shared the (+)-α-pinene chemotype while the 3-carene chemotype in the European populations was replaced by the (−)-limonene chemotype in the introduced range. Absence of (–)-limonene chemotype in European Scots pine foliage suggests that Scots pine populations in Canada likely originated from a wider geographical range in Europe than those trees sampled in Sweden and Finland in the current study (Tobolski and Hanover 1971; Muona et al. 1986; Yazdani and Nilsson 1986; Semiz et al. 2007). Alternatively, these results may reflect the adaptive capacity of Scots pine in its introduced range (Kännaste et al. 2013). Despite these differences, introduced and European Scots pine populations are chemically similar and share common monoterpenes such as α-pinene, myrcene, and terpinolene. Additionally, both introduced and European populations shared the same sesquiterpene chemotypes which co-occurred in all four provinces sampled in Canada, although it is unknown how sesquiterpenes affect MPB biology. Nevertheless, having the same sesquiterpene chemotypes in both Canada and Europe provides additional evidence for the chemical similarity between introduced and European Scots pine populations.

Second, MPB produced all four major pheromones including female and male aggregation pheromones, *cis/trans*-verbenol and *exo*-brevicomin, in Scots pine bolts, suggesting that beetles could find mates and overcome host defenses via mass aggregation on Scots pine trees, in agreement with earlier field studies (Furniss and Schenk 1969; Fries 2016). Furthermore, the amounts of pheromones produced were similar to those reported on the MPB historical hosts (Erbilgin et al. 2014). For example, during the first 24-hr of introduction, female beetles released 1.1 ± 0.2 (µg/ml) and 0.97 ± 0.2 (µg/ml) *cis/trans*-verbenol in the Scots pine and lodgepole pine bolts respectively. Such results were expected because both pine species contain similar amounts of (−)-α-pinene in their phloem (Erbilgin 2019), and (−)-α-pinene is the primary precursor of *trans*-verbenol production (Blomquist et al. 2010). In addition, *cis/trans*-verbenol production was similar among host chemotypes of phloem, suggesting that chemotypic differences among different Scots pine populations do not appear to be a biological barrier for MPB aggregation on Scots pine. However, small non-significant differences in the amounts of verbenone produced between

Table 5 Metrics of mountain pine beetle (*Dendroctonus ponderosae*) attack characteristics and development in artificially inoculated *Pinus sylvestris* bolts with or without the monoterpene limonene. All bolts were originated from New Brunswick

Chemical composition of bolts	Attack characteristics			Brood development (per maternal gallery)		
	Introduction success (%)	Maternal gallery length (cm)	Maternal galleries with larvae (%)	Mean number of larvae	Mean number of pupal chambers	Mean number of emergence holes
Bolts with limonene	75.0 ± 25.0	13.80 ± 9.35	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Bolts without limonene	87.5 ± 8.18	13.56 ± 2.81	50.0 ± 0.0	29.50 ± 10.50	27.50 ± 9.50	12.50 ± 6.50

Values are mean ± SE

the two chemotypes may differentially affect beetle host colonization dynamics. Furthermore, recently Chiu et al. (2018) showed that MPB larvae may accumulate the two monoterpenyl esters during their development in the natal host, suggesting that adult female beetles can release *trans*-verbenol upon landing on a new host, independent of access to α-pinene, and that the production of the female aggregation pheromone may be less influenced by the chemotypic classification of host trees.

Third, MPB completed development from egg to adult stage in bolts from all introduced populations and from both chemotypes, suggesting that beetle behaviors that govern host acceptance for mating, egg and larval development are similar to those in the historical hosts of MPB (Erbilgin et al. 2014; Rosenberger et al. 2018). In the current study, we used cult bolts to evaluate the effects of host tree quality on MPB pheromone production, brood production, and development. Although bolts are commonly used in bark beetle research (e.g., Erbilgin et al. 2014; Rosenberger et al. 2018) substrate quality of bolts differ from that of live trees. For instance Guevara-Rozo et al. (2019) reported that bolts contain higher concentration of monoterpenes and some nutrients including nitrogen relative to the standing live trees. Nevertheless, it is still considered the best approximate of host substrate to understand bark beetle performance on different host species (Rosenberger et al. 2018).

Finally, host preference by MPB to Scots pine over lodgepole pine was demonstrated in the field. Fries (2016) planted different populations of Scots pine and lodgepole pine seeds in the same common garden plots in B.C. and Yukon (Canada) in 1986 and conducted insect and disease surveys in 2010. The study found that 25-year old Scots pine trees were more frequently attacked and successfully colonized (50–95%) by MPB than lodgepole pine (20–57%) trees in the same cohort. In fact, those beetle attacks resulted in a much greater mortality of Scots pine (70–90%) than lodgepole pine (40–85%). Likewise, Furniss and Schenk (1969) reported that more than half of the Scots pine trees (age ranged from 24 to 51 years) were colonized and killed by MPB in an arboretum in Idaho (USA). Furthermore, in those trees killed by MPB, beetle progeny matured and emerged from them.

However, like the historical hosts, the quality of Scots pine as a host substrate influenced MPB reproduction. Particularly, Scots pine trees in New Brunswick (total emerged: 13) had the lowest beetle emergence in Alberta (38) and Nova Scotia (30). Interestingly, Scots pine populations in New Brunswick were also characterized by the high concentration of (−)-limonene in phloem while this monoterpene was largely undetected in the other two provinces. (−)-Limonene is the most toxic monoterpene to MPB and can impede beetle development (Chiu et al. 2017; Reid et al. 2017). Lodgepole pine trees also vary in limonene concentrations in their phloem. In fact, limonene seems to be a much more important component of phloem in lodgepole pine populations in the inland North America than those located along the coastal US (Forrest 1980). Furthermore, lodgepole pine trees resistant to MPB contain several fold higher amounts of limonene than susceptible pine trees (Erbilgin et al. 2017). Based on these results, we expect that Scots pine trees with high limonene amounts in their phloem may be less suitable to MPB in their native range.

Besides MPB, Scots pine is susceptible to other native pests in North America, most prominently the pinewood nematode, *Bursaphelenchus xylophilus*. Although native pine species are resistant to the nematode, Scots pine is the most affected host (Futai 2013). As a result, the nematode is widespread throughout the USA and Canada (Evans et al. 1996; CABI/EPPO 2019). This novel host-pest interaction is significant for three reasons: First, the presence of the nematode weakens Scots pine trees and could predispose them to attacks by other native insects; second, the nematode may use Scots pine to expand its range in other parts of North America where it is currently absent; third the nematode may pose a potential risk to Europe as it has already established in some parts of Europe (CABI/EPPO 2019).

In conclusion, Scots pine may serve as a conduit for the transcontinental invasion of pine forests by MPB in North America for at least three reasons. First, MPB can colonize and reproduce in many western North American pine species (Wood 1982). Colonization of chemically very distinct species suggests that MPB has a behavioral and physiological adaptability that could facilitate the colonization of eastern pine species (Cale et al. 2015; Rosenberger et al. 2017). In fact, the recent host expansion of MPB to jack pine suggests that beetles can colonize new tree species without going through any 'pre-adaptation phase' as long as the new species is chemically similar to that of the beetle's historical hosts (Erbilgin 2019).

Second, relative proportions of monoterpenes (i.e., chemotypes) in Scots pine and other conifer species are commonly used to describe the species' phenotypic and genotypic characteristics (Forrest 1980; Paule and Yazdani 1992; Sjödin et al. 2000; Thoss et al. 2007; Taft et al. 2015). We showed that European and Canadian Scots pine populations are chemotypically similar, and the Canadian populations are suitable for MPB. A recent review paper (Erbilgin 2019) provided extensive evidence for why particular chemical profiles (or chemotypes) of host trees matter in tree resistance to bark beetles. Briefly, the chemical profile of trees seems to be more important than individual monoterpenes as they play different roles in different stages of MPB host colonization, which was supported by the recent field studies (Erbilgin et al. 2017).

Finally, taxonomic relatedness between Scots pine and North American pine species may also support the suitability of this species to MPB as related plant species likely possess common functional traits that are involved in host selection by herbivores (Niemelä and Mattson 1996; Roques et al. 2006; Goßner et al. 2009; Pearse et al. 2013). Colonization of Scots pines in North America may provide behavioral and physiological flexibility for beetles that can potentially colonize the same or taxonomic related species in its introduced range (Niemelä and Mattson 1996; Paine 2006). In the current study we demonstrated that MPB is capable of producing its pheromones, reproducing, and completing its life cycle in all Canadian Scots pine populations which are chemotypically similar to those sampled in Europe.

Authors' contributions NE and MS conceived the ideas; NE designed the methodology; NE led the writing of the manuscript; JGK, GI, RR, and NE conducted chemical analysis; JAC and NE analysed the data; NE, GI, CB, TB, GJ, MH, CH, CJKM, and RS collected the data. All authors contributed critically to the drafts and gave final approval for publication.

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