J Chem Ecol (2011) 37:607–621
DOI 10.1007/s10886-011-9958-8

Ecosystem, Location, and Climate Effects on Foliar Secondary Metabolites of Lodgepole Pine Populations from Central British Columbia

Christopher M. Wallis · Dezene P. W. Huber · Kathy J. Lewis

Received: 15 September 2010 / Revised: 18 March 2011 / Accepted: 19 April 2011 / Published online: 3 May 2011

Abstract Lodgepole pines, *Pinus contorta* Douglas ex Louden var. *latifolia* Engelm. ex S. Watson, are encountering increased abiotic stress and pest activity due to recent increases in temperature and changes in precipitation throughout their range. This tree species counters these threats by producing secondary metabolites, including phenolics and terpenoids. We examined foliar levels of lignin, soluble phenolics, monoterpenoids, sesquiterpenoids, and diterpenoids in 12 stands in British Columbia, Canada. We used these data to assess associations among foliar secondary metabolite levels and ecosystem, geographic, and climatic variables. Regressions were also performed to observe which combinations of variables best explained secondary metabolite variance. Stands of *P. c. latifolia* in the Coastal Western Hemlock and Interior Cedar/Hemlock biogeoclimatic zones had consistently greater foliar levels of almost all measured secondary metabolites than did other stands. Lignin was present in greater amounts in Boreal White/Black Spruce ecosystem (i.e., northern) stands than in southern stands, suggesting a role for this metabolite in pine survival in the boreal forest. Attempts to develop regression models with geographic and climatic variables to explain foliar secondary metabolite levels resulted in multiple models with similar predictive capability. Since foliar secondary metabolite levels appeared to vary most between stand ecosystem types and not as much due to geographic and climatic variables, metabolic profiles appeared best matched to the stress levels within local environments. It is unknown if differences in secondary metabolite levels are the result of genetic adaptation or phenotypic plasticity, but results from this and other studies suggest that both are important. These results are interpreted in light of ongoing efforts to assist in the migration of certain populations of *P. c. latifolia* northward in an effort to counter predicted effects of climate change.

Key Words Climate change · Gas chromatography–mass spectrometry (GC-MS) · Lignin · Lodgepole pine · Monoterpenoid · Phenolics · *Pinus contorta latifolia* · Sesquiterpenoid · Diterpenoid

Introduction

Lodgepole pine, *Pinus contorta* Douglas ex Louden var. *latifolia* Engelm. ex S. Watson (http://www.itis.gov/), is a valuable and widespread species found throughout western North America, where it is widely planted as a part of sustainable forestry practice (Wu et al., 2005; Ying and Yanchuk, 2006). However, in recent years many plantations of *P. c. latifolia* throughout its range have been severely impacted by generally warmer temperatures (especially in winter) and localized changes in precipitation (Folke et al., 2004). In addition to climate adversely affecting tree physiology, and in turn productivity, there has been an increase in pest activity, including widespread mortality caused by the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae) (Walther et

C. M. Wallis · D. P. W. Huber · K. J. Lewis
Ecosystem Science and Management Program,
University of Northern British Columbia,
3333 University Drive,
Prince George, BC V2N 4Z9, Canada

Present Address:
C. M. Wallis (✉)
USDA-ARS San Joaquin Valley Agricultural Sciences Center,
9611 S. Riverbend Ave,
Parlier, CA 93648, USA
e-mail: christopher.wallis@ars.usda.gov

al., 2002; Kurz et al., 2008; Bentz et al., 2010) and regionally important outbreaks of *Dothistroma* needle cast [causal agent: *Mycosphaerella pini* Rost. in Munk; anamorph *Dothistroma septospora* (Dorog.) Morelet] (Ascomycota: Mycosphaerellaceae) (Woods et al., 2005). It is hypothesized that in the future, climate change will continue and even intensify, which will further impact pine growth and result in more frequent pine pest and disease outbreaks (Coakley et al., 1999; Logan et al., 2003; Desprez-Loustau et al., 2007; Bentz et al., 2010).

To counter the potential negative effects of climate change, efforts have begun to move individuals from particular pine populations to areas whose future climates are predicted to be optimal for their growth in a process called assisted migration (O'Neill et al., 2008). Assisted migration involves moving pine populations to higher latitudinal or elevational sites because it is hypothesized that certain populations of trees are better-suited to areas which, although also warming, will remain cooler overall (Coakley et al., 1999; O'Neill et al., 2008; Marris, 2009).

It remains unclear what impacts the assisted migration of certain populations of *P. c. latifolia* into new areas will have on their ability to thrive in the near- and long-terms (Marris, 2009). Although attempts have been made to find suitable locations to minimize climate change effects on *P. c. latifolia* physiology (Cumming and Burton, 1996; Hamann and Wang, 2006; O'Neill et al., 2008), our understanding of this process is in its infancy (Wu et al., 2005; Wallis et al., 2010). Populations of *P. c. latifolia* that originated from different ecosystems, or that were far apart geographically, have been found to vary not only in growth and reproduction, but also in their susceptibility to diseases (Rehfeldt et al., 1999; Wu et al., 2005; Heineman et al., 2010; Wallis et al., 2010). Such distinct populations also were observed to vary in foliar secondary metabolite (SM) levels, at least when they were planted together at one seed orchard site (Wallis et al., 2010). Overall differences in the ability of populations of *P. c. latifolia* to synthesize SMs, such as phenolics and terpenoids, are likely to have led to observed variations in growth rates and susceptibility to pests, because such compounds have a variety of roles including photoprotection, relieving oxidative stress, reducing water stress, and protection from insect and pathogen pests (Keeling and Bohlmann, 2006; Seybold et al., 2006; Whitzell and Martin, 2008).

Populations of *P. c. latifolia* from lower latitudes and from warmer, wetter stands will need increased protection from biotic stresses, and therefore the possession of greater pest defense-associated SM levels (both phenolics and terpenoids) will confer fitness advantages (e.g., Franceschi et al., 2005; Wallis et al., 2008; Adams et al., 2009). In contrast, *P. c. latifolia* from northern, colder, and drier locales will gain a fitness advantage by putting more resources into primary metabolites, presumably at the cost of producing certain SMs, in order to deal with a shorter growing season and to outcompete neighboring plants for scarce resources (Siska et al., 2002; Andrew and Hughes, 2005; Adams et al., 2009).

However, other SM classes might play larger roles in conferring resistance to abiotic stresses than in their abilities to combat pests; for instance, *P. c. latifolia* from boreal forest locations may possess greater cell-wall thickening phenolics such as lignin that could confer fitness advantages in overcoming abiotic stresses such as extreme cold (Smallwood and Bowles, 2002; Wei et al., 2006). Levels of other SMs, in particularly flavonols, are positively associated with latitude presumably because they are involved in resisting abiotic stresses (Lätti et al., 2008; Stark et al., 2008; Martz et al., 2009, 2010). Day lengths, lowered temperatures, and UV exposure are all implicated in the relationship between latitude and levels of certain SMs (reviewed by Jaakola and Hohtola, 2010).

Therefore, levels of biotic and abiotic stresses imparted on populations can positively or negatively affect distinct groups of SMs, depending on the class of the compounds and the particular roles that they play in resistance to stress. The aim of our study was to quantify the differences in foliar SM levels among stands of naturally regenerating *P. c. latifolia* that should be exposed to a variety of abiotic and biotic stresses. We examined variations in SM levels—including total soluble phenolics, lignin, and terpenoids—in the foliage from 12 distinct stands of *P. c. latifolia*. Ecological, geographical, and climatic differences among these sites were examined to observe which variables might influence the levels of these compound classes. We hypothesized that associations among SM classes and ecological, geographic, or climatic variables will be due, in part, to the particular roles they play in countering abiotic or biotic stresses.

Methods and Materials

Stand Descriptions and Sampling A total of 12 mature stands of *P. c. latifolia* of natural origin were assessed across central British Columbia (Fig. 1). These stands were from a variety of habitats and climates comprising five distinct ecosystems as delineated by British Columbia's biogeoclimatic (BEC) zone system. They included the very cold boreal white and black spruce (BWBS) zone; the very wet Coastal Western Hemlock (CWH) zone; the high elevation Englemann Spruce/Subalpine Fir (ESSF) zone; the very wet Interior Cedar/Hemlock zone (ICH); and the widespread Sub-boreal Spruce (SBS) zone (Meidinger and Pojar, 1991). Climate data for each sampling site [mean summer (June through August), mean winter (December through February) and mean annual temperatures; and

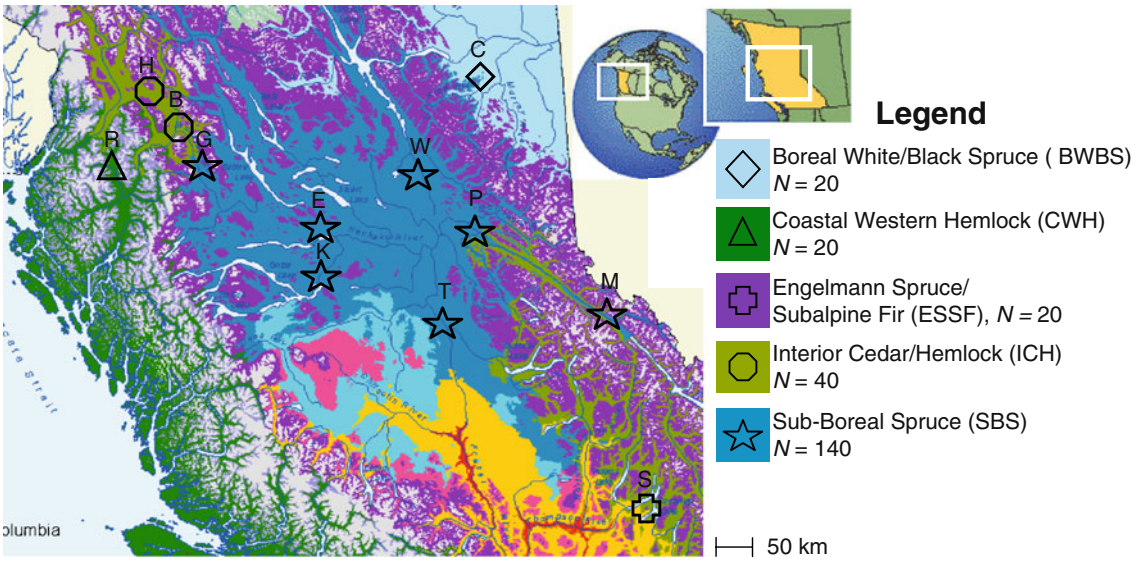

Fig. 1 Locations of populations of *Pinus contorta latifolia* in British Columbia, Canada identified by letter corresponding to site names in Table 1; and their British Columbia biogeoclimatic (BEC) classification and sample size. Map courtesy of the BC Ministry of Forests and Range (http://www.for.gov.bc.ca/hre/becweb/)

mean summer (June through August) and mean annual precipitation levels] were determined by using the Climate BC program, based upon measured temperature and precipitation data from 1961 to 1990 (Wang et al., 2006) (Table 1). Sampling occurred between June 15, and July 15, 2009, and at each stand the trees were in the same phenological stage (near the completion of needle expansion from newly-formed candles). At each site, approximately 5 g of apparently insect- and disease-free, second-year needles (i.e., those that were formed in 2008) were collected from three

Table 1 Coordinates and site characteristics for the *Pinus contorta latifolia* stands assessed for foliar secondary metabolites

Site Name	Elev. (m)	Zone	Easting	Northing	Biogeoclimatic (BEC) Zone	Mean Summer Temperature (°C)	Mean Winter Temperature (°C)	Mean Annual Temperature (°C)	Mean Summer Prec.(cm)	Mean Annual Prec. (cm)
Chetwynd (C)	925	10U	587,673	6,179,135	Boreal White/ Black Spruce (BWBS)	13.6	−8.3	1.7	33.9	57.8
Rosswood (R)	185	9U	514,695	6,075,002	Coastal Western Hemlock (CWH)	13.9	−3.9	5.1	34.8	114.1
Salmon Arm (S)	1,424	11U	327,518	5,621,723	Engelmann Spruce/ Subalpine Fir (ESSF)	13.0	−7.1	3.0	34.5	84.7
Bulkley Canyon (B)	396	9U	598,078	6,120,028	Interior Cedar Hemlock (ICH)	13.8	−6.8	3.8	26.0	58.5
Cullon Creek (H)	509	9U	564,007	6,165,179	ICH	13.4	−7.3	3.4	32.8	65.8
Burns Lake (E)	860	10U	322,846	6,013,982	Sub-boreal Spruce (SBS)	12.2	−9.0	2.0	22.2	49.7
Ganokwa Creek(G)	851	9U	631,887	6,074,792	SBS	12.5	−8.0	2.3	22.0	51.7
Kenny Dam (K)	898	10U	374,880	5,937,816	SBS	12.2	−9.1	2.1	23.6	50.6
McBride (M)	759	11U	324,779	5,882,146	SBS	14.4	−8.3	3.4	34.2	80.9
Purden (P)	729	10U	566,464	5,972,171	SBS	13.4	−8.2	3.0	34.3	78.5
Ten Mile Lake (T)	733	10U	537,587	5,880,356	SBS	14.6	−7.4	4.1	27.5	58.3
Whiskers Point (W)	687	10U	503,894	6,084,477	SBS	13.7	−9.7	2.4	26.6	75.4

randomly chosen branches from each of 20 naturally regenerating trees ranging from 15 to 25 year-old. The samples were immediately placed onto dry ice for transport to the lab, where they were stored at −20°C until processed for phytochemical analyses roughly 1 month later.

Phytochemical Analyses Needle samples were ground by mortar and pestle in liquid nitrogen, with 100 mg of ground tissue twice-extracted in 1 ml of methanol (Thermo-Fisher Scientific, Pittsburg, PA, USA) and another 100 mg of tissue twice-extracted in 1 ml of dichloromethane (methylene chloride) (Sigma-Aldrich, St. Louis, MO, USA) according to the protocols of Wallis et al. (2008). The methanol extracts were assessed for total phenolics by using the Folin-Ciocalteu reagent spectrophotometric method (with an instrument from Perkin-Elmer, Waltham, MA, USA) for quantifying soluble phenolics, and the pellet left behind following the methanol extraction was further processed to quantify total lignin (bound to the cell walls) according to the procedures of Wallis et al. (2008) with all reagents from Sigma-Aldrich. In brief, the pellets were washed once with 1 ml water, then 1 ml methanol, and finally 1 ml of petroleum ether and were allowed to dry overnight. To the pellet, 200 μl of 1 N NaOH were added, and this was left in a shaker at 40°C for 21 h, after which 200 μl of 1.5 M formic acid and 400 μl of methanol were added. The mixture was centrifuged at 10,000 x *g* for 5 min, and the supernatant removed. The pellet then was washed in 1 ml of water and re-suspended in 800 μl of 2 N HCl and 300 μl of thioglycolic (mercaptoacetic) acid heated at 86°C for 4 h. The tubes were spun down for 5 min at 10,000 x *g*, and the supernatant removed, with the pellet then twice-washed in 1 ml water. The pellet was then re-suspended in 1 ml of 0.5 M NaOH and left for 18 h on a shaker. Following centrifugation, the supernatant was transferred to a clean tube and the pellet re-suspended in 0.5 ml of 0.5 M NaOH. Following centrifugation, the supernatant was combined with that from the previous step to total a 1.5 ml of 0.5 M NaOH solution. To this, 300 μl of concentrated (12 N) HCl were added and allowed to stand at room at room temperature for 4 h. The solution was spun down for 5 min at 10,000 x *g*, and the supernatant discard. The pellet was re-suspended in 1 ml of 0.5 M NaOH, and read on a spectrophotometer (Perkin-Elmer) at 280 nm. Lignin concentrations in mg were determined by using a standard curve made from a dilution series of commercially available lignin standard from spruce (Sigma-Aldrich).

The dichloromethane extracts were analyzed for terpenoid content (monoterpenoids, sesquiterpenoids, and diterpenoids) with a Varian Model 3800 gas chromatograph with a flame-ionization detector and a split line to a Saturn 2200 Ion Trap MS-MS (Agilent, Palo Alto, CA, USA) and *n*-pentadecane (Sigma-Aldrich) as an internal standard (Wallis et al., 2010). The column was a 30 m×0.25 mm i.d.×0.25 μm film thickness Agilent Ultra 2.5% phenylmethylsiloxane capillary column; the carrier gas was helium running at a constant flow of 2.1 ml/min; the split/splitless injection port was in split mode (10:1 ratio) with the temperature set at 320°C; and the detector/transfer line temperatures were set at 320°C. The temperature program started with a hold of 2 min at 40°C; ramped at 10°C/min to 200°C; and finally ramped at 50°C/min to a final temperature of 300°C (with a 5 min final hold) (total run time of 28.20 min). All peak areas of identified terpenes were summed together with others in their class (monoterpenoids, sesquiterpenoids, or diterpenoids) to give an overall value for each terpenoid group. Compound identifications were based on retention time matching to known standards and matching to mass spectral libraries with reverse search indices higher than 850 (Wallis et al., 2008). Stereoisomers of certain compounds (e.g., α-pinene, β-pinene, and limonene) went unresolved, as this study stressed changes in total terpenoid group production (i.e., the regulation upstream of individual monoterpenes, sesquiterpenes, and diterpenes). The toxicity of terpenoids to pathogens is thought to not depend on the enantiomeric composition (Wallis et al., 2008). Putative diterpenoids, labeled in this work as unknown diterpenoids, were compounds whose matches to mass spectral libraries contained a typical diterpenoid skeleton of C_{20} and for which the top five library matches were different diterpenoids. However, for these putative diterpenoids no single compound exceeded the reverse search index threshold set at 850.

Statistical Analyses All statistical tests were performed with either SPSS ver. 17 (SPSS Inc., Chicago, IL, USA) or R ver. 2.10.0 (CRAN, Wirtschaftsuniversität Wien, Austria) at a threshold of $\alpha=0.05$. Normality was assessed by examining residual plots and histograms, and Levene's tests for variance heterogeneity were performed. Pearson's correlations were used to find associations between groups of foliar compounds. Univariate ANOVAs were used to find differences in phytochemistry between trees grown in different BEC zones. Tukey HSD tests were used to separate individual BEC zone means.

Coordinates were first converted from UTM to BC Albers, a metric that is continuous throughout British Columbia (http://geobc.gov.bc.ca) for all analyses that examined relationships between geographical variables and SM levels. Specifically, the BC Albers has an x-coordinate that represents longitude (with increasing numbers representing movement eastward) and a y-coordinate that represents latitude (with increasing numbers representing movement northward).

Pearson's correlations were used to examine potential relationships between geographic or climatic variables and

foliar SM levels. One analysis compared the mean levels of the SMs for each site with these variables, whereas another used the SM values for individual trees. The first analysis was performed to emphasize the geographic or climatic effects on a typical individual in the tree's population, whereas the latter emphasized effects on multiple individuals in the same stand of trees. This was also performed in part due to concerns over the large sample size (greater than 250 replicates overall) of SM levels potentially skewing these associations, considering that 12 estimates were made for each of the geographical and climatic variables. An additional analysis compared the levels of SMs from individual trees vs. geographic or climatic variables with data from the SBS zone only, as this zone was represented far more (7 times) than the other sites and is arguably the primary ecosystem type in which *P. c. latifolia* is found in British Columbia.

Linear regression analyses were performed to observe which combination of variables best explained the variance in foliar secondary metabolites. Using the "Rcmdr" package within R, a linear regression model for each SM group as explained by all the geographic and climatic variables was loaded. These models then underwent the "subset model selection…" application under Rcmdr's "Model" menu, which output a list of the variables included in the models that had the lowest Schwarz's Bayesian Information Criteria (BIC) values (with the lowest values representing the best models) (Schwarz, 1978). The strongest model matched that selected via Stepwise Regression Analysis based on BIC; however, the next two top models also were selected for comparison as they could not be dismissed due to their possessing similar BIC values (within ~2) to the top model (Burnham and Anderson, 2002). For additional evaluation, the Akaike Information Criteria (AIC) values also were assessed (Akaike, 1974). These regressions were performed on the mean SM levels at each site; on SM levels for all of the individual trees; and on the SM levels for all individual trees for the SBS zone alone.

Results

Secondary Metabolite Identities and Associations In this study, we assessed a total of 14 monoterpenoids (sabinene, α-pinene, camphene, β-pinene, β-myrcene, α-phellandrene, δ-3-carene, *p*-cymene, β-phellandrene, δ-2-carene, linalool, camphor, bornyl acetate, and verbanone), seven sesquiterpenoids (α-cubenene, α-copanene, aristolene, β-cadinene, τ-cadinol, germacrenol, and an unidentified sesquiterpenoid), and five diterpenoids (all of which were unable to be identified to specific compounds). We also assessed total phenolic (with Folin-Ciocalteu reagent) and total lignin content.

Foliar phenolic levels were significantly and positively associated with the foliar levels of all three groups of terpenoids; and sesquiterpenoid levels were positively associated with levels of both monoterpenoids and diterpenoids (Table 2). No other significant correlations were observed.

BEC Zone and Subspecies Effects on Foliar Secondary Metabolites Populations of *P. c. latifolia* from the Coastal Western Hemlock (CWH) and Interior Cedar Hemlock (ICH) zones had greater levels of soluble phenolics (41% to 45% more) than the Boreal White/Black Spruce (BWBS) zone (F=8.348, P<0.001, N=237) (Fig. 2a). Lignin levels were greater in the BWBS zone (11% to 22% more) than the SBS or Engelmann Spruce/Subalpine Fir (ESSF) zones, and trees from the ICH zone had greater lignin levels (14% more) than those in the ESSF zone (F=4.591, P=0.001, N=238). Total monoterpenoid levels were greater in *P. c. latifolia* from the ICH zone (72% to 242% more) than from any other zone (F=10.105, P<0.001, N=226) (Fig. 2b). Total sesquiterpenoid levels were greater in the CWH zone (60% to 147% more) than any other zone, and levels were greater in the ICH (38% more) and BWBS (37% more) zones than the SBS zone (F=38.467, P<0.001, N=233). Total diterpenoid levels were greater in the CWH population (39% to 857% more) than any other population, and diterpenoid levels were also greater in populations in the BWBS and ICH zones (93% to 588% more) than in the ESSF or SBS zones (F=38.763, P<0.001, N=224).

The foliar levels of almost every individual compound, with the exceptions of sabinene, β-pinene, and linalool, were significantly related to BEC zone (Table 3). Pines from the ICH zone possessed greater levels of camphene, δ-3-carene, and β-phellandrene than pines from the ESSF zone (F>4.088, P<0.010, N=233). Pines in the CWH zone had greater levels of α-cubenene, aristolene, β-cadinene,

Table 2 correlations among the different classes of secondary metabolites analyzed from the foliage of *Pinus contorta latifolia*

	Phenolics *r (P)*	Lignin *r (P)*	Monoterpenoids *r (P)*	Sesquiterpenoids *r (P)*
Lignin, N=237	0.063			
Monoterpenoids, N=224	0.197*	−0.047		
Sesquiterpenoids, N=226–231	0.105	−0.006	0.123	
Diterpenoids, N=217–224	0.124	0.081	0.030	0.800**

* P<0.010; ** P<0.001

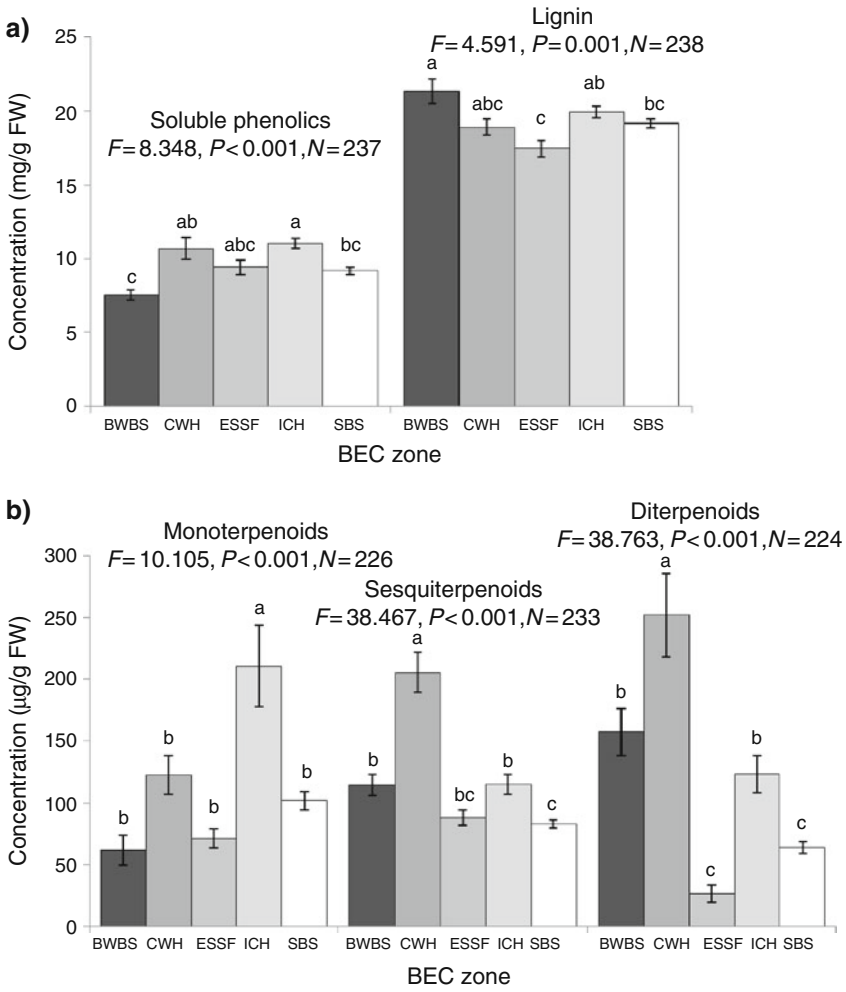

Fig. 2 Concentrations (mean ± s.e.) of **a** phenolic and **b** terpene secondary metabolites in the foliage of *Pinus contorta latifolia* collected from various British Columbia biogeoclimatic (BEC) zones. ANOVA statistics are given, and letters indicate significant differences by Tukey HSD tests. BEC zones are as follows: Boreal White/Black Spruce (BWBS); Coastal Western Hemlock (CWH); Engelmann Spruce/Subalpine Fir (ESSF); Interior Cedar Hemlock (ICH); and Sub-Boreal Spruce (SBS)

and germacrenol than those growing in either the ESSF, ICH, or SBS zones ($F \geq 5.247$, $P<0.001$, $N=233$). Pines growing in the BWBS zone possessed greater levels of the unidentified sesquiterpenoid than those growing in the ESSF or SBS zones ($F=5.355$, $P<0.001$, $N=233$). Pines growing in the CWH zone possessed greater levels of all five diterpenes than pines growing in the ESSF or SBS zones ($F \geq 4.766$, $P<0.001$, $N=233$).

Geographic and Climatic Variables and Secondary Metabolite Levels By using the mean compound levels from each site for the correlation analyses, foliar levels of phenolics were negatively associated with longitude (i.e., phenolic levels were lower in more easterly sites); levels of lignin were positively associated with latitude (i.e., lignin levels were higher in more northerly stands); levels of sesquiterpenoids were positively associated with winter temperature and annual precipitation; and levels of diterpenoids were negatively associated with longitude but positively associated with latitude and winter temperature (Table 4). No other significant correlations were observed.

Considering the foliar levels of SMs in each tree and not using site means for the correlation analyses, levels of phenolics were negatively associated with elevation, longitude, summer temperature, and summer precipitation but positively associated with latitude and winter temperature; levels of lignin were negatively associated with longitude, summer temperature, annual temperature, and annual precipitation but positively associated with latitude; levels of all terpenoid classes were negatively associated with elevation and longitude but positively associated with latitude, summer temperature (with the exception of the diterpenoids), winter temperature, annual temperature, and annual precipitation (with the exception of the monoterpenoids); and levels of monoterpenoids were negatively associated with summer precipitation, whereas levels of sesquiterpenoids and diterpenoids were positively associated with summer precipitation (Table 4). No other significant correlations were observed.

For trees in the SBS zone only, total phenolics were negatively associated with elevation, longitude, summer temperatures, annual temperature, summer precipitation,

Table 3 Mean compound levels from foliage of *Pinus contorta latifolia* (μg/g FW) and ANOVA statistics showing British Columbia's biogeoclimatic zone differences, with letters indicating separation by Tukey HSD tests

Class	Compound	British Columbia Biogeoclimatic Zone					
		Boreal White/ Black Spruce	Coastal Western Hemlock	Engelmann Spruce/ Subalpine Fir	Interior Cedar/ Hemlock	Sub-Boreal Spruce	*F*
Phenolics	total phenolics	7.56±0.36 c	10.71±0.73 ab	9.42±0.49 abc	11.04±0.34 a	9.16±0.23 bc	8.348***
	lignin	21.34±0.86 a	19.00±0.52 abc	17.46±0.58 c	19.92±0.40 ab	19.15±0.27 bc	4.591**
Monoterpenoids	sabinene	0.000±0.000	0.201±0.161	0.008±0.008	0.018±0.018	0.712±0.491	0.339
	α-pinene	2.97±1.03 ab	3.64±0.41 ab	1.14±0.21 b	5.52±1.30 a	4.23±0.43 ab	2.560*
	camphene	2.59±0.54 ab	2.65±0.40 ab	1.18±0.14 b	2.93±0.18 a	2.11±0.16 ab	4.088**
	β-pinene	14.56±4.94	6.11±2.37	11.83±2.72	11.30±4.03	20.93±2.72	2.127
	β-myrcene	1.21±0.49 b	2.38±0.51 ab	1.72±0.22 ab	6.37±1.00 a	3.83±0.66 ab	3.133*
	α-phellandrene	6.43±0.65 a	4.24±0.54 ab	3.68±0.34 b	5.02±0.48 ab	3.97±0.24 b	4.464**
	δ-3-carene	8.01±0.78 ab	6.65±0.52 ab	4.84±0.37 b	9.07±1.13 a	5.31±0.31 b	7.515***
	p-cymene	0.85±0.20 b	2.41±0.43 a	0.98±0.12 b	2.17±0.16 a	1.00±0.10 b	12.994***
	β-phellandrene	20.9±5.2 b	80.1±14.1 b	35.5±5.7 b	156.3±26.6 a	64.9±6.3 b	11.134***
	δ-2-carene	0.17±0.13 b	1.98±0.26 a	0.26±0.12 b	1.40±0.28 a	0.40±0.09 b	13.772***
	linalool	0.85±0.41	2.58±0.72	3.24±0.58	3.39±0.72	3.45±0.44	1.555
	camphor	1.28±0.28 b	2.54±0.32 ab	2.10±0.25 ab	2.62±0.18 a	1.45±0.17 b	5.218***
	bornyl acetate	0.01±0.01 b	1.68±0.45 ab	1.72±0.65 a	1.48±0.36 ab	0.90±0.16 ab	3.194*
	verbanone	1.73±0.38 b	3.85±0.07 a	3.01±0.25 a	3.00±0.18 a	2.00±0.12 b	13.513***
Sesquiterpenoids	unidentified sesquiterpenoid	13.2±3.6 a	11.6±0.8 ab	6.5±0.6 bc	9.2±0.5 abc	7.1±0.5 c	5.355***
	α-cubenene	55.1±5.6 b	110.9±12.7 a	34.9±2.3 bc	50.3±4.7 b	34.9±1.6 c	38.814***
	α-copanene	22.3±1.5 a	23.4±5.0 a	20.2±1.5 ab	20.3±1.8 ab	15.2±0.7 b	5.292***
	aristolene	7.9±1.1 b	27.4±3.3 a	8.9±1.0 b	7.6±0.8 b	6.5±0.4 b	48.630***
	β-cadinene	5.3±1.3 b	13.4±1.3 a	3.3±1.0 b	5.6±0.9 b	7.5±0.7 b	6.091***
	τ-cadinol	8.7±2.6 b	16.1±4.1 ab	11.7±2.0 ab	20.0±1.9 a	10.6±1.0 b	5.247***
	germacrenol	1.46±1.06 ab	4.93±1.44 a	2.35±0.60 b	1.56±0.30 b	1.25±0.25 b	5.416***
Diterpenoids	unidentified diterpenoid A	2.64±0.69 bc	7.91±1.95 a	0.56±0.33 c	4.67±0.80 ab	2.75±0.36 bc	8.037***
	unidentified diterpenoid B	10.91±1.42 a	8.76±1.38 ab	1.38±0.45 d	6.28±0.64 bc	4.94±0.38 c	14.430***
	unidentified diterpenoid C	64.7±13.8 a	40.4±6.5 ab	9.5±2.8 c	20.1±2.6 bc	19.9±2.4 c	12.781***
	unidentified diterpenoid D	29.8±3.0 bc	119.9±30.6 a	9.4±3.1 c	62.9±9.5 b	25.8±2.3 c	18.936***
	unidentified diterpenoid E	49.4±11.4 ab	82.9±10.2 a	5.5±1.8 b	31.4±5.8 b	29.1±6.4 b	4.766**

For lignin and total phenolics, N=238; for all other compounds, N=233.

* P<0.050; ** P<0.010; *** P<0.001.

and annual precipitation but positively associated with latitude; lignin levels were negatively associated with elevation, longitude, summer temperatures, annual temperatures, and summer precipitation but positively associated with latitude; monoterpenoid and sesquiterpenoid levels were positively associated with latitude; and diterpenoid levels were negatively associated with elevation, longitude, summer precipitation, and annual precipitation but positively associated with latitude (Table 4). No other significant correlations were observed.

Linear Regression Modeling to Explain Variance in Foliar Secondary Metabolite Levels Whereas the correlation analyses related individual climatic and geographic variables with the observed levels of the SMs, linear regressions were made to combine multiple factors together to explain the observed levels. Mean foliar phenolic levels at each site were best explained [by having the lowest Bayesian Information Criterion (BIC)] by longitude; lignin levels were best explained by elevation, mean annual temperature, and mean winter temperature; monoterpenoid levels were

Table 4 Pearson's correlations between geographic or climatic variables and mean compound levels per site or individual tree compound levels from an analysis of foliage metabolites from *Pinus contorta latifolia*

Analysis Unit	Compound Class	Elevation	Latitude S→N	Longitude W→E	Summer Temp.	Winter Temp.	Annual Temp.	Summer Prec.	Annual Prec.
Site Means, *N*=12	Phenolics	−0.308	0.249	**−0.706****	−0.337	0.380	−0.218	−0.290	−0.019
	Lignin	−0.120	**0.661***	−0.417	−0.198	−0.150	−0.489	−0.475	−0.306
	Monoterpenoids	−0.483	0.320	−0.478	−0.359	0.274	0.349	0.213	−0.138
	Sesquiterpenoids	−0.552	0.412	−0.501	0.397	**0.687***	0.516	0.258	**0.600***
	Diterpenoids	−0.570	**0.608***	**−0.606***	0.277	**0.581***	0.336	0.702	0.384
Individual Trees	Phenolics, *N*=238	**−0.169****	**0.138***	**−0.395*****	**−0.164***	**0.211****	0.119	**−0.191****	−0.016
	Lignin, *N*=238	−0.059	**0.326*****	**−0.206****	**−0.235*****	−0.074	**−0.151***	−0.098	**−0.151***
	Monoterpenoids, *N*=226	**−0.299*****	**0.191****	**−0.290*****	**0.138***	**0.162***	**0.218****	**−0.220****	−0.082
	Sesquiterpenoids, *N*=233	**−0.414*****	**0.304*****	**−0.374*****	**0.200****	**0.516*****	**0.390*****	**0.307*****	**0.461*****
	Diterpenoids, *N*=224	**−0.441*****	**0.462*****	**−0.465*****	0.100	**0.445*****	**0.261*****	**0.216****	**0.297*****
Individual TreesSBS Zone Only	Phenolics, *N*=138	**−0.394*****	**0.288****	**−0.394*****	**−0.279****	0.008	**−0.279****	**−0.308*****	**−0.308*****
	Lignin, *N*=138	**−0.306*****	**0.335*****	**−0.306*****	**−0.394*****	−0.132	**−0.394*****	**−0.182***	−0.121
	Monoterpenoids, *N*=127	−0.145	**0.207***	−0.145	0.072	0.085	0.051	−0.164	−0.077
	Sesquiterpenoids, *N*=134	−0.069	**0.289****	−0.069	0.143	−0.047	0.033	−0.124	0.042
	Diterpenoids, *N*=127	**−0.372*****	**0.422*****	**−0.372*****	−0.140	−0.036	−0.162	**−0.355*****	**−0.224***

Bold text represents a significant association.

* $P<0.050$; ** $P<0.010$; *** $P<0.001$.

best explained by mean summer precipitation, mean summer temperature, and mean winter temperature; sesquiterpenoid levels were best explained elevation, longitude, mean annual precipitation, mean annual temperature, and mean summer precipitation; and diterpene levels were best explained by elevation, latitude, longitude, mean annual precipitation, and mean summer precipitation (Table 5).

By using the chemical levels found in foliage from individual trees and not using their site means for analyses, foliar soluble phenolic levels were best explained by longitude, elevation, and mean annual temperature; lignin levels were best explained by elevation, mean annual temperature, and mean winter temperature; monoterpenoid levels were best explained by mean summer precipitation, mean summer temperature, and mean winter temperature; sesquiterpenoid levels were best explained by longitude, elevation, mean annual precipitation, mean annual temperature, and mean summer temperature; and diterpenoids were best explained by longitude, latitude, elevation, mean annual precipitation, and mean summer temperature (Table 6).

By using the chemical levels found in foliage from individual trees from the SBS zone only, foliar soluble phenolic levels were best explained by latitude, elevation, and mean summer temperature; lignin levels were best explained by mean annual precipitation, mean annual temperature, and mean winter temperature; monoterpenoid levels were best explained by longitude and mean summer temperature; sesquiterpenoid levels were best explained by elevation and mean summer precipitation; and diterpenoids were best explained by longitude and elevation (Table 7).

It should be noted that in all cases the top three models for each variable had close BIC and AIC values, meaning that one model cannot be considered necessarily better than the others. Additional studies and data collection would be necessary to assess which of the top models would be best for explaining the levels of foliar SMs.

Discussion

Ecosystem type (represented by the BEC zone classifications), geographical location, and climate all significantly impacted at least some of the foliar secondary metabolite levels in the *P. c. latifolia* that were analyzed in this study. Thus, *P. c. latifolia* foliar SMs, which likely contribute to overall fitness, varied with ecosystem, locale, and climate.

BEC zones were associated with differential levels of almost every SM that we analyzed. Trees from the CWH and ICH zones often possessed greater foliar SM levels than trees from other zones, albeit significant differences varied depending on the compound. The CWH and ICH are both defined, in part, as rainforests

Table 5 Top three models as selected by lowest Bayesian Information Criterion (with Akaike's Information Criterion also given) explaining secondary metabolite response variables from an analysis of the foliage of *Pinus contorta latifolia* with the means from each site

Response Variable	Model	R^2	BIC	AIC	Explanatory Variables	β	SE
Phenolics	1	0.498*	42.898	41.425	intercept	15.220***	1.851
					longitude	−5.10E-06*	1.62E-06
	2	0.577*	43.303	41.364	intercept	21.100**	4.871
					latitude	−3.82E-06	2.94E-06
					longitude	−6.86E-06**	2.07E-06
	3	0.572*	43.446	41.506	intercept	15.980***	1.900
					elevation	1.96E-03	1.57E-03
					longitude	−7.07E-06*	2.23E-06
Lignin	1	0.781**	38.604	36.178	intercept	38.121***	4.197
					elevation	−0.004**	0.001
					mean annual temp.	−2.750**	0.540
					mean winter temp.	0.992*	0.327
	2	0.765**	39.453	37.028	intercept	24.110**	4.808
					latitude	5.89E-06*	1.85E-06
					mean annual temp.	−1.654*	0.517
					mean winter temp.	0.749	0.344
	3	0.807*	39.618	36.709	intercept	27.440**	5.846
					latitude	1.22E-05**	3.01E-06
					longitude	5.34E-06	2.60E-06
					mean summer prec.	−1.732*	4.946
					mean winter temp.	0.440	0.283
Monoterpenoids	1	0.538	83.875	81.450	intercept	3.018	36.490
					mean summer prec.	4.571	2.665
					mean summer temp.	−1.179*	0.424
					mean winter temp.	2.341	1.331
	2	0.423	84.043	82.103	intercept	23.087	10.686
					mean annual temp.	−2.750**	2.030
					mean summer temp.	−0.844	0.389
	3	0.515	84.450	82.025	intercept	−24.910	31.730
					longitude	−1.45E-05	9.04E-06
					mean summer prec.	5.506	2.705
					mean summer temp.	−0.717	0.431
Sesquiterpenoids	1	0.889**	59.119	55.724	intercept	−58.900*	21.330
					elevation	1.69E-02*	5.91E-03
					longitude	−3.91E-05**	8.92E-06
					mean annual prec.	0.244**	0.050
					mean annual temp.	−2.750**	1.301
					mean summer prec.	7.019*	2.032
	2	0.909*	59.224	55.345	intercept	−23.560	14.150
					latitude	−7.51E-05*	2.72E-05
					longitude	−8.94E-05*	2.64E-05
					mean annual prec.	0.212*	0.065
					mean annual temp.	−2.750**	5.499
					mean summer prec.	16.570*	5.474
					mean summer temp.	0.858*	0.292
	3	0.925*	59.374	55.009	intercept	−42.290	24.780
					elevation	8.58E-03	9.25E-03
					latitude	−4.52E-05	4.25E-05

Table 5 (continued)

Response Variable	Model	R^2	BIC	AIC	Explanatory Variables	β	SE
					longitude	−7.22E-05	3.26E-05
					mean annual prec.	0.220*	0.066
					mean annual temp.	−2.750**	7.937
					mean summer prec.	13.150	6.661
					mean summer temp.	0.576	0.425
Diterpenoids	1	0.834*	77.258	73.864	intercept	−99.790	44.870
					elevation	0.030	0.013
					latitude	3.87E-05*	1.34E-05
					longitude	−4.07E-05*	1.57E-05
					mean annual prec.	0.297*	0.087
					mean summer prec.	5.399	3.249
	2	0.690**	77.339	75.399	intercept	1.529	12.080
					latitude	2.94E-05*	9.18E-06
					mean winter temp.	2.851*	0.936
	3	0.831*	77.487	74.093	intercept	−89.460	40.600
					elevation	3.10E-02	1.38E-02
					latitude	6.30E-05*	2.13E-05
					longitude	−1.83E-05	9.30E-06
					mean annual prec.	0.230	0.095
					mean annual temp.	−2.750**	3.316

Increasing latitude represents movement from south to north, and increasing longitude represents movement from west to east.
* $P<0.050$; ** $P<0.010$; *** $P<0.001$.

(Meidinger and Pojar, 1991). These ecosystems likely allow *P. c. latifolia* to have a longer growing season and a climate where certain abiotic stresses (e.g., drought) are reduced. Both longer growing season and reduced stress could have resulted in additional resources for the pines to invest in their secondary metabolism (Herms and Mattson, 1992). Furthermore, these ecosystems could have been more favorable to pest development (Allen et al., 1996), which would, due to more frequent pest encounters, impose selective pressure on the lodgepole pines to produce more defense-associated compounds in their foliage (Wallis et al., 2010). This latter hypothesis could be overtly tested by attempting to find associations between foliar pest outbreaks and SMs, potentially with dendrochronological techniques to estimate historical pest outbreak frequency (Welsh et al., 2009). Regardless, it would appear that the need to counter biotic stresses in these ecosystems resulted in selection pressure leading to greater levels of defense-associated SMs, namely monoterpenoids for the Interior Cedar-Hemlock ecosystem and both sesquiterpenoids and diterpenoids for the Cedar-Western Hemlock ecosystem.

Precipitation likely had some influence on the measured levels of SMs because these compounds were extracted from 0.1 g of fresh and not dried material. However, the differences in terpenoid levels from the wettest sites would presumably be even greater than those from drier sites if samples were dried first, as wetter sites (i.e., those from the CWH and ICH) would likely have greater water content in their tissues.

Regardless of ecosystem type, stands in more northerly and westerly locations, and at lower elevations, were associated with higher foliar SM levels. These results do not support the hypothesis that more southerly stands would contain greater levels of SMs (Siska et al., 2002; Andrew and Hughes, 2005; Adams et al., 2009). However, in this study, northern, western, and lower elevation sites included some of the wettest and warmest sites, including the CWH and ICH zones. Indeed, prior studies observed that greater pest activity at lower elevations was associated with plants having increased levels of SMs (e.g., Hobbs and Partridge, 1979; Hengxiao et al., 1999; Heineman et al., 2010), and the CWH and ICH sites were at the lowest elevations of all the sites sampled.

In contrast to the other SMs, levels of lignin were highest in the boreal BWBS ecosystem type, whereas the other metabolites were often at the lowest levels in trees from that zone. Lignin is involved with cell wall thickening and strengthening, which contributes to cold temperature tolerance (Smallwood and Bowles, 2002; Wei et al., 2006). However, lignin was not associated with lower winter temperatures, so other selection pressures might be at play.

Table 6 Top three models as selected by lowest Bayesian Information Criterion (with Akaike's Information Criterion also given) explaining secondary metabolite response variables from an analysis of the foliage of *Pinus contorta latifolia* with data from each individual tree

Response Variable	Model	R^2	BIC	AIC	Explanatory Variables	β	SE
Phenolics	1	0.203***	1117.74	1100.38	intercept	14.170***	1.163
					longitude	−8.12E-06***	1.14E-06
					elevation	0.004***	0.001
					mean annual temp.	0.575**	0.220
	2	0.181***	1118.84	1104.95	intercept	21.200***	2.378
					longitude	−6.92E-06***	1.02E-06
					latitude	−3.85E-06**	1.43E-06
	3	0.180***	1119.09	1105.20	intercept	16.040***	0.927
					longitude	−7.17E-06***	1.09E-06
					elevation	0.002**	0.001
Lignin	1	0.191***	1196.74	1179.38	intercept	38.254***	3.041
					elevation	−0.004***	0.001
					mean annual temp.	−2.767***	0.392
					mean winter temp.	1.002***	0.237
	2	0.169***	1197.66	1183.77	intercept	25.840***	3.511
					latitude	7.24E-06***	1.28E-06
					mean summer temp.	−1.040***	0.248
	3	0.187***	1197.85	1180.49	intercept	24.220***	3.362
					latitude	5.90E-06***	1.29E-06
					mean annual temp.	−1.670***	0.362
					mean winter temp.	0.760**	0.241
Monoterpenoids	1	0.199***	2769.22	2752.11	intercept	37.638	152.368
					mean summer prec.	−1.198***	0.177
					mean summer temp.	45.611***	10.987
					mean winter temp.	23.440***	5.464
	2	0.190***	2771.72	2754.62	intercept	−0.024	0.013
					longitude	−1.44E-04***	3.64E-05
					mean summer prec.	−0.072***	0.017
					mean summer temp.	54.740***	10.940
	3	0.147***	2783.48	2766.38	intercept	−390.100**	137.700
					latitude	9.54E-05	4.96E-05
					mean summer prec.	−0.863***	0.174
					mean summer temp.	49.390***	11.340
Sesquiterpenoids	1	0.502***	2405.02	2380.87	intercept	−591.100***	97.790
					longitude	−3.93E-04***	4.15E-05
					elevation	0.170***	0.027
					mean annual prec.	0.245***	0.023
					mean annual temp.	−34.170***	5.909
					mean summer temp.	70.400***	9.337
	2	0.514***	2405.17	2377.56	intercept	−238.300***	64.690
					longitude	−8.95E-04***	1.22E-04
					latitude	−7.52E-04***	1.25E-04
					mean annual prec.	0.211***	0.029
					mean annual temp.	−167.400***	25.280
					mean summer prec.	0.861***	0.134
					mean summer temp.	165.900***	25.180
	3	0.500***	2406.21	2382.05	intercept	−573.300***	96.810
					longitude	−2.62E-04***	3.44E-05

Table 6 (continued)

Response Variable	Model	R^2	BIC	AIC	Explanatory Variables	β	SE
					latitude	1.62E-04***	2.87E-05
					elevation	0.186***	0.029
					mean annual prec.	0.204***	0.019
					mean summer temp.	39.150***	7.016
Diterpenoids	1	0.481***	2574.87	2550.99	intercept	−1013.000***	177.600
					longitude	−4.09E-04***	6.25E-05
					latitude	3.92E-04***	5.20E-05
					elevation	0.304***	0.052
					mean annual prec.	0.297***	0.034
					mean summer temp.	55.000***	12.910
	2	0.492***	2575.48	2548.18	intercept	−816.800***	185.100
					longitude	−6.84E-04***	7.50E-05
					elevation	0.223***	0.050
					mean annual prec.	0.249***	0.054
					mean annual temp.	−66.360***	11.060
					mean summer prec.	0.594***	0.165
					mean summer temp.	102.200***	17.990
	3	0.480***	2575.60	2551.72	intercept	−905.900***	158.700
					longitude	−1.81E-04***	3.64E-05
					latitude	6.37E-04***	8.32E-05
					elevation	0.310***	0.054
					mean annual prec.	0.228***	0.037
					mean annual temp.	54.430***	13.050

Increasing latitude represents movement from south to north, and increasing longitude represents movement from west to east.
* $P<0.050$; ** $P<0.010$; *** $P<0.001$.

When only the SBS *P. c. latifolia* populations were considered (because most pine plantations are found in that ecosystem type), increases in total soluble phenolics and lignin levels were associated with lower temperatures and rainfall averages, suggesting greater abiotic stress. By contrast, monoterpenoids and sesquiterpenoids were not associated with climatic variables at all, perhaps implying low variance in biological stresses that influence their production in the SBS zone. Increasing latitude was positively associated with the production of all SMs for trees in the SBS zone. Studies are needed to determine the specific factors that might explain this trend, such as increased summer day length, decreased growing season duration, or increases in cold temperature extremes that occur with higher latitude that imparts a selection pressure for greater SM production in pine populations.

Although we observed that geographic and climatic variables did affect foliar SM levels in *P. c. latifolia* populations, the physiological and genetic mechanisms behind these observations remain unclear. Our attempts to form testable regression models proved inconclusive, as the top models did not vary much according to their information criteria (both BIC and AIC values), likely because of the inter-relatedness between all of these variables. This also suggests that these geographic and extrapolated climatic variables are perhaps not the best explanatory variables to use for the levels of foliar SMs.

Future studies should attempt to correlate historic and current abiotic and biotic stress levels of stands of *P. c. latifolia* with their secondary metabolites. Associations with current stress levels would indicate that populations might have responded to local abiotic and biotic stresses by shifting SM production or responding via systemic induced responses (Herms and Mattson, 1992; Koricheva et al., 1998). Alternatively, associations with historical stress levels might reveal that different pine populations may have evolved to produce greater SM levels in response to selection pressures caused by adverse abiotic and biotic stressors (Wu et al., 1996; Hodkinson, 2005; Wallis et al., 2010).

Viewed in light of current efforts to assist the migration of *P. c. latifolia* populations from warmer, southerly areas, to colder, northerly areas (O'Neill et al., 2008), these results are not in and of themselves sufficient to suggest that such efforts are or are not worthwhile. However, these results reveal that variation exists between different populations of

Table 7 Top three models as selected by lowest Bayesian Information Criterion (with Akaike's Information Criterion also given) explaining secondary metabolite response variables from an analysis of the foliage of *Pinus contorta latifolia* with data from each individual trees from the SBS zone only

Response Variables	Model	R2	BIC	AIC	Explanatory Variables	B	SE
Phenolics	1	0.314***	639.21	624.53	intercept	−95.450***	17.490
					latitude	2.867E-05***	4.492E-06
					elevation	0.044***	0.007
					mean summer temp.	3.119***	0.630
	2	0.321***	642.83	625.22	intercept	−85.740***	19.470
					latitude	2.870E-05***	4.487E-06
					elevation	0.041***	0.007
					mean summer temp.	2.804***	0.689
					mean winter temp.	0.380	0.337
	3	0.319***	643.06	625.46	intercept	9.569	4.931
					elevation	0.027***	0.005
					mean annual prec.	0.038***	0.007
					mean summer prec.	−0.094***	0.015
					mean winter temp.	2.327***	0.443
Lignin	1	0.260***	696.44	681.80	intercept	47.823***	5.808
					mean annual prec.	0.008**	0.002
					mean annual temp.	−4.221***	0.659
					mean winter temp.	2.569***	0.596
	2	0.259***	696.74	682.11	intercept	−30.680***	8.470
					latitude	2.288E-05***	4.007E-06
					elevation	0.024***	0.005
					mean summer prec.	0.029***	0.008
	3	0.253***	697.91	683.28	intercept	30.851***	6.086
					mean annual temp.	−7.558***	1.744
					mean summer temp.	2.843**	1.011
					mean winter temp.	3.358***	0.822
Monoterpenoids	1	0.152***	1480.66	1469.29	intercept	−225.200*	109.800
					longitude	−3.899E-04***	8.416E-05
					mean summer temp.	58.710***	13.420
	2	0.173***	1482.29	1468.07	intercept	−479.000**	179.600
					longitude	−3.335E-04***	8.927E-05
					latitude	1.931E-04	1.087E-04
					mean summer temp.	58.360***	13.310
	3	0.173***	1482.30	1468.08	intercept	−218.400*	108.900
					longitude	−5.354E-0***4	1.171E-04
					mean annual prec.	0.173	0.098
					mean summer temp.	62.690***	13.490
Sesquiterpenoids	1	0.207***	1334.92	1323.33	intercept	410.870***	56.091
					elevation	−0.284***	0.050
					mean summer prec.	−0.384***	0.079
	2	0.236***	1334.96	1320.48	intercept	−333.200***	74.930
					latitude	1.964E-04***	4.335E-05
					mean summer prec.	−0.253**	0.083
					mean summer temp.	21.750***	4.281
	3	0.234***	1335.22	1320.73	intercept	−450.100***	86.250
					latitude	2.720E-04***	4.571E-05
					mean annual prec.	−0.094**	0.031
					mean summer temp.	24.160***	4.842

Table 7 (continued)

Response Variables	Model	R2	BIC	AIC	Explanatory Variables	B	SE
Diterpenoids	1	0.296***	1344.34	1332.96	intercept	681.500***	93.760
					longitude	−2.618E-04***	3.627E-05
					elevation	−0.397***	0.075
	2	0.308***	1347.07	1332.85	intercept	−459.200***	90.570
					latitude	5.178E-04***	7.736E-05
					mean annual prec.	−0.147***	0.035
					mean annual temp.	35.980***	8.710
	3	0.307***	1347.33	1333.11	intercept	493.500**	168.000
					longitude	−3.047E-04***	4.820E-05
					elevation	−0.320***	0.094
					mean summer temp.	13.340	9.906

Increasing latitude represents movement from south to north, and increasing longitude represents movement from west to east.
* $P<0.050$; ** $P<0.010$; *** $P<0.001$.

P. c. latifolia, especially those in different broadly-defined ecosystem types. Previous studies have shown that when grown in the same location, pines originating from different stands and ecosystems still retain their variance in both SM levels and pest resistance (Wu et al., 1996, 2005; Wallis et al., 2010). Therefore, the variance in SM concentrations observed in pine populations in this study could be predicted to remain for at least one generation if trees undergo assisted migration.

Climate change is predicted to increase both biotic and abiotic stresses on pine populations. Bark beetle populations are the major cause of area wide mortality for *P. c. latifolia*, and these populations are predicted to increase regionally with climate change (Bentz et al., 2010). Coupled with changes in temperature and rainfall patterns, bark beetles could force pine ecosystems to higher latitudes and elevations (Bentz et al., 2010). In this study, terpenoid levels, and to some extent the soluble phenolics and lignin, were greater in ecosystems that were wetter and warmer (the CWH and ICH *versus* the SBS and ESSF), i.e., those likely facing greater biological stresses (Allen et al., 1996). Therefore, migrant pines from these populations would be expected to have a fitness advantage over native pines (i.e., trees whose ancestors originate from the same area) at higher latitude or elevation sites when predicted bark beetle, and possibly pathogen, outbreaks expand into those areas (Bentz et al., 2010). Hypothetically, migrant pines would possess a greater capacity to produce defense-associated compounds. These pines might also be more tolerant of certain abiotic stresses, such as increased temperature extremes and more frequent droughts. However, only longer-term monitoring of the variation of secondary metabolites in multiple stands can confirm if this is the case, or if the native pines might fare little better than migrant trees.

Acknowledgments The authors thank Nick Ukraintz, Alex Woods, Brian Aukema, Sam Barnes, and Rhiannon Montgomery for planning and technical help in executing this study. We also acknowledge the University of Northern British Columbia, the Forest Genetic Council of British Columbia, the British Columbia Ministry of Forests and Range, the Natural Science and Engineering Research Council of Canada, the Canada Research Chairs Program, the Canada Foundation for Innovation, and the British Columbia Knowledge Development Fund for funding and other support in conducting this research.

References

ADAMS, J. M., REHILL, B., ZHANG, Y., and GOWER, J. 2009. A test of the latitudinal defense hypothesis: herbivory, tannins and total phenolics in four North American tree species. *Ecol. Res.* 24:697–704.

AKAIKE, H. 1974. A new look at the statistical model identification. *IEEE Trans. Automat. Contr.* 19:716–723.

ALLEN, E., MORRISON, D., and WALLIS, G. 1996. Common Tree Diseases of British Columbia. Canadian Forest Service-Pacific Forestry Centre: Victoria, BC, Canada, pp 178.

ANDREW, N. R. and HUGHES, L. 2005. Herbivore damage along a latitudinal gradient: relative impacts of different feeding guilds. *Oikos* 108:176–182.

BENTZ, B. J., RÉGNIÈRE, J., FETTIG, C .J., HANSEN, E. M., HAYES, J. L., HICKE, J. A., KELSEY, R. G., NEGRÓN, J. F., and SEYBOLD, S. J. 2010. Climate change and bark beetles of the western US and Canada: Direct and indirect effects. *BioScience* 60:602–613.

BURNHAM, K. P. and ANDERSON, D. R. 2002. Model Selection and Multi-model Inference: A Practical Information-Theoretic Approach, 2nd ed. Springer-Verlag, New York, NY, USA, pp 488.

COAKLEY, S. M., SCHERM, H., and CHAKRABORTY, S. 1999. Climate change and plant disease management. *Annu. Rev. Phytopathol.* 37:399–426.

CUMMING, S. G. and BURTON, P. G. 1996. Phenology-mediated effects of climate change on some simulated British Columbia forests. *Clim. Change* 34:213–222.

DESPREZ-LOUSTAU, M. L., ROBIN, C., REYNAUD, G., DEQUE, M., BADEAU, V., PION, D., HUSSON, C., and MARCAIS, B. 2007.

Simulating the effects of a climate-change scenario on the geographical range and activity of forest-pathogenic fungi. *Can. J. Plant Pathol.* 29:101–120.

FOLKE, C., CARPENTER, S., WALKER, B., SCHEFFER, M., ELMQVIST, T., GUNDERSON, L., and HOLLING, C. S. 2004. Regime shifts, resilience, and biodiversity in ecosystem management. *Annul. Rev. Ecol. Evol. Syst.* 35:557–581.

FRANCESCHI, V. R., KROKENE, P., CHRISTIANSEN, E., and KRECKLING, T. 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytol.* 167:353–376.

HAMANN, A. and WANG, T. 2006. Potential effects of climate change on ecosystem and tree species distribution in British Columbia. *Ecology* 87:2773–2786.

HEINEMAN, J., SACHS, D. L., MATHER, W. J., and SIMARD, S. W. 2010. Investigating the influence of climate, site, location, and treatment factors on damage to young lodgepole pine stands in southern British Columbia. *Can. J. For. Res.* 40:1109–1127.

HENGXIAO, G., MCMILLIN, J. D., WAGNER, M. R., ZHOU, J., ZHOU, Z., and XU, X. 1999. Altitudinal variation in foliar chemistry and anatomy of Yunnan pine, *Pinus yunnanensis*, and pine sawfly (Hym., Diprionidae) performance. *J. Appl. Ent.* 123:465–471.

HERMS, D. A. and MATTSON, W. J. 1992. The dilemma of plants—to grow or defend. *Quart. Rev. Biol.* 67:283–335.

HOBBS, S. D. and PARTRIDGE, A. D. 1979. Wood decays, root rots, and stand composition along an elevation gradient. *For. Sci.* 25:31–42.

HODKINSON, I. D. 2005. Terrestrial insects along elevation gradients: species and community responses to altitude. *Bio. Rev.* 80:489–513.

JAAKOLA, L. and HOHTOLA, A. 2010. Effect of latitude on flavonoid biosynthesis in plants. *Plant Cell Environ*. 33:1239–1247.

KEELING, C. I. and BOHLMANN, J. 2006. Diterpene resin acids in conifers. *Phytochemistry* 67:2415–2423.

KORICHEVA, J., LARSSON, S., HAUKIOJA, E., and KEINANEN, M. 1998. Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. *Oikos* 83:212–226.

KURZ, W. A., DYMOND, C. C., STINSON, G., RAMPLEY, G. J., NEILSON, E. T., CARROLL, A. L., EBATA, T., and SAFRANYIK, L. 2008. Mountain pine beetle and forest Carbon feedback to climate change. *Nature* 452:987–990.

LÄTTI, A. K., JAAKOLA, L., RIIHINEN, K. R., and KAINULAINEN, P. S. 2008. Anthocyanin and flavonol variation in bog bilberries (*Vaccinium uliginosum* L.) in Finland. *J. Agric. Food Chem.* 58:427–433.

LOGAN, J. A., RÉGNIÈRE, J., and POWELL, J. A. 2003. Assessing the impacts of global warming on forest pest dynamics. *Front. Ecol. Environ.* 1:130–137.

MARRIS, E. 2009. Planting the forest of the future. *Nature* 459:906–908.

MARTZ, F., PELTOLA, R., FONTANAY, S., DUVAL, R. E., JULKUNEN-TIITTO, R., and STARK, S. 2009. Effect of latitude and altitude on the terpenoid and soluble phenolic composition of juniper (*Juniperus communis*) needles and evaluation of their antibacterial activity in the boreal zone. *J. Agri. Food Chem*. 57:9575–9584.

MARTZ, F., JAAKOLA, L., JULKUNEN-TIITTO, R., and STARK, S. 2010. Phenolic composition and antioxidant capacity of bilberry (*Vaccinium myrtillus*) leaves in northern Europe following foliar development and along environmental gradients. *J. Chem. Ecol.* doi 10.1007/s10886-010-9836-9

MEIDINGER, D. and POJAR, J. 1991. Ecosystems of British Columbia. BC Min. For., Res. Br. Victoria, BC, pp 330.

O'NEILL, G. A., HAMANN, A., and WANG, T. 2008. Accounting for population variation improves estimates of the impact of climate change on species' growth and distribution. *J. Applied Ecol.* 45:1040–1049.

REHFELDT, G. E., YING, C. C., SPITTLEHOUSE, D. L., and HAMILTON, D. A. 1999. Genetic responses to climate in *Pinus contorta*: Niche breadth, climate change, and reforestation. *Ecol. Monogr.* 69:375–407.

SCHWARZ, G. E. 1978. Estimating the dimension of a model. *Ann. Stat.* 6:461–464.

SEYBOLD, S. J., HUBER, D. P. W., LEE, J. C., GRAVES, A. D., and BOHLMANN, J. 2006. Pine monoterpenes and pine bark beetles: A marriage of convenience for defense and chemical communication. *Phytochem. Rev.* 5:143–178.

SISKA, E. L., PENNINGS, S. C., BUCK, T. L., and HANISAK, M. D. 2002. Latitudinal variation in palatability of salt-marsh plants: which traits are responsible. *Ecology* 83:3369–3381.

SMALLWOOD, M. and BOWLES, D. 2002. Plants in a cold climate. *Phil. Trans. R. Soc. Lond. B* 357:831–847.

STARK, S., JULKUNEN-TIITTO, R., HOLAPPA, E., MIKKOLA, K., and NIKULA, A. 2008. Concentrations of foliar quercetin in natural populations of white birch (*Betula pubescens*) increase with latitude. *J. Chem. Ecol.* 34:1382–1391.

WALLIS, C., EYLES, A., CHORBADJIAN, R., MCSPADDEN GARDENER, B., HANSEN, R., CIPOLLINI, D., HERMS, D. A., and BONELLO, P. 2008. Systemic induction of phloem secondary metabolism and its relationship to resistance to a canker pathogen in Austrian pine. *New Phytol.* 177:767–778.

WALLIS, C. M., REICH, R., LEWIS, K. J., and HUBER, D. P. W. 2010. Lodgepole pine provenances differ in chemical defense capacities against foliage and stem diseases. *Can. J For. Res.* 40:2333–2344.

WALTHER, G. R., POST, E., CONVEY, P., MENZEL, A., PARMESAN, C., BEEBEE, T. J. C., FROMENTIN, J.-M., HOEGH-GULDBERG, O., and BAIRLEIN, F. 2002. Ecological responses to recent climate change. *Nature* 416:389–395.

WANG, T., HAMANN, A., SPITTLEHOUSE, D., and AITKEN, S. N. 2006. Development of scale-free climate data for western Canada for use in resource management. *Int. J. Climatol.* 26:383–397.

WEI, H., DHANARAJ, A. L., ARORA, R., ROWLAND, L. J., FU, Y., and SUN, L. 2006. Identification of cold acclimation-responsive *Rhododendron* gene for lipid metabolism, membrane transport and lignin biosynthesis: importance of moderately abundant ESTs in genomic studies. *Plant Cell Env.* 29:558–570.

WELSH, C., LEWIS, K., and WOODS, A. 2009. The outbreak history of *Dothistroma* needle blight: an emerging forest disease in northwestern British Columbia, Canada. *Can. J. For. Res.* 39:2505–2519.

WHITZELL, J. and MARTIN, J. A. 2008. Phenolic metabolites in the resistance of northern forest trees to pathogens—past experiences and future prospects. *Can. J. For. Res.* 38:2711–2727.

WOODS, A., COATES, K. D., and HAMANN, A. 2005. Is an unprecedented *Dothistroma* needle blight epidemic related to climate change? *Bioscience* 55:761–769.

WU, H. X., YING, C. C., and MUIR, J. A. 1996. Effect of geographic variation and jack pine introgression on disease and insect resistance in lodgepole pine. *Can. J. For. Res.* 26:711–726.

WU, H. X., YING, C. C., and JU, H. B. 2005. Predicting site productivity and pest hazard in lodgepole pine using biogeoclimatic system and geographic variables in British Columbia. *Ann. For. Sci.* 62:31–42.

YING, C. C. and YANCHUK, A. D. 2006. The development of British Columbia's tree seed transfer guidelines: purpose, concept, methodology, and implementation. *For. Ecol. Manage.* 227:1–13.