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Foliar phenols in beech (*Fagus sylvatica* L.) seedlings of six European provenances from a latitudinal gradient grown in three common gardens in Norway

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Forest science

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Abstract

With progressive climate change, the total concentration and composition of the chemical defense in European beech (*Fagus sylvatica* L.) is likely to change, which might impact its fitness and ability to establish beyond its current northernmost distribution limit in South-East Norway. In this master thesis, I aimed to explore the phenolic defense in European beech provenances grown in three Norwegian common gardens, and how the phenol concentration changed from the first to the second year after planting. The question whether the phenol concentration depends more on environment or genetics has so far been little studied.

The concentration of low molecular weight phenols in beech seedling leaves were analyzed using high-performance liquid chromatography (HPLC), whereas the condensed tannins were analyzed in an acid-butanol assay followed by spectrophotometry. I identified 19 low molecular weight phenols. I grouped the individual compounds into chlorogenic acid derivatives, quercetin 3-glycosides, kaempferol 3-glycosides, and calculated total flavonoids and total low molecular weight phenols. The explanatory power of the complete mixed linear model was about 50% for low molecular weight phenols as a group, and about 15% for the condensed tannins.

Environment (growing location) explained 5 % of the variation in total low molecular weight phenols, whereas genetic background (provenance) only explained 0.8%. Year explained most of the variation in low molecular weight phenols (31%). After the first year in the field, the northernmost growing location (Munkrøstad) had about 70% higher concentration of low molecular weight phenols than the southern growing locations, Ås and Re. The concentrations of methanol-soluble condensed tannins were 13% lower in Munkrøstad than in Ås and the methanol-insoluble condensed tannins were 33% lower in Munkrøstad than in Ås and Re. The mean concentration of total low molecular weight phenols was twice as high after the first year compared to the second year. After the second year, the differences between the growing locations were nonsignificant in many cases. I attribute the decrease in low molecular weight phenols from the first to the second year mainly to decreasing water and nutrient stress, as the root systems had had more time to establish and grow. My results suggest that intraspecific variations in concentration of phenolic compounds in beech are caused by differences in environment rather than genetic background, which may mean that changes in chemical defense with climate change will be faster.

Sammendrag

Klimaendringene fører sannsynligvis til at både den totale mengden og sammensetningen av kjemiske forsvarstoffer i bøk (*Fagus sylvatica* L.) forandres. Dette kan endre artens fitness og evne til å etablere seg nord for dagens utbredelsesgrense i Sør-Øst-Norge. I denne masteroppgaven var målet å utforske fenoler som bidrar til det kjemiske forsvaret i europeiske bøkeprovenienser i et proveniensforsøk på tre lokaliteter i Norge. Jeg undersøkte også hvordan fenolkonsentrasjonen endret seg i løpet av de to første årene i felt etter utplanting. Spørsmålet om hvorvidt fenolkonsentrasjonen styres mest av arv eller miljø har så langt vært lite studert.

Konsentrasjonen av fenoler med lav molekylmasse ble analysert ved hjelp av væskekromatografi (HPLC), mens kondenserte tanniner ble kvantifisert ved syre-butanol-analyse og spektrofotometri. Jeg identifiserte 19 fenoler med lav molekylmasse, og grupperte dem i klorogensyrederivativer, quercetin 3-glykosider og kaempferol 3-glykosider. Fra disse gruppene regnet jeg ut konsentrasjonen av totale flavonoider og alle fenoler med lav molekylmasse. De blanda lineære modellene forklarte omtrent 50% av variasjonen i total konsentrasjon av fenoler med lav molekylmasse og rundt 15% av variasjoner i kondenserte tanniner.

Miljø (voksested) forklarte 5% av variasjonen i fenoler med lav molekylmasse, mens genetisk bakgrunn (proveniens) bare forklarte 0.8%. År forklarte aller mest av variasjonen i små fenoler (31%). Etter ett år i felt fantes den høyeste konsentrasjonen av fenoler med lav molekylmasse på det nordligste voksestedet (Munkrøstad), hvor konsentrasjonen var 63% høyere enn i Ås og 83% høyere enn i Re. Konsentrasjonen av metanolløselige kondenserte tanniner var derimot 13% lavere i Munkrøstad enn i Ås og konsentrasjonen av metanoluløselige kondenserte tanniner var 33% lavere i Munkrøstad enn i Ås og Re. Den gjennomsnittlige konsentrasjonen av fenoler med lav molekylmasse var dobbelt så høy etter det første året som etter det andre året. Etter det andre året var forskjellene mellom voksesteder i mange tilfeller ikke statistisk signifikante. Jeg forklarer forskjellene mellom voksesteder hovedsakelig med avtakende stressnivå som følge av at røttene har fått tid til å etablere seg på voksestedet, slik at opptaket av vann og næringsstoffer ble forbedret. Resultatene mine tyder på at variasjoner i fenoler i bøk i større grad skyldes miljø enn arv, noe som betyr at forandringer i kjemisk forsvar som følge av klimaendringene vil skje raskere.

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Johanna Sætherø Steen

1 Introduction

Global warming will have a pronounced effect on European forest ecosystems and might cause a shift in the competitiveness and dominance of species (Alkemade et al., 2011; Kellomäki et al., 2001). The global mean temperature is predicted to increase by 0.3°C-4.0°C by the end of this century, and the temperature at the northernmost latitudes is expected to increase even more than the global average (IPCC, 2014). The frequency and severity of summer droughts is increasing (IPCC, 2014), as well as the risk and intensity of pathogen infections and insect attacks (La Porta et al., 2008). More biotic and abiotic stresses pose threats to European tree species, which makes plant resistance more important than ever. Climate change will bring changes in drought frequency and extent, changed UV-radiation, elevated temperatures and CO_2, which may all impact the chemical and physical defense of plants (Julkunen-Tiitto et al., 2015; Zvereva & Kozlov, 2006).

As the mean temperature in the northern hemisphere increases, keystone species in European forest ecosystems, like beech and spruce, shift northwards (Kramer et al., 2010; Bradshaw et al., 2000). European Beech (*Fagus sylvatica* L.), hereafter beech, is considered to be a native tree species in Southern Norway (Bjune et al., 2013) with its core area in Vestfold and a separate population in Seim in Western Norway. Plantings and natural regeneration from plantings are widespread along the coastline from the Swedish border to Levanger in Trøndelag county, and beech is found as far north as in Harstad in the northernmost county in Norway (Artsdatabanken, 2020). Due to less intensive forestry practices and warmer climate, some models predict beech to expand its range in Norway (Kramer et al., 2010) but see (Saltré et al., 2015), and it is rapidly establishing in spruce monocultures (Kolstad et al., 2016).

This expansion will likely increase the economic and ecological importance of beech in Norway, particularly as spruce monocultures may become less stable due to warming and severe insect attacks (Bradshaw et al., 2000; Marini et al., 2017). Admixture of beech in spruce stands reduces the risk of stand mortality in a climate change scenario (Neuner et al., 2015), while maintaining the revenue at small beech percentages (Neuner & Knoke, 2017). In parts of Vestfold, the local beech provenance has poor growth form, and is currently sold as energy wood (Bingen, 2020). Introduction of foreign provenances with better stem form and higher productivity could increase the profitability of beech in Norway. Norwegian beeches are also susceptible to frost damage and infection by *Phytophtora* species (Hanssen, 2015).

For foreign provenances to be successful long term, it is important that they are adapted to the planting site conditions. An important aspect of adaptation and long-term survival is the chemical defense. Phenols are a large and highly abundant group of defense chemicals which occurs across the plant kingdom, but they can also be found in fungi (Gutierrez-Uribe & Verduzco-Oliva, 2019) and in certain prokaryotes (e.g. Nishioka et al., 1989). Phenols play a vital role in plant defense against fungi (Ganthaler et al., 2017), insect and mammalian herbivores (Barbehenn & Constabel, 2011), other plants (e.g. González et al., 2015) and UV light (e.g. Contreras et al., 2019). At unfavorable site conditions, such as low soil pH, fertility and moisture, we often find high levels of phenols (Bussotti et al., 1998; Northup et al., 1998). They also affect the belowground part of the ecosystem through their delaying effect on decomposition (reviewed by Min et al., 2015). Phenolic composition and concentration vary between species, but the intraspecific variation is considerable as well (Moreira et al., 2014; Sampedro et al., 2011). It also varies with season and age (Bussotti et al., 1998; Wam et al., 2017), plant compartment and even between the leaves of the same individual (Petrakis et al., 2011). Phenols have in common that they contain one or more aromatic rings with a hydroxyl group attached to the ring and are often grouped according to their size and number of aromatic rings. We often distinguish between low molecular weight phenols, which are smaller, less complex phenols and the larger and more complex condensed tannins, which consist of several flavonoid subunits. The condensed tannins can be subdivided into methanol (MeOH)-soluble and MeOH-insoluble tannins (e.g. by Wam et al., 2017).

The phenolic composition and concentration of an individual has both a genetic and an environmental component, but few studies have attempted to quantify which of these factors explains more of the variation in phenolic compounds in beech (but see Zimmer et al., 2015). Researchers often use common garden experiments to untangle the effects of genetics and environment on plant phenotypes (de Villemereuil et al., 2016). Several common garden experiments exist for beech, studying important aspects of fitness and survival, like drought resistance, growth and phenology. To my knowledge, there has been only a few common garden experiments studying foliar phenols in beech. Zimmer et al. (2015) included only continental European provenances and growing locations, which might not be representative for beeches growing at the edges of the distribution range of the species, where environmental constraints are more severe. Furthermore, Zimmer et al. (2015) only included data from one growing season, and their method for determining total phenolic content has been criticized (Everette et al., 2010). Aranda et al. (2017) compared foliar phenols in three provenances, but

used only one growing location, which makes it difficult to draw conclusions to how the environment influences the phenol concentration. Bussotti et al. (1998) investigated foliar phenols in natural beech populations along an environmental gradient, but the study design did not allow for separation of the genetic and the environmental effects on foliar phenols. Some studies have looked at the effect of environmental and genetic factors on foliar phenols in other tree species like birch e.g. (Deepak et al., 2018; Laitinen et al., 2005), and aspen (e.g. Osier & Lindroth, 2006), but these studies might not be representative for beech.

In this thesis, I explored the composition of phenolic compounds in European beech seedlings of six European provenances originating from a latitudinal gradient, planted in three common gardens in Norway. The main goal was to quantify if the environment (growing location) or the genetic background (provenance) affect the concentration and composition of phenolic compounds more. If the phenol concentration is under strong genetic control, adjustment to new growing locations will be slower, and the changes in foliar phenols due to climate change might be smaller. In this thesis, I addressed the following research questions.

1) Do the concentrations of condensed tannins and low molecular weight phenols differ between planting locations and between provenances in the same planting location?
2) Do the concentrations of condensed tannins and low molecular weight phenols change between the first and the second growing season in the field, indicating phenotypic plasticity?
3) Is there a difference in the phenolic footprint, i.e. in the composition of low molecular weight phenolic compounds between provenances, planting regions and sampling years?

By answering these research questions, I aimed to fill some of the gaps in the understanding of the factors influencing the concentration and composition of the phenolic defense in beech. This is a small, but important part of the knowledge base needed to predict how beech will respond to climate change and the predicted increase in insect attacks, fungal pathogens and severe weather events. As beech is a widespread tree species, this topic is of considerable importance to European ecosystems and forestry.

2 Materials and methods

2.1 Study area and study species

The study areas were located in Ås, Viken county in SE Norway, Re, Vestfold and Telemark county in SE Norway and Munkrøstad, Trøndelag county in central Norway (Figure 1, Table 1). All three planting locations were located on former agricultural land, and the sites in Ås and Munkrøstad had had one rotation of spruce before the experiment started. Re belongs to the core area of natural beech forests in Norway, whereas the beech population in Ås originates from natural regeneration from earlier plantings. There is also an older beech planting near Munkrøstad. The study species was European beech (*Fagus sylvatica* L.) of six European provenances: Vestfold (Norway), Stenderup (Denmark), Albjershus (Sweden), De Soignes (lowland, Belgium), Ardennes (Belgium) and Massif Armoricain (France) (Figure 2, Table 2). The provenance origins form an approximate latitudinal gradient, with Massif Armoricain being the southernmost origin and Vestfold the northernmost origin.

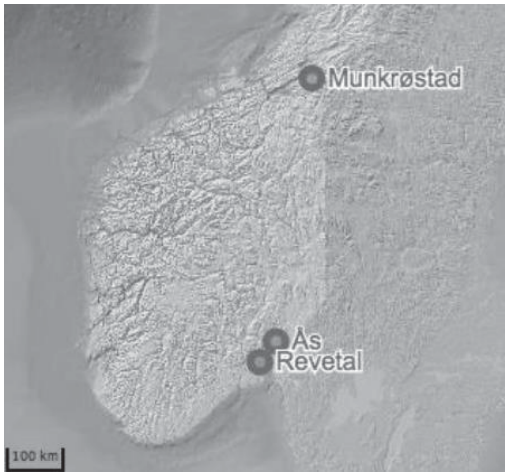

Figure 1. Location of the three common gardens used in this experiment. Reproduced with permission from Hagalid (2017)

Table 1. Coordinates, elevation, exposition, mean annual temperature (MAT), mean annual precipitation (MAP) (Hagalid, 2017) and soil type (Geological Survey of Norway, 2020) for the three common gardens in this experiment. Mean annual temperature and precipitation are calculated from the period 1961-1990. Growing season average temperature (April-October) (GST) and growing season average precipitation (GSP) for the years 2016 and 2017 were obtained from NIBIOs nearest climate station: Ramnes for Revetal, Ås for Ås and Kvithamar for Munkrøstad (NIBIO, 2020).

	Location		
	Revetal (Re)	Ås	Munkrøstad
Latitude	59°21’40.2’’N	59°41’36.6’’N	63°44’17.4’’N
Longitude	10°18’50.7’’E	10°45’06.9’’E	11°24’01.3’’E
Elevation (m a.s.l.)	Appr. 80	Appr. 90	Appr. 100
Soil type	Silty loam	Silty loam	Gravelly sand
Exposition	West/east	East/west	West/south
MAT (°C)	5.3	5.3	5.0
GST 2016 (°C)	12.1	11.8	10.5
GST 2017(°C)	11.4	11.0	10.5
MAP (mm)	1060	785	900
GSP2016 (mm)	520.6	511.5	501.0
GSP2017 (mm)	619.4	577.6	697.7

The beech seedlings from Sweden, Belgium and France were cultivated in a greenhouse from seeds, whereas the seedlings from Norway and Denmark were purchased from a nursery. The seedlings were planted in Re and Ås at 0.5-1.5 years of age in September and October 2015, and in Munkrøstad in May 2016. The Danish plants were one year older than the other provenances and suffered from an insect attack in 2015. For more details on the plant material, see Hagalid (2017). The planting scheme was equal in all three locations: a 25x12 matrix with a set mixture of provenances, keeping two plants of the same provenance next to each other on the same row. The plantings were fenced, and ground vegetation kept down to decrease browsing pressure and competition from other vegetation. Between sampling in 2016 and 2017, many plants were killed, likely by European water vole (*Arvicola terrestris*), and these plants were excluded from the analysis (Hagalid, 2017).

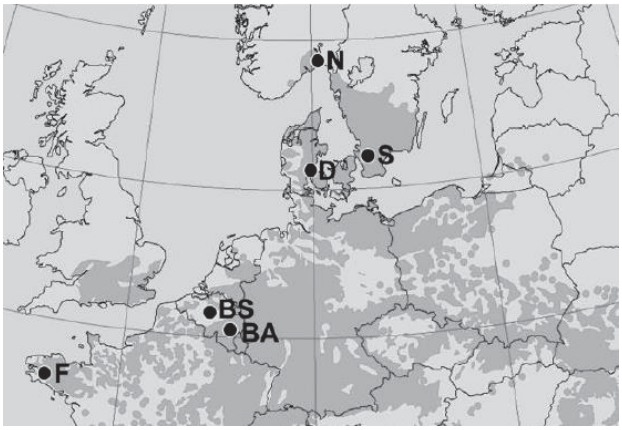

Figure 2. The natural distribution range of beech in gray. The black points annotate the origins of beech seedlings used in the experiment: Norway (N), Sweden (S), Denmark (D), Belgium (de Soignes, BS), Belgium (Ardennes, BA) and France (F). See table 1 for more details on the provenance origins. Reproduced with permission from Hagalid (2017).

Table 2. Coordinates and climate data for the beech provenance origins in the experiment (Hagalid, 2017). MAT stands for mean annual temperature and MAP for mean annual precipitation.

Country	Provenance	Latitude	Longitude	MAT (°C)	MAP (mm)
Norway	Vestfold	59°18'N	10°08'E	5.3	1060
Sweden	Albjershus	55°56'N	13°15'E	7.8	668
Denmark	Stenderup	55°47'N	9°48'E	7.7	725
Belgium	De Soignes	50°46'N	4°24'E	10.3	785
Belgium	Ardennes	50°15'N	5°40'E	7.5	1095
France	Massif Armoricain	48°06'N	4°10'W	10.4	1142

2.2 Harvest

Foliage sampling took place during the first week of September in 2016 and 2017. Two fully developed leaves about 5 cm below the top shoot were sampled from each beech sapling. The leaves were put into paper bags with silica, transported to the lab on the same day and dried for two days at 30°C in an oven. The dried leaves were ground to fine powder using a Retsch MM400 ball mill (Retsch, Haag, Germany).

2.3 Extraction of MeOH-soluble phenolic compounds

From each powdered sample, 10 mg plant material was transferred into a Precellys vial together with 2-3 zirconium oxide balls. The vials were stored in the freezer (-20°C) until extraction. For the extraction, we added 400 µL methanol (MeOH) to the vial and

homogenized the samples for 20 s at 5000 rpm, using a Precellys 24 homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France).

The vial was put in an ice bath for 15 min, and thereafter centrifuged for 3 minutes at 15000 rpm, using an Eppendorf 5417C centrifuge (Eppendorf, Hamburg, Germany). The supernatant was transferred to plastic tubes using a pasteur pipette. 400 µL MeOH was then added to the residue and the vial was homogenized for 20 s and then centrifuged for 3 min. The supernatant was again transferred into the same plastic tube. We repeated the process four times, so that MeOH was added 5 times to the vial in total. The plastic tube with the collected supernatants was afterwards dried in a vacuum centrifuge (Concentrator plus, Eppendorf, Hamburg, Germany) using the vacofuge-alcohol setting 30°C at 1400 rpm for about 3 hours until all the MeOH had evaporated. The plastic tubes were stored in the freezer until the HPLC analysis, and the Precellys vials which contained MeOH-insoluble condensed tannins were air dried and then stored at -18°C until further analysis.

2.4 HPLC-analysis

The low molecular weight phenols were identified and quantified using HPLC, which is an established method for analysis of phenols in plants. We removed the frozen extracts from the freezer and allowed them to thaw before adding 200µL MeOH to each tube. The tubes were put in an ultrasound bath (mod. no. USC200TH, VWR International LLC, Randor, US) until the dried extract dissolved. 200 µL ultrapure water (USF ELGA Maxima HPLC; Veolia Water Technologies, Saint-Maurice, France) was added to the tube, and the tube was put in the ultrasound bath for a few seconds to allow the ingredients to mix. The liquid was then poured into Eppendorf vials and centrifuged for 3 minutes at 15000 rpm. We transferred the supernatant into a HPLC vial using a Pasteur pipette, and analyzed it using a HPLC system (Agilent 1200 series, Agilent Technologies Inc., Santa Clara, USA) comprised of a G1379B degasser, a G1312A binary pump, a G1329 autosampler, a G1316A thermoregulated column heater, and a G1315D diode array detector. The mobile phases were methanol and a solution of 5 mL orthophosphoric acid 30 mL tetrahydrofuran diluted to 2 L using ultrapure water (A-solution), which eluted the samples using the same gradient as Nybakken et al. (2012) and an injection volume of 20 µL. The stationary phase was a Thermo Scientific column (Thermo Fisher Scientific Inc., Waltham, USA) which has a 50 x 4.6 mm internal diameter and filled with 3 µm ODS Hypersil particles. We used the retention times at 320 nm to identify the low molecular weight phenolic compounds, after having cross-referenced the retention times against other samples with known absorbance spectra, identifying 19 peaks. The

concentrations were calculated from the area of the peaks using compound-specific response factors based on commercial standards.

2.5 Condensed tannins

The condensed tannins were quantified using acid-butanol assay with spectrophotometry, which is the most widespread method for quantitative analysis of condensed tannins (Schofield et al., 2001).We analyzed the MeOH-soluble condensed tannins from HPLC extracts within 48h of the HPLC analysis using an acid-butanol assay. 50 µL of sample was transferred to a closable glass test tube together with 3 mL butanolic acid (95 % butanol and 5% HCl), 450 µL MeOH and 100 µL iron reagent (2% ferric ammonium sulfate in 2N HCl). The test tube was closed with a plastic cap and stirred, and then boiled in water for 50 mins. After the test tube had cooled down, some of the liquid was transferred to a plastic cuvette and the absorption was measured at 550 nm using a UV-spectrophotometer (UV-1800, Shimadzu Corp., Kyoto, Japan). It was prepared two duplicates of every sample, to obtain an average value, as well as ensuring at least one useable reading for every sample. From a few test tubes, some of the sample evaporated, and these were discarded, as they gave artificially high absorption values. We analyzed the amount of MeOH-insoluble condensed tannins using the dried residues left after the extraction. The MeOH-insoluble condensed tannins from 2017 were not included in this thesis. 2x 1-3 mg residue from each sample was transferred to 25 mL closable glass test tubes together with 3 mL butanolic acid, 500 µL MeOH and 100 µL iron reagent, and then treated the same way as the MeOH-soluble condensed tannins. Calculation of concentration was based on standard curves of purified spruce tannins.

2.6 Data analysis

I performed the data analysis using the statistical programming language R, version 3.6.1 (R core team, 2019). I made the graphics using the R-package ggplot2 (Wickham, 2016) and Inkscape, version 0.92.4 (Inkscape project contributors, 2007). The individual compounds were first grouped into three groups: chlorogenic acid derivatives, quercetin 3-glycosides and kaempferol 3-glycosides. The quercetin 3-glycosides and kaempferol 3-glycosides were further grouped into total flavonoids. I also analyzed all low molecular weight phenols as one group. One compound was hence categorized into more than one group.

I developed linear mixed-effects models using the R-package lme4 (Bates et al., 2015). The models were tested for significance in the R-package lmerTest (Kuznetsova et al., 2017). To check if the condition homogeneity of residuals was fulfilled, the residuals were plotted on a

qq-plot, and a Shapiro-Wilk test was performed. If this condition was violated, the data was log-transformed. In some cases, p-values of the Shapiro-Wilk test was below 0.05 even after log-transformation and other alternative transformations, and in these cases, I chose to still use the log-transformed model, as linear mixed effect models are robust against violations of the requirement of normal residuals. To identify which groups differed significantly from other groups, the R-package emmeans was used (Lenth, 2020). For the grouped models, R^2-values for fixed effects only were calculated according to Nakagawa and Schielzeth (2013) using the r.squaredGLMM function from the package MuMIn (Barton, 2019). I developed separate models using only provenance, year or growing location to quantify how much of the variation in phenolic compound concentration was explained by provenance, year and location separately and combined.

To analyze the composition of phenolic substances in different growing locations and provenances, I created a present/absent table where all non-zero values were transformed to 1s. For this analysis, only the 2017 data set was used. I developed general linear models using the function glm in the stats package (R core team, 2019), using provenance and location as explanatory variables. To identify which of the explanatory variables was significant for each substance, the Anova function from R's car package was used (Fox & Weisberg, 2019), followed by calculating the estimated marginal means to identify which provenances/locations were significantly different from other provenances/locations.

3 Results

The total amount of phenolic compounds (condensed tannins + low molecular weight phenols) in beech leaves varied between 110 mg g^{-1} (without MeOH-insoluble condensed tannins) and 240 mg g^{-1} dry weight. I identified in total 19 low molecular weight phenolic peaks on the chromatograms, but I also analyzed the individual compounds in different groupings (Table 3) and note that a compound fit into multiple groups. The explanatory power of the complete mixed linear model was about 50% for low molecular weight phenols as a group, and about 15% for the condensed tannins (Table 3).

Table 3. R^2-values (variance explained by the fixed effects) from r.squaredGLMM function from the R package MuMIn performed on the grouped models, incorporating each explanatory variable separately and all together (whole model). The models were developed from groupings of individual phenolic compounds in beech seedlings included in this experiment.

Substance	Provenance	Year	Location	Whole model
MeOH-insoluble condensed tannins	1.64%	N.A.	10.21%	15.80%
MeOH-soluble condensed tannins	1.28%	2.95%	1.27%	15.54%
Low molecular weight phenols	0.77%	30.59%	5.04%	50.25%
Chlorogenic acid derivatives	2.19%	2.51%	11.81%	24.23%
Quercetin 3-glycosides	4.37%	26.13%	1.94%	50.25%
Kaempferol 3-glycosides	0.54%	43.00%	1.73%	54.88%
Total flavonoids	2.22%	38.83%	1.91%	53.99%

3.1 Effect of location and provenance

The total concentration of low molecular weight phenols differed significantly between growing locations in 2016 (Figure 3A), but the differences were much smaller in 2017 (Figure 3B). A general trend across all phenolic groups in 2016, except from condensed tannins (Figure 3,4 and 5), was that the highest concentrations were found in plants from Munkrøstad, the northernmost location. Munkrøstad had 63% higher concentration of low molecular weight phenols than Ås, and 83% higher than Re in 2016 (88 mg g^{-1}, 54 mg g^{-1} and 48 mg g^{-1}, respectively, $p<0.001$). In contrast, there were only minor differences between locations in 2017. The concentration of MeOH-soluble condensed tannins were 13% lower in Munkrøstad than in Ås (117 mg g^{-1} vs 132 mg g^{-1}, $p=0.016$), and the MeOH-insoluble condensed tannins 33% were lower in Munkrøstad than in Ås and 44% lower than in Re (9 mg g^{-1}, 12 mg g^{-1} and 13 mg g^{-1}, respectively, $p<0.001$). However, growing site explained only 5% of variation in total low molecular weight phenols (Table 3). In the mixed linear models, growing location was highly significant for most compounds with a few exceptions (Table 4). The interaction

term between year and location was significant in all models except for dicoumaroylastragallin 2 (Table 4), as many compounds exhibited significant differences between locations in 2016, but not in 2017. The interaction factor between year, location and provenance was insignificant in all models.

Different provenances differed significantly from each other only in certain compounds and growing locations (e.g. Figure 4 E). There was no clear trend among the provenances, but Denmark stood out as the provenance with the highest concentration of low molecular weight phenols in many cases, whereas Belgian provenances often had the lowest concentration (Figure 4 and 5). In the mixed linear models, provenance was significant in 17 out of 26 compounds or groups of compounds (Table 4), but often only within one growing location. Provenance only explained 0.77% of the variance in total low molecular weight phenols (Table 3).

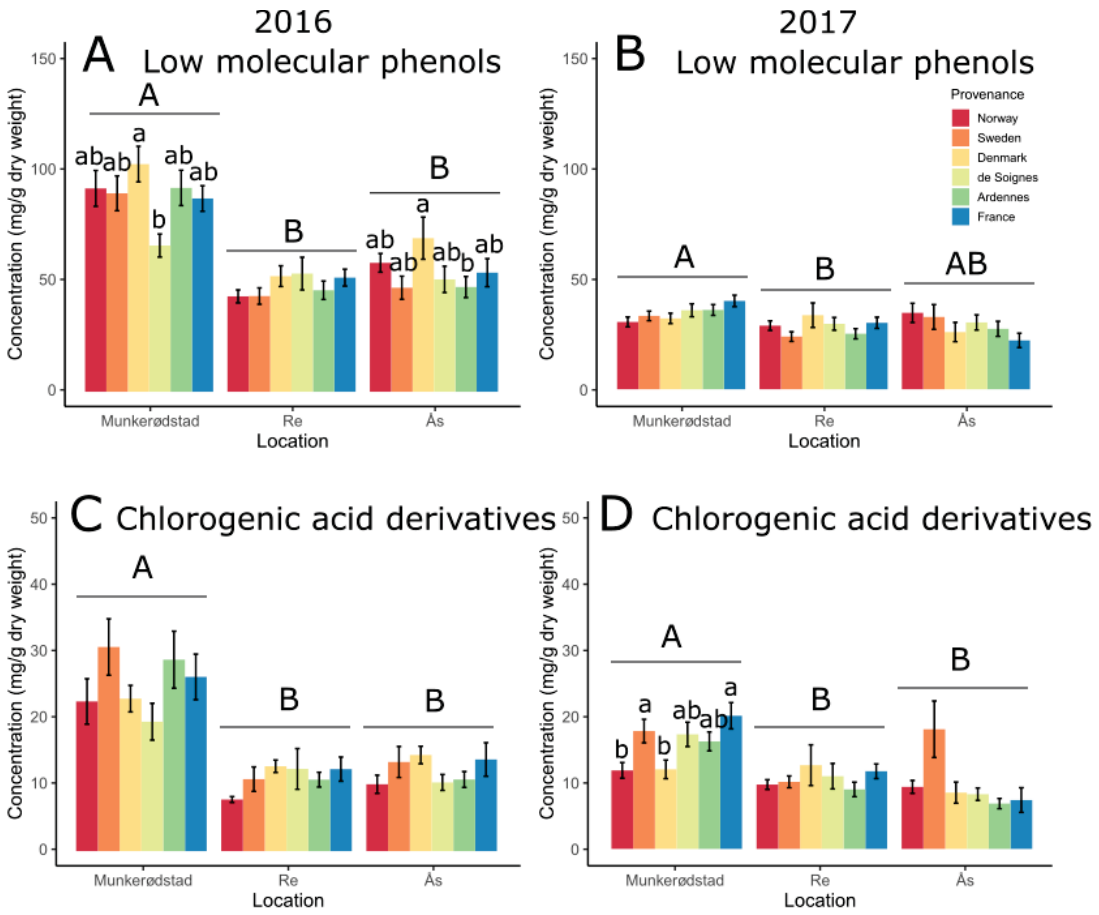

Figure 3. Variation in total low molecular weight phenols and the subgroup chlorogenic acid derivatives in beech seedling leaves between growing location, provenances and years. Colors designate provenance, ordered from north to south. Data from 2016 is shown on the left (A and C) and data from 2017 on the right (B and D). Error bars depict the standard error of the mean. Capital letters above the horizontal lines indicate statistical significance ($p<0.05$) between the growing location. Lowercase letters show statistical significance ($p<0.05$) between provenances within a growing location. If no provenances were statistically significant from the others, no letters are shown. Statistical significance was calculated using ANOVA on log-transformed linear mixed effects models in R.

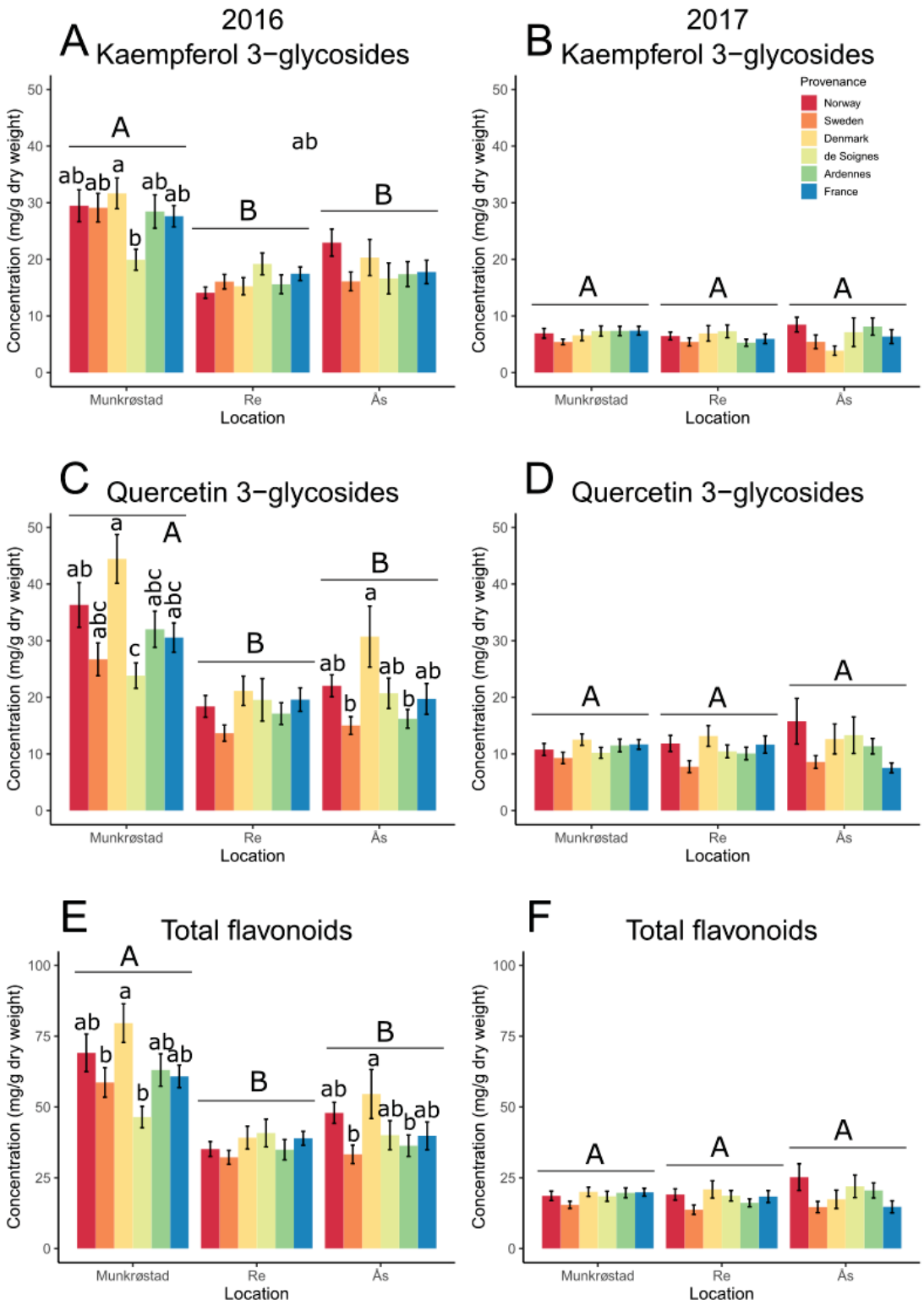

Figure 4. Variation in kaempferol 3-glycosides, quercetin 3-glycosides and total flavonoids (kaempferol 3-glycosides + quercetin 3-glycosides) in beech seedling leaves between growing location, provenances and years. Colors designate provenance, ordered from north to south. Data from 2016 is shown on the left (A, C and E) and data from 2017 on the right (B, D and F). Error bars depict the standard error of the mean. Capital letters above the horizontal lines indicate statistical significance ($p<0.05$) between the growing location. Lowercase letters show statistical significance ($p<0.05$) between provenances within a growing location. If no provenances were statistically significant, no letters are shown. Statistical significance was calculated using ANOVA on log-transformed linear mixed effects models in R.

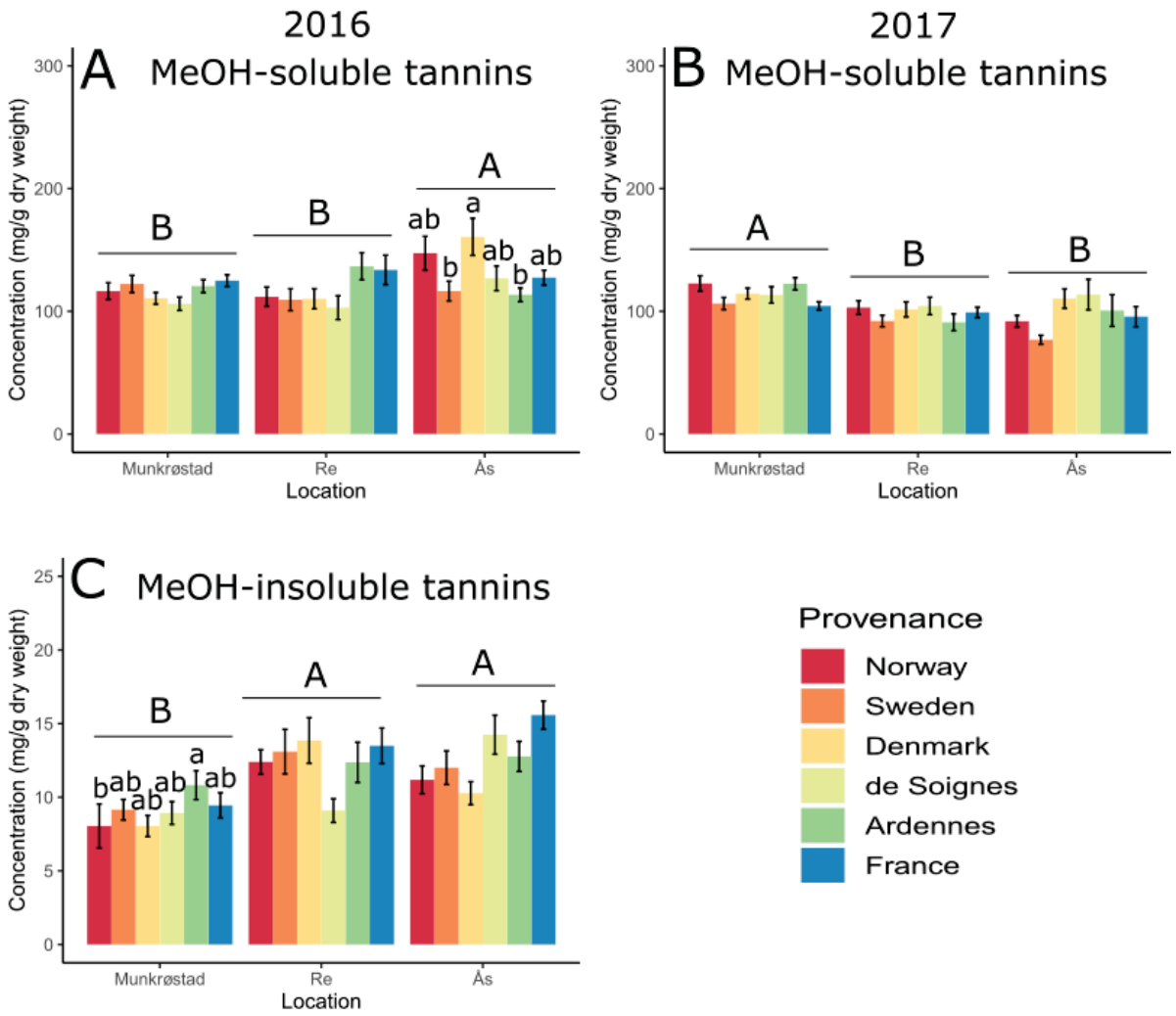

Figure 5. Variation in MeOH-soluble and MeOH-insoluble condensed tannins in beech seedling leaves between growing location, provenances and years. Note the difference in scale between MeOH-soluble and insoluble condensed tannins. Colors designate provenance, ordered from north to south. Data from 2016 is shown on the left (A and C) and data from 2017 on the right (B). Data on MeOH-insoluble condensed tannins in leaves collected in 2017 is missing. Error bars depict the standard error of the mean. Capital letters above the horizontal lines indicate statistical significance ($p<0.05$) between the growing location. Lowercase letters show statistical significance ($p<0.05$) between provenances within a growing location. If no provenances were statistically significant, no letters are shown. Statistical significance was calculated using ANOVA on log-transformed linear mixed effects models in R.

3.2 Effect of year

The mean concentration of total low molecular weight phenols was twice as high in 2016 (63.8 mg g^{-1}) compared to 2017 (30.6 mg g^{-1}, $p<0.001$). The absolute differences between growing locations were also smaller in 2017, whereas the relative differences showed no clear trend. The average concentration of MeOH-soluble condensed tannins across provenances and planting sites also decreased from 2016 to 2017, from 123 mg g^{-1} to 104 mg g^{-1} ($p<0.001$). The concentration of condensed tannins did not behave in the same way as the low molecular weight phenols. In 2016, Munkrøstad had the highest concentration of low molecular weight

phenols, whereas the highest amounts of MeOH-soluble condensed tannins were found in Ås (Figures 3 and 5). There was also no significant decrease in MeOH-soluble condensed tannins between the years in Munkrøstad ($p=0.360$), but the amount of MeOH-soluble condensed tannins in Ås decreased from 2016 to 2017 ($p<0.001$).

Year was the variable which explained the largest part of the variance in low molecular weight phenols, both in total and by group (Table 3) and was also a highly significant variable in the linear mixed models of almost all phenolic compounds (Table 4). Year explained almost 31% of variance in the concentration of low molecular weight phenols, but only 3% of the variance in MeOH-soluble condensed tannins (Table 3). There was also a large variation in the explanatory power of year between the groups of low molecular weight phenols. Whereas year explained 43% of the variation in kaempferol 3-glycosides, the corresponding number for chlorogenic acid derivatives was only 2.5% (Table 3).

Table 4. ANOVA output of the linear mixed effects models of variation in phenolic composition in beech seedling leaves between growing locations, provenances and years. F-values (p-values). Low molecular weight phenols, chlorogenic acid derivatives, quercetin 3-glycosides, kaempferol 3-glycosides and total flavonoids are composed of data from the individual phenolic compounds shown in the list below in entries 8-26 (below the line). Statistically significant results ($p<0.05$) are printed in bold. P:Y stands for the interaction between provenance and year. MeOH-insoluble condensed tannins and MeOH-soluble condensed tannins were measured using UV-spectrometry and the other compounds using HPLC.

	Substance	Provenance	Year	Location	P:Y	P:L	Y:L	P:Y:L
1	MeOH-insoluble condensed tannins	2.71(0.141)	N.A.	**23.69(0.002)**	N.A.	1.88(0.242)	N.A.	N.A.
2	MeOH-soluble condensed tannins	**2.49(0.030)**	**39.13(<0.001)**	**6.26(0.002)**	1.85(0.101)	**1.95(0.036)**	**10.23(<0.001)**	1.62(0.097)
3	Low molecular weight phenols	1.12(0.348)	**372.06(<0.001)**	**55.28(<0.001)**	1.53(0.178)	1.61(0.100)	**18.26(<0.001)**	1.52(0.129)
4	Chlorogenic acid derivatives	**2.64(0.023)**	**27.69(<0.001)**	**71.49(<0.001)**	2.19(0.054)	**1.65(<0.001)**	**9.92(<0.001)**	1.45(0.156)
5	Quercetin 3-glycosides	**6.99(<0.001)**	**237.72(<0.001)**	**13.23(<0.001)**	0.84(0.518)	1.05(0.401)	**20.29(<0.001)**	0.66(0.760)
6	Kaempferol 3-glycosides	1.35(0.242)	**626.81(<0.001)**	**14.79(<0.001)**	2.11(0.063)	1.39(0.181)	**12.69(<0.001)**	1.25(0.253)
7	Total flavonoids	**3.91(0.002)**	**498.07(<0.001)**	**17.76(<0.001)**	1.42(0.213)	1.35(0.199)	**19.84(<0.001)**	0.83(0.599)
8	Neochlorogenic acid	**4.47(<0.001)**	**17.31(<0.001)**	**9.79(<0.001)**	0.83(0.525)	0.69(0.738)	**15.34(<0.001)**	0.52(0.873)
9	Chlorogenic acid der. 1	1.37(0.233)	**430.07(<0.001)**	**3.02(0.049)**	**3.08(0.009)**	**2.41(0.008)**	**3.62(0.027)**	0.78(0.644)
10	Chlorogenic acid der. 2	0.19(0.968)	**342.54(<0.001)**	**6.28(0.002)**	**2.42(0.035)**	0.40(0.946)	**14.78(<0.001)**	0.77(0.655)
11	Chlorogenic acid der. 3	**3.62(0.003)**	0.05(0.826)	0.52(0.592)	**2.98(0.011)**	0.87(0.563)	**10.21(<0.001)**	0.99(0.446)
12	Chlorogenic acid	2.17(0.056)	**13.49(<0.001)**	**39.49(<0.001)**	1.06(0.379)	1.54(0.120)	0.66(0.520)	0.61(0.803)
13	Chlorogenic acid der. 4	**4.03(0.001)**	**133.93(<0.001)**	**24.82(<0.001)**	1.51(0.184)	1.39(0.179)	**32.06(<0.001)**	1.21(0.280)
14	Dihydroquercetin der. 1	**6.06(<0.001)**	**168.76(<0.001)**	0.25(0.777)	**2.47(0.031)**	0.56(0.845)	**3.77(0.024)**	0.47(0.907)
15	Quercetin 3-glycoside 1	**6.49(<0.001)**	**293.79(<0.001)**	**6.87(0.001)**	**4.40(<0.001)**	1.55(0.120)	**16.90(<0.001)**	1.47(0.147)
16	Quercetin 3-glycoside 2	**3.56(0.004)**	**182.21(<0.001)**	**5.82(0.003)**	1.10(0.360)	0.90(0.532)	**7.16(<0.001)**	0.96(0.476)
17	Quercetin 3-glycoside 3	**4.30(<0.001)**	**442.24(<0.001)**	**14.15(<0.001)**	1.87(0.097)	1.33(0.210)	**7.80(<0.001)**	1.39(0.182)
18	Chlorogenic acid der. 5	**4.10(0.001)**	**516.61(<0.001)**	**13.72(<0.001)**	**2.90(0.013)**	1.05(0.402)	**4.04(0.018)**	0.97(0.467)
19	Kaempferol 3-glycoside 1	**2.56(0.026)**	**224.50(<0.001)**	**7.04(<0.001)**	1.16(0.328)	0.60(0.812)	**28.45(<0.001)**	0.63(0.791)
20	Kaempferol 3-glycoside 2	1.98(0.080)	**474.44(<0.001)**	**26.51(<0.001)**	2.22(0.050)	1.51(0.132)	**3.50(0.031)**	1.54(0.121)
21	Chlorogenic acid der. 6	**7.24(<0.001)**	**35.35(<0.001)**	**18.10(<0.001)**	**3.39(0.005)**	1.62(0.096)	**6.62(0.001)**	1.78(0.060)
22	Kaempferol 3-glycoside 4	**3.47(0.004)**	**34.31(<0.001)**	**6.00(0.003)**	**7.72(<0.001)**	1.56(0.115)	**29.25(<0.001)**	1.50(0.136)
23	Kaempferol 3-glycoside 5	1.98(0.080)	**486.52(<0.001)**	**31.67(<0.001)**	**3.22(0.007)**	1.60(0.103)	**36.92(<0.001)**	0.93(0.501)
24	Monocoumaroylastragallin	**8.02(<0.001)**	**185.40(<0.001)**	**10.96(<0.001)**	1.64(0.146)	0.86(0.574)	**4.21(0.015)**	0.94(0.494)
25	Dicoumaroylastragallin 1	2.23(0.05)	**167.71(<0.001)**	0.62(0.540)	1.83(0.105)	1.05(0.397)	2.08(0.125)	1.22(0.272)
26	Dicoumaroylastragallin 2	**5.66(<0.001)**	**63.74(<0.001)**	1.72(0.180)	0.53(0.751)	0.32(0.977)	0.63(0.531)	0.63(0.788)

3.3 Phenolic footprint

Not a single combination of provenance and location contained all 19 identified compounds in all samples, and some compounds occurred significantly more frequently in some locations and provenances than others, as determined by generalized linear models on the present/absent table followed by ANOVA (Table 5). Most of the compounds identified, however, were present in almost all samples. Exceptions were chlorogenic acid derivative 1, 2 and 4, kaempferol 3-glycoside 3 and monocoumaroylastragallin, which were missing from many samples in the 2017 dataset. One example is chlorogenic acid derivative 1, which in 2017 occurred more often in detectable amounts in the samples grown in Re (63% of samples) than in Ås (43% of samples, p=0.041, as determined by estimated marginal means). Chlorogenic acid derivative 2 was also missing from many of the 2016 samples. In general, the 2016 dataset contained a lot fewer 0-entries.

Table 5. χ^2 values (p-values) from Anova-function in the R-package car, performed on the generalized linear models of presence/absence of individual compounds. A significant p-value indicates that the compound occurs significantly more frequent in one growing location or provenance of beech seedling leaves harvested in 2017. Statistically significant results ($p<0.05$) are printed in bold.

Substance (2017)	Location	Provenance	L:P
Neochlorogenic acid	**10.65 (0.005)**	**11.41 (0.044)**	1.38 (0.999)
Chlorogenic acid der. 1	**8.28 (0.016)**	**13.50 (0.019)**	15.96 (0.101)
Chlorogenic acid der. 2	**22.78 (<0.001)**	9.40 (0.094)	7.83 (0.646)
Chlorogenic acid der. 3	4.01 (0.135)	4.85 (0.435)	4.60 (0.916)
Chlorogenic acid	0.95 (0.623)	9.63 (0.087)	0.69 (1.000)
Chlorogenic acid der. 4	**44.56 (<0.001)**	11.01 (0.051)	17.52 (0.064)
Dihydroquercetin der. 1	**6.43 (0.040)**	**14.11 (0.015)**	8.40 (0.590)
Quercetin 3-glycoside 1	2.04 (0.361)	**27.08 (<0.001)**	7.17 (0.709)
Quercetin 3-glycoside 2	3.90 (0.143)	7.62 (0.179)	10.52 (0.397)
Quercetin 3-glycoside 3	**27.05 (<0.001)**	7.94 (0.160)	7.91 (0.638)
Chlorogenic acid der. 5	4.30 (0.117)	3.10 (0.684)	**19.12 (0.039)**
Kaempferol 3-glycoside 1	2.58 (0.275)	3.91 (0.563)	3.78 (0.957)
Kaempferol 3-glycoside 2	3.09 (0.214)	3.87 (0.568)	0.00 (1.000)
Chlorogenic acid der. 6	0.83 (0.661)	10.93 (0.053)	12.74 (0.238)
Kaempferol 3-glycoside 4	4.17 (0.124)	**15.51 (0.008)**	12.00 (0.285)
Kaempferol 3-glycoside 5	**58.83 (<0.001)**	7.44 (0.190)	15.20 (0.125)
Monocoumaroylastragallin	**8.27 (0.016)**	10.03 (0.074)	15.03 (0.131)
Dicoumaroylastragallin 1	1.10 (0.578)	2.31 (0.805)	**18.56 (0.046)**
Dicoumaroylastragallin 2	1.15 (0.563)	**12.10 (0.033)**	5.20 (0.878)

4 Discussion

The main aim of this thesis was to explore and quantify the factors influencing the concentration of phenolic defense chemicals in beech seedlings, which were planted in three common gardens in Norway. Leaves were sampled from two consecutive years after planting. Year was the factor which explained most variation in total low molecular weight phenol concentration. Six provenances were tested, representing a large variety of site condition origins, but I found mostly only small differences between provenances. The highest phenol concentrations were found in the northernmost growing location, and growing location explained more of the variation in foliar phenols than provenance in this experiment. My finding that growing location (environment) affected the concentration of foliar phenols more than the provenance (genetic background) contrasts with previous findings in beech.

4.1 Variation between growing locations

Growing location explained 5% of the variation in low molecular weight phenols as a group (Table 3), and there was a significant difference in concentration between locations for most individual compounds (Table 4). After one growing season in the field, the highest concentration of all groups of low molecular weight phenols were found in Munkrøstad, which is the northernmost growing location. In 2017, the concentration of chlorogenic acid derivatives was significantly higher in Munkrøstad, whereas there was no significant difference between growing location in concentration of total flavonoids. Munkrøstad has lower mean annual temperature than Ås and Re, whereas the annual precipitation is lower than in Re while higher than in Ås (Table 2). The light conditions are similar, as all growing locations are open and west- or east-facing. All common gardens were established on former agricultural land. Silty loam, which is the soil type in Re and Ås, is generally more fertile than the gravelly sand in Munkrøstad and also more drought resistant (Ingels, 2015). Competition from vegetation was minimized through mowing in all locations. Animals lethally browsed some plants, but since these plants were removed from the data material, I do not expect browsing to have affected the results. Bolte et al. (2007) defined climate restraints for beech, and all three locations got much more precipitation than the minimum requirements, whereas none of the locations meet Bolte et al.'s minimum requirement of 217 days per year with a mean temperature above 7°C, neither in 2016 nor in 2016. Re was closest, with 172 days in 2017, whereas Munkrøstad had 161 days above 7°C (NIBIO; 2020). Therefore, I assume that temperature is the most important physical difference between the growing sites, but the sites also differ in soil type.

A number of previous studies have concluded that foliar phenol concentration depends on environmental variables. Pahlsson (1989) reported that the concentration of foliar phenols increased in beeches deficient in several mineral nutrients. Covelo and Gallardo (2001) found higher levels of foliar phenols in oak seedlings on clearings than under a pine canopy. Bussotti et al. (1998) also found more foliar phenols, mainly tannins, at high altitudes in mature beeches. As these were natural beech populations, the populations differed not only in growing site, but also in genetic background. Bussotti et al. (1998) also attributed the differences mostly to higher water stress and poor nutrient status in the mountain ridge population. By contrast, Zimmer et al. (2015) found that the total concentration of foliar phenols depended more on provenance than on environment, but hypothesized that the difference in concentration of individual compounds was mainly due to the temperature difference between the growing sites. The low predicative power of growing location (5%) in this experiment contrasts the findings of Covelo and Gallardo (2001), who found that growing site explained 76% of foliage phenol variation in oak seedlings. However, in their study, different growing site also meant a difference in light availability and genetic background.

Another important environmental factor is temperature. To my knowledge, no study has so far studied the effect of temperature on foliar phenols in beech, but there are a few studies on other tree species, which are reviewed in Julkunen-Tiitto et al. (2015). In a study on dark-leaved willow (*Salix myrsinifolia*), Nybakken et al. (2012) found a decrease in foliar phenols, including tannins, with increasing temperature. The decrease of phenols at higher temperatures is likely caused by temperature-dependent regulation of genes involved in the phenol biosynthesis, as well as faster degradation of kaempferol- and quercetin glycosides (Escobar-Bravo et al., 2017; Olsen et al., 2009). Also in spruce seedling needles, elevated temperature decreased the concentration of low molecular weight phenols, but not condensed tannins (Virjamo et al., 2014). These results agree with my finding that the growing site with the lowest temperature has the highest concentration of low molecular weight phenols. It is however in contrast to my finding that the phenol concentration was lower in 2017, which was a colder year than 2016 in Re and Ås. This indicates that temperature differences are not the main driver behind the observed variation in phenol concentration in this experiment.

4.2 Variation between provenances

I found significant differences in the total concentration of low molecular weight phenols in 2016 between some provenances (Figure 3), but nonetheless, provenance explained less than 1% of the variation (Table 3). The Danish provenance often had high phenol concentration,

which could be due to the insect attack the Danish seedlings suffered in 2015. Beech has high intraspecific genetic variability, and genetic differences between provenances exist (Konnert & Ruetz, 2001), which indicates that beech has developed local adaptation (Robson et al., 2012). The genetic variation within provenances is however also considerable (Konnert, 1995; Konnert & Ruetz, 2001). The provenances in this experiment originate from a range of climates, which may require different levels and composition of constitutive chemical defense. Beech also exhibits phenotypic plasticity (Bolte et al., 2007; Gárate-Escamilla et al., 2019), which decreases phenotypic differences between provenances when grown in a common garden. Common gardens are used to quantify the genetic basis of traits, as the effect of different environments is minimized (de Villemereuil et al., 2016). Hence, common gardens are valuable to quantify genetic differences between provenances.

Some tree species have shown significant differences in chemical defense between provenances in common gardens (e.g. Wallis et al., 2010 (lodgepole pine) and Enderle et al., 2013 (ash)). To my knowledge, only Zimmer et al. (2015) and Aranda et al. (2017) have quantified intraspecific variation in phenolic defense in beech in a common garden setup. Zimmer et al. (2015) found that provenance explained 25% of the total phenolic content in beech, which contrasts strongly with my results. There were, however, some noteworthy differences in the methods used. Zimmer et al. (2015) sampled senescent leaves in November, two months later than in this experiment. They measured the total phenolic content using a Folin-Ciocalteu assay, whereas I used HPLC and acid-butanol assay (for condensed tannins). Using Folin-Ciocalteu assay for determining total phenols is somehow controversial, as it also sensitive to other plant compounds, which gives a rough estimate of the total phenolic content (Everette et al., 2010). Aranda et al. (2017) also found a significant effect of provenance on foliar phenols in beech, which was larger than the effect of water stress. On the other hand, Baldwin et al. (1987) found no significant provenance effect on the total phenolic content in yellow birch (*Betula allegheniensis*) and sugar maple (*Acer saccharum*), and only one provenance showed significantly higher levels of condensed tannins. In a study on silver birch (*Betula pendula*), Deepak et al. (2018) found that 1-36% of variation in foliar phenols was explained by provenance (depending on compound). My results for the phenolic groups are in the lower part of this range (Table 3). In summary, I found little explanatory effect of provenance compared to previous studies in beech.

4.3 Variation between years

Between the first and the second year in the field, the concentration of all groups of phenols decreased, except MeOH-soluble condensed tannins. Year explained almost 31% of variance in the concentration of low molecular weight phenols, but only 3% of the variance in MeOH-soluble condensed tannins (Table 3). The average concentration of low molecular weight phenols was reduced by more than 50% between the first and second year in the field, and in Munkrøstad, the decrease was even larger. Differences in weather and aging of the seedlings can both cause variation in phenols between years, but in this experiment, the stress due to planting and establishment also contributes. Plants in Munkrøstad, which were planted half a year (spring 2016) later than the plants in Ås and Re (fall 2015), had the highest levels of phenols in the fall of 2016. One year after, the plants had had more time to acclimate to the growing location, and differences between growing locations were much smaller.

The variation between years seems to be unrelated to temperature differences. Although the mean temperature in Munkrøstad during the growing period stayed the same in 2016 and 2017, the seedlings growing in Munkrøstad had the largest decrease in low molecular weight phenols. Furthermore, previous studies on phenols and temperature found less phenols at higher temperatures (Nybakken et al., 2012; Zvereva & Kozlov, 2006), whereas my results showed less phenols at lower mean temperatures. In a study on young oaks, a significant variation in foliar phenolic was observed between the study years, and year explained 22% of the variation in total foliar phenols (Covelo & Gallardo, 2001), which is less than in this experiment.

To my knowledge, there are no studies on the effect of stress after planting on phenol concentration in tree seedlings, but studies on planting stress in general exist (reviewed by Grossnickle, 2005). Freshly planted seedlings have less roots, the roots are more restricted, and the root-soil contact is limited, which may result in planting stress because of poor nutrient uptake and restricted water availability (Burdett, 1990). This problem is further accentuated in coarse soils (Örlander & Due, 1986), such as the gravelly sandy soil in Munkrøstad. In colder climates, the planting stress is increased by slow root growth at low temperatures (Grossnickle, 2005; Nagelmüller et al., 2017). Newly planted seedlings have higher water stress than established seedlings under the same conditions (Grossnickle & Reid, 1984), which might explain the higher concentration of concentration of seedlings in Munkrøstad in 2016, as well as the large decrease in phenols in these plants between 2016 and 2017. Even for established seedlings, there might be a carry-over effect of the planting stress

for instance due to nutrient limitations (Grossnickle, 2005), which may cause a higher stress level over time in seedlings after planting. In conclusion, the planting stress was likely higher in Munkrøstad because of less favorable soil conditions and later planting, which contributed to the high phenol level in Munkrøstad in the first year.

The difference between 2016 and 2017 may also partially be due to increasing age of the seedlings. Nissinen et al. (2018) reported no change in foliar phenol concentration in *Salix myrsinifolia* individuals over a seven-year period. Wam et al. (2017) observed a decrease in foliar low molecular weight phenols with age in young birches, but the relative magnitude of the yearly change was much lower than the substantial decrease from one year to the next which was observed in this experiment. These findings imply that age is not driving the observed change in phenols in this experiment. It is important to note, however, that willow and birch species are pioneers, which may have a different phenolic development over time than late successional species like beech. As discussed, the decrease in foliar phenols between the consecutive years in the field is likely caused by a combination of the factors discussed above, but mainly reduced planting stress.

4.4 Phenolic footprint

Growing locations and provenances displayed significant differences between the frequencies of certain, but not all, individual low molecular weight phenolic compounds (Table 4). Location was a significant factor for eight out of 19 compounds, whereas provenance was significant for six of them (Table 5). The p-values were, on average, also lower for location than for provenance. The results indicate that environment is more important than genetics in explaining the foliar low molecular phenolic footprint in beech seedlings, which is also Zimmer et al. (2015)'s conclusion. Another noteworthy difference was that the 2016 dataset contained a lot fewer 0-entries than the 2017 dataset. It is uncertain whether this represents a real difference in phenolic composition between the years, or because the 2016 dataset was run on another HPLC-machine using a different acquisition software. It seems like the software used to analyze the 2017 dataset had a higher detection limit, which could explain the larger amount of 0-entries in the 2017 dataset. If the observed difference represents real variation between the years, the reason could be that the seedlings needed less complex phenolic defense due to reduced stress levels, as discussed above.

4.5 Implications

My results indicate that total low molecular phenol concentration in beech seedlings depends more on the environment than on the seedlings' genetic background, which has implications for how beech reacts to climate change. Traits which responds to changes in the environment without a change in genotype, exhibit phenotypic plasticity, whereas local adaptation produces new genotypes which are better adapted to the site conditions through natural selection (Gárate-Escamilla et al., 2019). Phenotypic plasticity results in acclimation, which is a more rapid adjustment to environmental change than genetic adaptation, especially in organisms with long generation times such as beech. Global warming will cause substantial changes in environmental factors in European forests such as higher mean temperatures, more uneven precipitation patterns, higher CO_2-concentration in the air and more frequent pathogen infections (IPCC, 2014; La Porta et al., 2008). Beech at the edge of its range is more sensitive to climatic conditions than core populations, and it has become more sensitive over the last decades (Farahat & Linderholm, 2018). It remains to be seen if the acclimation can keep up with the speed at which the climate changes. Saltré et al. (2015) did not think it will and predicted a decrease in the distribution area of European beech by 36-61% by 2100. The future climate, with more frequent weather extremes might cause a threat to beech populations at the northernmost distribution limit in Norway.

Even though increasing temperatures decrease the phenol concentration in beech, it is not necessarily true that global change will result in poorer chemical defense and increased susceptibility to pathogens. A meta-study by Zvereva and Kozlov (2006) concluded that the effect of elevated temperature and CO_2 on phenols cancel each other out. The general pattern was that, when studied alone, elevated CO_2 increased the phenol levels in plants, whereas elevated temperature caused a decrease in phenols (Zvereva & Kozlov, 2006 and references therein). No studies on beech were included in their meta-analysis, but the tested deciduous tree species obtained similar results, which implies small interspecific differences (Zvereva & Kozlov, 2006). Whereas the atmospheric CO_2-concentration increases steadily, the annual temperature varies between years. Higher temperatures in one year will already have an effect on the growth and phenolic concentration of plants, but the effect possibly decreases over time (Nybakken et al., 2012). In conclusion, it is difficult to predict how the phenol concentrations in beech will change in the future.

The processes influencing the northwards spread of beech are complex, and phenol concentration is only one component. According to simulations by Saltré et al. (2015), coastal areas as far north as Troms and Finnmark county in northern Norway will become suitable habitat for beech by the end of this century. However, Saltré et al. (2015) predicted that new potential habitats outside of Vestfold will remain uncolonized in 2100, mainly due to limited dispersal ability. It is also not given that higher concentrations of phenols and other defense chemicals will result in higher fitness and survival. Chemical defense is costly, and as plants have limited resources, allocation of resources to chemical defense means less resources to growth and reproduction (Fernandez et al., 2016; Karasov et al., 2017). Most likely, other factors such as phenology and dispersal rate will be more limiting for the future distribution range of European beech than phenolic defense. In her master thesis, using the same plant material, Hagalid (2017) found that provenance explained more variation in phenology than environment, and that the Norwegian provenance performed better than the foreign provenances in terms of growth. Given the small and mostly non-significant differences between provenances in this experiment, my results do not point towards some provenances being better suited than others in terms of chemical defense. If we want to promote the spread of beech in Norway, it might therefore be best to allow natural regeneration of local provenances and plant Norwegian provenances in new, suitable habitats.

5 Conclusions

This thesis adds to our understanding of how genes and environment affect the chemical defense in beech seedlings. My findings indicate that the concentration of phenolic compounds in beech leaves depends more on environment than on genetic background of the seedlings. Hence, changes in the environmental conditions such as changed precipitation patterns and higher temperatures will cause changes in the concentration and composition of foliar phenols in beech. This thesis does not quantify the direction nor the magnitude of the change in phenols, as the experiment only went on for two years. This is also a highly complex process, which is influenced by a multitude of factors, which in part counteract each other. Furthermore, I observed a large decrease in phenols between the first and the second year in the field, which may be attributed to decreased stress from planting and establishment on the site. This is an indication of acclimation to changes in the environment and hence phenotypic plasticity. There was also a difference in the phenolic footprint between planting locations, provenances and years, and it seems like the environment influences the phenolic footprint more than the genetic background.

Our knowledge of how the phenolic composition and concentration of phenolic defense compounds in beech relate to genetic background and environment is still incomplete, particularly as existing results are contradictory. Several common garden provenance trials exist for beech, but few of these experiments include studies of chemical defense. Sampling leaves to quantify defense chemicals from existing common gardens will minimize stress due to planting and establishment, and cut costs compared to establishing new common garden experiments. Experiments where seedlings are subjected to controlled temperatures and precipitation are also needed to untangle the effects of different climatic aspects on chemical defense in beech. We have yet to see the full extent and speed of global change, and the knowledge of how beech reacts to rapid environmental change is still insufficient for making reliable forecasts about the future fitness of the species.

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7 Appendix

Table 6. Concentration of phenolic compounds in mg g^{-1} dry weight (S.E.) in beech seedling leaves which were harvested in 2016 from plants grown in Munkrøstad by provenance.

Munkrøstad	2016					
Substance	Norway	Sweden	Denmark	De Soignes	Ardennes	France
MeOH-insoluble condensed tannins	8.05±1.56	9.15±0.7	8.05±0.74	8.93±0.78	10.82±1.0	9.45±0.89
MeOH-soluble condensed tannins	116.48±6.8	122.31±6.96	110.59±4.77	126.13±5.41	120.53±5.25	124.98±4.72
Low molecular weight phenols	92.07±8.11	89.84±7.89	103.07±8.07	66.2±5.22	92.3±8	87.51±5.77
Chlorogenic acid der.	22.59±3.43	30.8±4.26	23.01±2.01	19.53±2.78	28.89±4.3	26.3±3.44
Quercetin 3-glycosides	36.32±3.95	26.7±2.89	44.44±4.3	23.83±2.24	32.02±3.19	30.54±2.59
Kaempferol 3-glycosides	29.46±2.82	29.11±2.52	31.65±2.71	19.93±1.85	28.44±2.94	27.62±1.88
Flavonoids	69.11±6.64	58.67±5.21	79.61±6.81	46.43±3.77	63.06±5.72	60.83±3.97
Neochlorogenic acid	0.9±0.24	2.69±0.55	1.49±0.51	1.36±0.39	2.43±0.67	1.73±0.54
Chlorogenic acid der. 1	0.65±0.09	0.8±0.12	0.99±0.15	0.74±0.16	0.92±0.1	0.8±0.16
Chlorogenic acid der. 2	0.28±0.07	0.48±0.11	0.41±0.09	0.32±0.11	0.33±0.08	0.3±0.09
Chlorogenic acid der.3	2.37±0.25	3.42±0.35	3.64±0.24	2.96±0.32	3.1±0.2	3.44±0.25
Chlorogenic acid	11.14±2.95	17.48±3.43	9.86±1.61	9.72±2.23	16.08±3.33	14.24±2.98
Chlorogenic acid der. 4	0.3±0.05	0.51±0.1	0.54±0.05	0.4±0.07	0.45±0.1	0.46±0.07
Dihydroquercetin der. 1	0.71±0.11	1.13±0.19	0.89±0.09	0.81±0.1	0.98±0.17	1.02±0.17
Quercetin 3-glycoside 1	2.78±0.36	2.97±0.36	3.74±0.59	2.1±0.22	2.75±0.39	3.04±0.35
Quercetin 3-glycoside 2	16.69±2.2	12.15±1.41	21.37±2.41	10.23±1.02	16.33±1.7	14.96±1.47
Quercetin 3-glycoside 3	16.61±1.82	10.8±1.31	18.67±1.87	11.34±1.23	12.57±1.37	12.13±1.13
Chlorogenic acid der. 5	2.04±0.26	1.33±0.19	2.32±0.24	1.26±0.16	1.74±0.23	1.4±0.21
Kaempferol 3-glycoside 1	6.38±0.71	5.38±0.52	6.31±0.63	3.75±0.36	6.05±0.59	5.44±0.48
Kaempferol 3-glycoside 2	20.21±2.16	20.18±1.97	21.5±2	13.97±1.51	19.11±2.37	19.45±1.48
Chlorogenic acid der. 6	4.91±0.55	4.09±0.35	3.76±0.38	2.77±0.31	3.84±0.51	3.93±0.31
Kaempferol 3-glycoside 4	0.76±0.08	0.92±0.12	1.13±0.16	0.52±0.06	1.15±0.21	0.7±0.08
Kaempferol 3-glycoside 5	1.06±0.15	1.66±0.38	1.45±0.29	1.03±0.15	1.18±0.2	1.25±0.21
Monocoumaroylastragallin	0.59±0.09	0.28±0.06	0.53±0.07	0.29±0.04	0.33±0.06	0.27±0.06
Dicoumaroylastragallin 1	0.9±0.12	0.49±0.08	0.8±0.09	0.6±0.07	0.49±0.06	0.56±0.07
Dicoumaroylastragallin 2	0.63±0.07	0.27±0.05	0.43±0.05	0.38±0.04	0.32±0.04	0.32±0.04

Table 7. Concentration of phenolic compounds in mg g^{-1} dry weight (S.E.) in beech seedling leaves which were harvested in 2017 from plants grown in Munkrøstad by provenance.

Munkrøstad	2017					
Substance	Norway	Sweden	Denmark	De Soignes	Ardennes	France
MeOH-soluble condensed tannins	122.67±6.27	106.34±4.94	114.44±4.48	113.38±6.72	122.43±5	104.4±3.36
Low molecular weight phenols	30.47±2.25	33.21±2.19	32.07±2.34	35.74±2.93	35.89±2.44	40±2.61
Chlorogenic acid der.	11.85±1.18	17.79±1.77	12.01±1.41	17.29±1.84	16.22±1.43	20.1±1.98
Quercetin 3-glycosides	10.79±1.07	9.29±1.01	12.53±1.02	10.19±0.96	11.49±1.12	11.69±0.86
Kaempferol 3-glycosides	6.94±0.86	5.41±0.49	6.58±0.93	7.33±0.9	7.35±0.82	7.39±0.78
Flavonoids	18.62±1.68	15.42±1.26	20.06±1.64	18.45±1.79	19.68±1.75	19.91±1.4
Neochlorogenic acid	0.75±0.13	1.19±0.13	0.79±0.16	1.17±0.21	1.12±0.21	0.94±0.12
Chlorogenic acid der. 1	0.19±0.04	0.22±0.06	0.18±0.03	0.14±0.04	0.61±0.27	0.2±0.05
Chlorogenic acid der. 2	0.15±0.05	0.15±0.04	0.11±0.05	0.44±0.13	0.18±0.08	0.15±0.06
Chlorogenic acid der.3	1.89±0.19	2.82±0.22	1.99±0.16	2.89±0.26	2.31±0.2	3.39±0.21
Chlorogenic acid	5.34±1.04	10.33±1.54	6.05±1.19	10.03±1.57	8.72±1.25	11.91±1.72
Chlorogenic acid der. 4	0.07±0.02	0.09±0.02	0.14±0.02	0.04±0.01	0.22±0.12	0.09±0.02
Dihydroquercetin der. 1	0.22±0.03	0.23±0.02	0.39±0.05	0.35±0.05	0.34±0.05	0.29±0.05
Quercetin 3-glycoside 1	0.55±0.09	0.55±0.1	0.44±0.07	0.67±0.09	0.57±0.08	0.81±0.11
Quercetin 3-glycoside 2	3.51±0.59	3.14±0.67	4.55±0.65	4.15±0.81	4.49±0.72	4.38±0.62
Quercetin 3-glycoside 3	2.79±0.3	1.66±0.25	2.09±0.27	2.21±0.31	1.77±0.28	1.7±0.26
Chlorogenic acid der. 5	0.25±0.04	0.22±0.03	0.34±0.05	0.34±0.07	0.28±0.05	0.26±0.04
Kaempferol 3-glycoside 1	1.15±0.14	1.01±0.12	1.09±0.11	1.15±0.17	1.38±0.14	1.4±0.14
Kaempferol 3-glycoside 2	5.34±0.71	3.9±0.41	5.14±0.84	5.7±0.74	5.46±0.67	5.49±0.64
Chlorogenic acid der. 6	3.21±0.35	2.77±0.33	2.4±0.29	2.23±0.31	2.78±0.33	3.17±0.38
Kaempferol 3-glycoside 4	0.38±0.05	0.43±0.05	0.23±0.03	0.42±0.07	0.44±0.05	0.42±0.05
Kaempferol 3-glycoside 5	0.08±0.01	0.07±0.01	0.11±0.02	0.06±0.02	0.07±0.01	0.09±0.02
Monocoumaroylastragallin	0.12±0.02	0.07±0.02	0.12±0.02	0.11±0.02	0.09±0.02	0.1±0.02
Dicoumaroylastragallin 1	0.29±0.03	0.25±0.02	0.25±0.04	0.29±0.04	0.24±0.04	0.26±0.03
Dicoumaroylastragallin 2	0.26±0.03	0.17±0.02	0.2±0.02	0.18±0.03	0.17±0.02	0.18±0.02

Table 8. Concentration of phenolic compounds in mg g^{-1} dry weight (S.E.) in beech seedling leaves which were harvested in 2016 from plants grown in Re, by provenance.

Re	2016					
Substance	Norway	Sweden	Denmark	De Soignes	Ardennes	France
MeOH-insoluble condensed tannins	12.4±0.85	13.1±1.55	13.85±1.68	9.09±0.89	12.37±1.4	13.49±1.26
MeOH-soluble condensed tannins	111.95±7.88	109.45±8.94	110.18±8.18	102.97±9.71	136.71±11.01	133.72±12
Low molecular weight phenols	43.2±2.94	43.32±3.71	52.34±4.7	53.5±7.4	45.95±4.19	51.68±3.83
Chlorogenic acid der.	7.79±0.44	10.86±1.85	12.81±0.94	12.4±3.08	10.78±1.12	12.38±1.82
Quercetin 3-glycosides	18.41±1.92	13.68±1.43	21.15±2.58	19.55±3.76	17.12±1.92	19.6±2.07
Kaempferol 3-glycosides	14.11±0.99	16.06±1.28	15.25±1.52	19.19±1.92	15.6±1.67	17.46±1.2
Flavonoids	35.16±2.62	32.21±2.44	39.18±4.04	40.79±4.89	34.91±3.59	38.94±2.46
Neochlorogenic acid	0.27±0.05	0.53±0.13	0.61±0.14	0.3±0.12	0.59±0.22	0.45±0.17
Chlorogenic acid der. 1	0.33±0.05	0.71±0.14	0.69±0.09	0.77±0.26	0.69±0.1	0.79±0.13
Chlorogenic acid der. 2	0.33±0.06	0.3±0.04	0.33±0.05	0.19±0.04	0.38±0.06	0.48±0.08
Chlorogenic acid der.3	1.38±0.13	2.35±0.26	2.64±0.15	2.41±0.36	2.27±0.21	2.01±0.16
Chlorogenic acid	2.15±0.23	3.82±1.31	4.92±0.68	5.52±2.42	3.94±0.74	5.45±1.3
Chlorogenic acid der. 4	0.25±0.02	0.26±0.03	0.37±0.02	0.26±0.04	0.27±0.03	0.33±0.03
Dihydroquercetin der. 1	0.67±0.07	0.73±0.08	0.82±0.08	0.68±0.08	0.76±0.06	0.56±0.11
Quercetin 3-glycoside 1	1.01±0.13	1.09±0.19	1.37±0.2	1.79±0.3	1.02±0.18	2.18±0.28
Quercetin 3-glycoside 2	7.6±0.97	5.71±0.69	9.4±1.56	8.29±1.78	7.66±0.97	8.52±1.15
Quercetin 3-glycoside 3	9.59±0.95	6.11±0.81	9.79±1.13	8.1±1.41	7.83±0.9	8.33±0.97
Chlorogenic acid der. 5	1.1±0.08	0.75±0.08	1.18±0.13	1.01±0.26	0.95±0.15	1.01±0.16
Kaempferol 3-glycoside 1	3.03±0.28	3.13±0.31	3.16±0.37	3.06±0.57	2.92±0.39	3.33±0.35
Kaempferol 3-glycoside 2	9.15±0.79	10.37±0.96	10.01±1.15	12.71±1.61	10.5±1.32	11±0.94
Chlorogenic acid der. 6	1.98±0.11	2.14±0.26	2.06±0.19	1.95±0.29	1.69±0.2	1.85±0.19
Kaempferol 3-glycoside 4	0.51±0.08	0.44±0.06	0.79±0.16	0.52±0.1	0.47±0.05	0.6±0.04
Kaempferol 3-glycoside 5	0.55±0.07	1.07±0.18	0.78±0.11	1.14±0.25	0.8±0.1	1.37±0.18
Monocoumaroylastragallin	0.42±0.03	0.18±0.02	0.38±0.06	0.22±0.03	0.23±0.03	0.25±0.04
Dicoumaroylastragallin 1	0.62±0.05	0.67±0.17	0.63±0.07	0.48±0.03	0.44±0.07	0.57±0.06
Dicoumaroylastragallin 2	0.37±0.03	0.21±0.02	0.3±0.02	0.24±0.02	0.24±0.03	0.24±0.02

Table 9. Concentration of phenolic compounds in mg g^{-1} dry weight (S.E.) in beech seedling leaves which were harvested in 2017 from plants grown in Re, by provenance

Re	2017					
Substance	Norway	Sweden	Denmark	De Soignes	Ardennes	France
MeOH-soluble condensed tannins	103.06±5.61	92.08±4.71	101.56±6.13	104.31±7.09	91.01±6.82	99.1±4.29
Low molecular weight phenols	28.8±2.16	23.84±2.2	33.52±5.55	29.61±2.91	25.12±2.31	30.09±2.54
Chlorogenic acid der.	9.71±0.73	10.12±0.89	12.64±3.07	10.97±1.92	8.98±1.1	11.72±1.11
Quercetin 3-glycosides	11.85±1.42	7.75±1.06	13.17±1.83	10.45±1.14	10.08±1.11	11.66±1.52
Kaempferol 3-glycosides	6.46±0.69	5.42±0.71	6.92±1.36	7.28±1.13	5.27±0.63	5.96±0.84
Flavonoids	19.09±1.97	13.73±1.66	20.88±3.06	18.64±1.87	16.15±1.42	18.37±2.1
Neochlorogenic acid	0.68±0.06	1.08±0.25	0.77±0.18	0.79±0.15	0.66±0.09	0.85±0.04
Chlorogenic acid der. 1	0.15±0.05	0.05±0.02	0.13±0.04	0.17±0.07	0.16±0.05	0.24±0.07
Chlorogenic acid der. 2	0.01±0.01	0.01±0.01	0.03±0.02	0.07±0.05	0.02±0.02	0.02±0.02
Chlorogenic acid der.3	3.1±0.22	4.24±0.35	3.13±0.49	2.95±0.37	2.87±0.37	3.44±0.34
Chlorogenic acid	1.96±0.47	1.93±0.29	6.08±2.6	4.73±1.51	2.97±0.65	4.41±1
Chlorogenic acid der. 4	0.23±0.04	0.26±0.04	0.25±0.07	0.2±0.04	0.28±0.05	0.3±0.05
Dihydroquercetin der. 1	0.23±0.05	0.22±0.04	0.31±0.06	0.47±0.09	0.41±0.04	0.31±0.05
Quercetin 3-glycoside 1	0.52±0.14	0.41±0.08	0.53±0.13	0.96±0.19	0.44±0.1	1.16±0.26
Quercetin 3-glycoside 2	3.54±0.84	2.26±0.51	5.33±1.4	3.32±0.62	3.09±0.43	3.91±1.06
Quercetin 3-glycoside 3	2.6±0.53	1.61±0.31	2.3±0.62	1.88±0.47	1.66±0.41	2.61±0.85
Chlorogenic acid der. 5	0.14±0.05	0.13±0.03	0.24±0.04	0.23±0.04	0.19±0.02	0.17±0.04
Kaempferol 3-glycoside 1	1.53±0.19	1.28±0.18	1.53±0.2	1.66±0.22	1.29±0.17	1.49±0.17
Kaempferol 3-glycoside 2	4.01±0.48	3.02±0.46	4.62±1.14	4.36±0.9	3.02±0.41	3.15±0.53
Chlorogenic acid der. 6	3.45±0.54	2.41±0.44	2.01±0.39	1.84±0.34	1.84±0.34	2.3±0.42
Kaempferol 3-glycoside 4	0.72±0.13	0.93±0.16	0.57±0.09	1.04±0.23	0.71±0.13	1.03±0.23
Kaempferol 3-glycoside 5	0.21±0.03	0.19±0.04	0.19±0.05	0.22±0.04	0.25±0.04	0.29±0.04
Monocoumaroylastragallin	0.07±0.03	0.04±0.02	0.09±0.03	0.09±0.02	0.07±0.02	0.06±0.02
Dicoumaroylastragallin 1	0.21±0.03	0.14±0.03	0.22±0.04	0.19±0.05	0.17±0.03	0.22±0.04
Dicoumaroylastragallin 2	0.26±0.04	0.15±0.04	0.18±0.02	0.17±0.04	0.15±0.03	0.15±0.03

Table 10. Concentration of phenolic compounds in mg g^{-1} dry weight (S.E.) in beech seedling leaves which were harvested in 2016 from plants grown in Ås, by provenance.

Ås	2016					
Substance	Norway	Sweden	Denmark	De Soignes	Ardennes	France
MeOH-insoluble condensed tannins	11.18±0.98	12±1.17	10.28±0.77	14.25±1.4	12.77±1.02	17.82±0.96
MeOH-soluble condensed tannins	147.33±13.77	116.49±8.05	160.64±15.1	126.85±10.02	113.42±5.55	127.41±6.02
Low molecular weight phenols	58.38±4.24	47.1±5.23	69.54±9.56	50.87±5.95	47.42±4.78	53.91±6.35
Chlorogenic acid der.	10.09±1.36	13.45±2.34	14.51±1.32	10.36±1.2	10.81±1.2	13.83±2.53
Quercetin 3-glycosides	22.03±1.94	15.01±1.58	30.72±5.38	20.71±2.68	16.21±1.64	19.72±2.72
Kaempferol 3-glycosides	22.95±2.39	16.1±1.65	20.31±3.19	16.63±2.73	17.39±2.2	17.77±2.07
Flavonoids	47.91±3.72	33.28±3.25	54.58±8.66	40.03±5.14	36.3±3.79	39.8±4.9
Neochlorogenic acid	0.53±0.15	0.78±0.29	0.68±0.15	0.27±0.1	1.22±0.81	0.78±0.3
Chlorogenic acid der. 1	0.45±0.1	0.88±0.22	0.92±0.19	0.9±0.31	0.53±0.06	1.08±0.21
Chlorogenic acid der. 2	0.17±0.03	0.14±0.03	0.44±0.1	0.21±0.11	0.19±0.05	0.28±0.07
Chlorogenic acid der.3	1.58±0.17	2.69±0.28	2.49±0.24	2.65±0.22	2.3±0.23	2.47±0.32
Chlorogenic acid	3.17±0.99	6.05±1.54	5.3±0.88	2.7±0.64	3.3±0.48	5.74±1.83
Chlorogenic acid der. 4	0.25±0.03	0.29±0.03	0.53±0.07	0.46±0.07	0.36±0.05	0.36±0.07
Dihydroquercetin der. 1	0.67±0.05	0.71±0.09	0.94±0.14	0.71±0.13	0.97±0.13	0.7±0.07
Quercetin 3-glycoside 1	1.71±0.19	1.49±0.15	2.88±0.7	2.19±0.38	1.75±0.22	2.23±0.28
Quercetin 3-glycoside 2	8±0.73	6.68±0.95	13.45±2.68	6.58±0.9	6.81±0.92	7.89±1.28
Quercetin 3-glycoside 3	12.2±1.21	6.6±0.65	13.88±2.18	11.76±1.67	7.4±0.67	9.4±1.47
Chlorogenic acid der. 5	1.36±0.15	0.78±0.11	1.85±0.25	1.16±0.19	0.99±0.13	0.96±0.19
Kaempferol 3-glycoside 1	3.66±0.33	3.08±0.39	4.09±0.61	2.75±0.41	3.11±0.35	3.5±0.42
Kaempferol 3-glycoside 2	17.13±2.21	10.1±1.31	13.53±2.44	11.36±2.15	11.65±1.83	11.05±1.2
Chlorogenic acid der. 6	2.57±0.24	1.83±0.21	2.29±0.23	2.01±0.34	1.93±0.29	2.16±0.41
Kaempferol 3-glycoside 4	0.56±0.07	0.58±0.09	0.74±0.18	0.69±0.09	0.56±0.08	0.99±0.39
Kaempferol 3-glycoside 5	0.85±0.08	1.77±0.2	1.09±0.16	1.3±0.27	1.42±0.3	1.53±0.3
Monocoumaroylastragallin	0.53±0.06	0.25±0.05	0.65±0.11	0.36±0.06	0.29±0.04	0.35±0.08
Dicoumaroylastragallin 1	0.78±0.06	0.43±0.05	0.67±0.08	0.57±0.1	0.54±0.07	0.47±0.07
Dicoumaroylastragallin 2	0.49±0.04	0.24±0.03	0.36±0.05	0.31±0.05	0.27±0.03	0.28±0.04

Table 11. Concentration of phenolic compounds in mg g^{-1} dry weight (S.E.) in beech seedling leaves which were harvested in 2017 from plants grown in Ås, by provenance.

Ås	2017					
Substance	Norway	Sweden	Denmark	De Soignes	Ardennes	France
MeOH-soluble condensed tannins	91.9±4.71	76.81±3.65	110.34±7.91	113.63±12.45	100.64±12.95	95.64±8.24
Low molecular weight phenols	34.59±4.35	32.71±5.6	25.89±4.36	30.25±3.46	27.35±3.42	22.11±3.26
Chlorogenic acid der.	9.36±0.96	18.06±4.27	8.49±1.6	8.26±0.94	6.84±0.77	7.38±1.86
Quercetin 3-glycosides	15.78±4.03	8.58±1.12	12.64±2.64	13.31±3.23	11.37±1.37	7.53±0.87
Kaempferol 3-glycosides	8.47±1.3	5.44±1.21	3.88±0.82	7.14±2.53	8.13±1.53	6.36±1.24
Flavonoids	25.23±4.7	14.66±1.99	17.39±3.23	21.99±3.98	20.51±2.66	14.73±2.11
Neochlorogenic acid	0.59±0.15	1.64±0.44	0.56±0.08	0.59±0.21	0.6±0.05	0.59±0.1
Chlorogenic acid der. 1	0.11±0.03	0.03±0.02	0.14±0.1	0.08±0.04	0.01±0.01	0.07±0.03
Chlorogenic acid der. 2	0.09±0.07	0.03±0.02	0±0	0.04±0.04	0.02±0.02	0±0
Chlorogenic acid der.3	2.69±0.45	3.73±0.71	2.17±0.43	3.47±0.04	2.53±0.23	2.28±0.37
Chlorogenic acid	2.65±0.7	9.77±3.31	2.76±0.94	2.62±1.1	2.1±0.67	3.12±1.69
Chlorogenic acid der. 4	0.21±0.04	0.13±0.07	0.2±0.03	0.14±0.07	0.29±0.04	0.23±0.04
Dihydroquercetin der. 1	0.23±0.05	0.25±0.06	0.32±0.05	0.61±0.24	0.35±0.05	0.28±0.05
Quercetin 3-glycoside 1	0.68±0.13	0.55±0.14	0.18±0.05	0.73±0.25	0.67±0.21	0.76±0.28
Quercetin 3-glycoside 2	7.48±3.83	2.84±0.76	2.58±0.64	1.6±0.84	5.39±0.89	3.1±0.83
Quercetin 3-glycoside 3	1.39±0.56	1.23±0.4	3.87±1.12	2.06±2.06	0.59±0.4	0.81±0.5
Chlorogenic acid der. 5	0.43±0.11	0.34±0.1	0.22±0.05	0.35±0.1	0.45±0.09	0.26±0.07
Kaempferol 3-glycoside 1	1.75±0.25	1.03±0.19	1.09±0.15	1.07±0.31	1.69±0.24	1.25±0.21
Kaempferol 3-glycoside 2	5.9±1	3.76±1.07	2.38±0.75	5.45±2.12	5.55±1.24	4.27±1.02
Chlorogenic acid der. 6	2.59±0.54	2.38±0.51	2.45±0.75	0.95±0.95	0.84±0.23	0.83±0.19
Kaempferol 3-glycoside 4	0.55±0.15	0.5±0.08	0.19±0.08	0.38±0.23	0.56±0.1	0.52±0.05
Kaempferol 3-glycoside 5	0.27±0.06	0.14±0.05	0.23±0.07	0.23±0.13	0.33±0.04	0.32±0.07
Monocoumaroylastragallin	0.23±0.06	0.07±0.03	0.17±0.05	0.3±0.08	0.18±0.04	0.14±0.04
Dicoumaroylastragallin 1	0.29±0.05	0.16±0.04	0.16±0.05	0.35±0.11	0.3±0.04	0.26±0.05
Dicoumaroylastragallin 2	0.23±0.04	0.16±0.03	0.22±0.06	0.29±0.03	0.18±0.04	0.15±0.03

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