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Linking Changes in Fatty Acid Composition to Postharvest Needle Abscission Resistance in Balsam Fir Trees

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Abstract: Balsam fir needle retention and fatty acid profile changes due to cold acclimation throughout autumn, but little is known about the relationship between these two phenomena. The objective was to examine differences in FAs in contrasting needle abscission resistant balsam fir genotypes throughout autumn and early winter. Branches from genotypes with low and high needle abscission resistance (NAR) were collected from September to January and analyzed for FA composition. High NAR genotypes retained needles 120–130% longer than low NAR genotypes and NAR increased through autumn in both genotypes. There was approximately a 3:1 ratio of unsaturated: saturated FAs, which increased by 4% in favor of unsaturated fatty acids through autumn. Palmitic, palmitoleic, and linolenic acid content was significantly higher in high NAR versus low NAR genotypes; arachidic, oleic, linoleic, pinolenic, coniferonic, icosadienoic, and sciadonic acids were lower in high NAR genotypes versus low. Linolenic acid was of particular interest because it tended to decrease throughout autumn, to the point that high NAR genotypes were significantly lower in linolenic acid than low NAR genotypes in January. These changes may be linked to an increase in abscisic acid and/or jasmonic acid synthesis depleting linolenic acid stores and promoting postharvest needle abscission resistance.

Keywords: abies balsamea; christmas tree; cold acclimation; conifers; fatty acid profile; linolenic acid; saturated fatty acid; unsaturated fatty acid

Citation: MacDonald, G.E.; Lada, R.R.; Caldwell, C.D.; Udenigwe, C.C.; MacDonald, M.T. Linking Changes in Fatty Acid Composition to Postharvest Needle Abscission Resistance in Balsam Fir Trees. *Forests* **2022**, *13*, 800. https://doi.org/10.3390/f13050800

Academic Editors: Bert Cregg, Inger Sundheim Fløistad and Chloé Gendre

Received: 22 April 2022
Accepted: 19 May 2022
Published: 20 May 2022

1. Introduction

Natural cold acclimation tends to delay postharvest needle abscission [1]. Acclimation is an adaptive process where plants acquire an ability to withstand cold stress. This is often triggered by changes in the environment, such as temperature and photoperiod [2,3]. Needle abscission rates significantly decreased correspondingly with cooler temperatures and shorter day length in balsam fir in a 9-month study [4]. Postharvest needle retention in balsam fir is typically highest in November and December, but commercial harvest often begins in October [4,5]. It is suspected that earlier harvest practices combined with warmer autumn temperatures contribute to increased postharvest needle abscission [6].

Needle retention varies considerably within balsam fir genotypes and ecotypes. One study screened over 300 balsam fir genotypes and found that needle retention varied from 6 to 60 days [5]. Differences in needle retention after cold acclimation are not consistent with every genotype. Many trees with poor needle retention benefit greatly from cold acclimation postharvest, while those with superior needle retention receive significantly less benefit [5]. Some typical physiological changes associated with cold acclimation, such as accumulation of raffinose, galactose, or abscisic acid, increased during cold acclimation

but did not vary significantly among genotypes [5]. The exact reason for different genotypic responses to cold acclimation in balsam fir remains unknown.

A change in fatty acid (FA) profile is one important cold-induced effect in plants [7,8]. FAs are integral components of cellular membranes in amphiphilic lipids and influence the integrity of all cellular membranes [9,10]. FAs are formed through de nova synthesis, which forms long chain saturated FAs (i.e., palmitic and stearic acid) from Acetyl-CoA via carboxylase and FA synthase [11]. Unsaturated FAs are created via desaturases while very long chain FAs are formed via elongases (Figure 1). FA desaturation increases postharvest, with low needle abscission resistant genotypes shows a decline in unsaturated fatty acids and loss of membrane integrity sooner than high NRD clones [12]. This could be related to the stability of membranes prior to harvest or the action of a fatty acid signaling molecule. In other frost-resistant plants, it has been observed that unsaturated FAs have been known to increase to help maintain membrane integrity for proper protein function to survive cold [13].

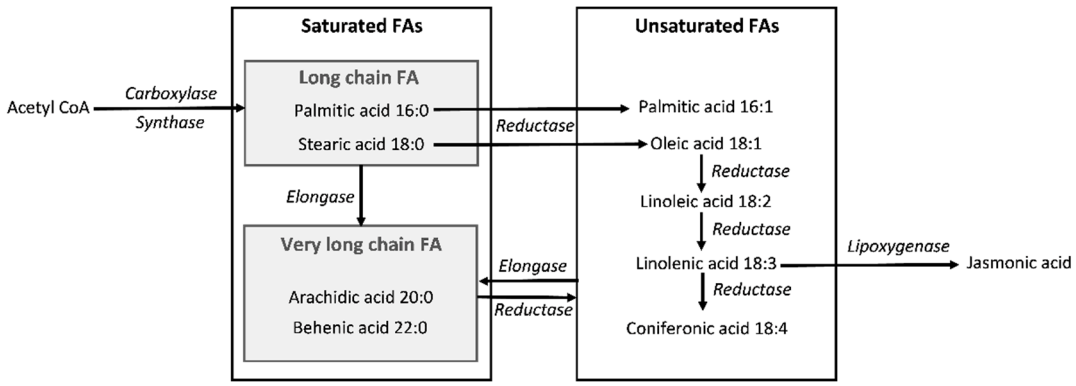


Figure 1. Simplified schematic of fatty acid synthesis adapted from [11,14]. Specific fatty acids are shown as examples and not meant to be a comprehensive list of all fatty acids. Enzymes are shown in italics.

It is postulated that changes in FA profile could help explain genotypic differences in cold acclimation of balsam fir. We know from previous studies that cold acclimation leads to a redistribution of lipids [15], but subtle specific FA changes were not investigated. It is the objective of this research to document the FA profiles in contrasting genotypes of low and high needle abscission resistance (NAR) during cold acclimation and link changes in FAs with needle abscission.

2. Materials and Methods

2.1. Sampling and Experimental Set-Up

Plant material for this investigation was collected from a clonal orchard located in Debert, NS, Canada (45°25′ N, 63°28′ W) that has over 220 genotypes of balsam fir. Genotypes were previously categorized according to NAR [16]. Branches were collected from low and high NAR genotypes in this current experiment.

Each sample consisted of a pair of parallel lateral branches, which had previously been shown to have almost identical needle abscission resistance, and 40 samples were collected each month. Lateral branches were cut from approximately 20-year-old trees at a height of 1 m from the ground level on the south facing side of the trees. Each branch was cut to include the most recent 2 full years of growth and was collected once per month from September to December (10 replicates each per NAR genotype per sampling period). One branch from a parallel pair was immediately frozen in liquid nitrogen and then stored at −80 °C until lipid extraction. The other branch from a parallel pair was placed in water and transferred to the lab to evaluate needle abscission resistance.

Once in the lab, hydrated branches were given a fresh cut 2.5 cm above the previous cut while stem ends were under water to reduce risk of cavitation, weighed, and then

placed in a 100 mL amber bottle filled with 100 mL of distilled water. The neck of each flask was plugged with cotton wool to prevent direct water evaporation and provide added stability to a branch. The branch and entire apparatus were weighed. The branches were kept at an average temperature of 20 °C with a light intensity of 90 μmol·m^{-2}·s^{-1} supplied by fluorescent lights to mimic household display conditions.

2.2. Needle Abscission

Needle abscission was measured by collecting the number of needles that would fall after a 'finger run' test each day [17] and the mass of those needles was measured fresh and after oven drying. The experiment was run until all branches reached peak abscission, which typically follows a sigmoidal curve [6]. Two time points from the abscission curves were used to evaluate needle abscission resistance: needle abscission commencement (NAC) and peak needle abscission (PNA). NAC was defined as the point where postharvest abscission began (1% needle loss), and PNA was defined as the point where needle abscission occurred at the highest rate [4].

2.3. FA Extraction

All fatty acids were extracted from branches that were immediately frozen after harvest using a protocol adapted from [18]. A total of 1g of frozen needles were ground, incubated in 1 mL of isopropanol with 0.01% butylated hydroxytoluene at 75 °C for 15 min, then 1.5 mL of chloroform and 0.6 mL of water were added. The solvent was transferred to a new glass tube with a Teflon-lined screw-cap after 1 h then 0.7 mL chloroform:methanol (2:1) was added and shaken for 30 min. The extraction was completed by adding 4mL of chloroform: methanol (2:1) ten times and collecting the solvent. The solvent extracts were washed once with 1 mL KCl (1.0 M) and once with 0.66 mL water. The solvent was evaporated under nitrogen and the lipid extract was quantified and dissolved in 1 mL chloroform. The tissues after lipid extraction were dried in an oven at 105 °C and dry weights were determined.

2.4. FA Analysis

Fatty acid methyl ester (FAME) was prepared using methanolic hydrochloric acid according to [19]. FAME was dissolved in 100–200 μL of hexane and placed in 2 mL gas chromatography (GC) vials with inserts. FAME were quantified using gas chromatography GC-FID analysis performed at the Kansas Lipdomic Center, Kansas State University, on an Agilent Technologies 6890N Network GC system equipped with a HP-88 capillary column (100 mm × 0.25 mm I.D., 0.2 μm film thickness) coupled with a flame ionization detector (FID). Injector and FID temperatures were set at 275 °C and 260 °C, respectively. The carrier gas (He) pressure was at 51.61PSI with a flow rate of 1.6 mL/min with continuous flow. For the detector, the H flow rate was 30.0 mL/min, air flow rate was 400 mL/min, and the He flow rate was 2 mL/min. The GC oven temperature ramp was operated as follows: initial temperature of 150 °C held for 1min, increased at 10 °C/min to 175 °C and held 10 min, increased at 5 °C/min to 210 °C and held for 5 min, then increased at 5 °C/min to a final temperature of 230 °C and held 15 min. The total run time was 44.5 min. The sampling rate of the FID was 20 Hz. The FAME solutions were injected (1 μL) using an autosampler Agilent Technologies 7683 Series Injector in splitless mode. The chromatograph was displayed with Agilent Technologies Enhanced Chemstation software. FAs were identified by comparison of relative retention times of the compounds in the sample with relative retention times of Supelco 37 component fatty acid methyl ester mix standards and quantified using internal standard pentadecanoic acid (15:0) and peak area.

2.5. Statistical Analysis

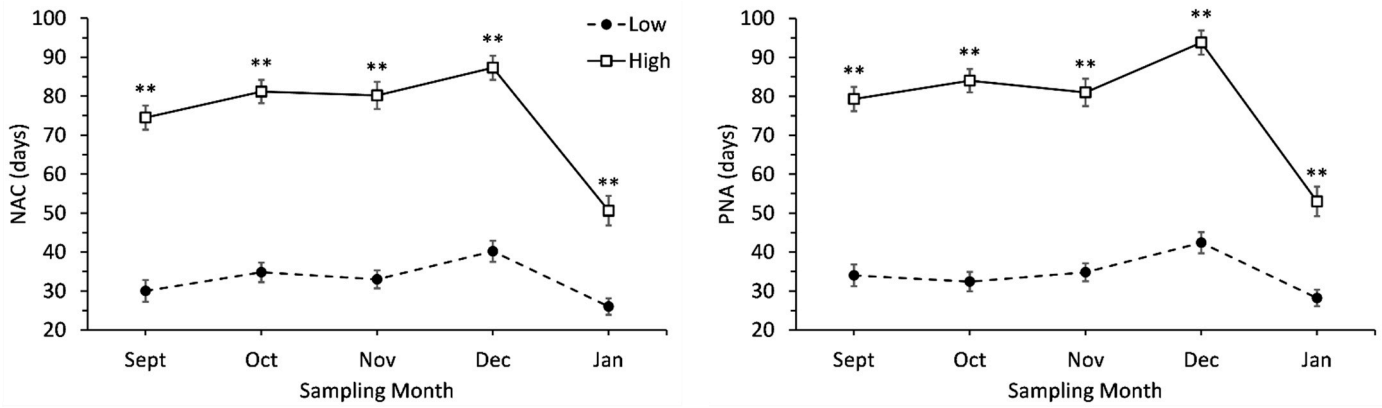
The experiment was designed as a 2 × 5 factorial with 2 genotypes and 5 sampling times. The experiment was replicated 10 times. The experimental design was chosen based on previous studies of significant changes in needle retention and polar lipids from

September to January [4,15] and identification of NAR genotypes [5]. As noted above, parallel lateral branches were used so that FAs and NAR could both be assessed, which resulted in a total of 200 branches sampled. All the experimental data was checked for the statistical assumptions of normality, independence, and constant variance prior to statistical analyses using SAS® (SAS Institute, Cary, NC, USA). The mixed procedure in SAS software was used to determine main and interaction effects between genotype and sampling times on NAR and FA responses. A software-based macro was used to separate means upon significant differences at 5% significance with Tukey's procedure. FAs were correlated to NAR using Minitab 19 software (Minitab, LLC, State College, PA, USA). Stepwise regression was used to create a model to describe NAR as a function of multiple FAs using $\alpha = 0.15$ to enter and exit the model. Correlation and regression were each calculated based on the 100 branches used for FA analysis.

3. Results

3.1. Needle Abscission Resistance

There were significant ($p < 0.001$) differences in NAR due to genotype and sampling month, but no significant interactions. The low NAR genotype had the lowest NAC at 32.8 days versus 75.4 days (130% increase) in high NAR genotype (Figure 2a). The low NAR genotype also had the lowest PNA at 36.4 days versus 80.2 days (120% increase) in high NAR genotype (Figure 2b). Both NAC and PNA had no difference between September through November but were highest in December and lowest in January (Figure 2a,b).



(**a**) (**b**)

Figure 2. Changes in (**a**) needle abscission commencement (NAC) and (**b**) peak needle abscission (PNA) in low and high NAR balsam fir genotypes over time. Data points are displayed as mean ± standard error as calculated from 10 branches each. The ** indicates 1% significance between genotypes at each month.

3.2. Unsaturated: Saturated FAs

Balsam fir needles had an unsaturated: saturated FA ratio of 3.12 in September. There was a small but statistically significant ($p < 0.001$) shift towards accumulation of unsaturated FAs in October, November, and December (Figure 3). The unsaturated: saturated ratio in January was not significantly higher than September, nor lower than October, November, and December.

3.3. Baseline FA Profiles

A FA profile of low and high NAR genotypes was established based on September sampling, which provided a baseline before cold acclimation. Both genotypes contained the same 20 FAs in September, with 9 saturated FAs and 11 unsaturated FAs. The highest percentages of unsaturated FAs were linolenic acid, linoleic acid, and oleic acid; the highest percentages of saturated FAs were palmitic acid, isopalmitic acid, and stearic acid (Table 1). There was no hexadecatrienoic acid (16:3) present in any genotypes, which is characteristic

of plants using the prokaryotic pathway for lipid synthesis [20]. It is also noteworthy that there were small amounts of isomargaric acid present, which is rarely found in plants outside of Pinaceae [21].

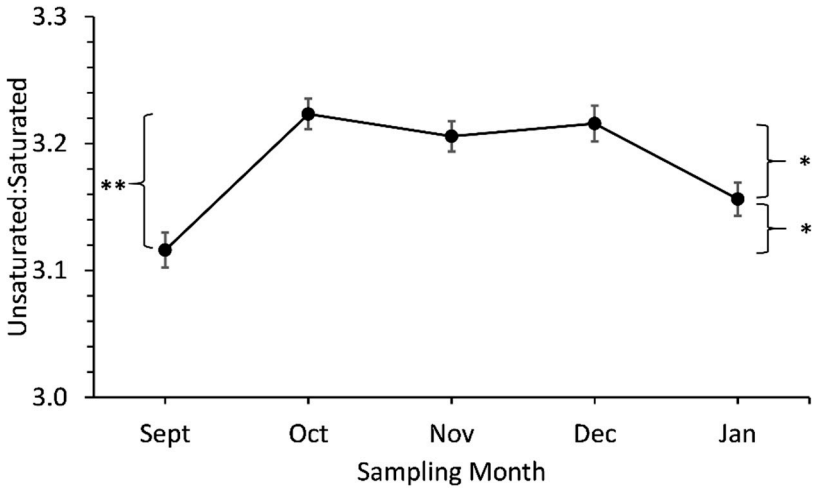


Figure 3. Unsaturated: saturated FA ratio of balsam fir branches based on sampling date. Data points represent mean ± standard error as calculated from 20 branches. The * represents 1% to 5% significance and ** represents less than 1% significance.

Table 1. Fatty acid relative percentages in balsam fir needles from low and high needle abscission resistance genotypes of balsam fir collected initially in September to establish a baseline fatty acid profile at the beginning of the experiment. Relative percentages are displayed as mean ± standard error as calculated from 20 branches. An * indicates 1–5% significance while ** indicates less than 1% significance.

Fatty Acid	Low NAR	High NAR	Sig.
TOTAL SATURATED	23.7 ± 0.5	23.7 ± 0.4	
Myristic acid (14:0)	1.4 ± 0.1	1.5 ± 0.1	
Palmitic acid (16:0)	9.1 ± 0.3	10.9 ± 0.4	**
Isopalmitic acid (14-methyl-16:0)	5.3 ± 0.3	4.8 ± 0.3	
Isomargaric acid (14-methyl-17:0)	0.4 ± 0.1	0.3 ± 0.1	
Stearic acid (18:0)	2.9 ± 0.2	2.5 ± 0.3	
Arachidic acid (20:0)	1.0 ± 0.1	0.4 ± 0.1	**
Behenic acid (22:0)	1.5 ± 0.2	1.3 ± 0.3	
Tricosylic acid (23:0)	1.0 ± 0.1	0.8 ± 0.1	
Lignoceric acid (24:0)	1.1 ± 0.1	1.2 ± 0.1	
TOTAL UNSATURATED	76.3 ± 0.5	76.3 ± 0.4	
Palmitoleic acid (9-*cis*-16:1)	0.8 ± 0.1	1.1 ± 0.1	*
Oleic acid (9-*cis*-18:1)	12.1 ± 0.2	11.2 ± 0.3	*
Taxoleic acid (5,9-*cis*-18:2)	3.0 ± 0.2	3.2 ± 0.2	
Linoleic acid (9,12-*cis*-18:2)	17.0 ± 0.4	14.5 ± 0.3	**
Pinolenic acid (5,9,12-*cis*-18:3)	7.0 ± 0.3	5.1 ± 0.3	**
Linolenic acid (9,12,15-*cis*-18:3)	24.3 ± 0.5	30.4 ± 0.7	**
Coniferonic acid (5,9,12,15-*cis*-18:4)	4.7 ± 0.1	4.4 ± 0.1	*
Icosadienoic acid (11,14-*cis*-20:2)	0.6 ± 0.1	0.3 ± 0.1	*
Sciadonic acid (5,11,14-*cis*-20:3)	4.5 ± 0.2	3.7 ± 0.1	**
Dihomolinolenic acid (11,14,17-*cis*-20:3)	0.4 ± 0.1	0.4 ± 0.1	
Juniperonic acid (5,11,14,17-*cis*-20:4)	1.9 ± 0.1	2.0 ± 0.2	

Genotype had a significant main effect in 2 of 9 saturated FAs and 8 of 11 unsaturated FAs for September (Table 1). Saturated FAs palmitic acid and arachidic acid were 20% higher and 60% lower, respectively, in high NAR genotypes versus low NAR genotypes.

(Table 1). Most unsaturated FAs were significantly lower in high NAR genotypes including a 7% decrease in oleic acid, 15% decrease in linoleic acid, 27% decrease in pinolenic acid, 6% decrease in coniferonic acid, 50% decrease in icosadienoic acid, and 18% decrease in sciadonic acid. The decreases in unsaturated FAs in high NAR genotypes were largely offset by a 25% increase in linolenic acid and 38% increase in palmitoleic acid, ultimately resulting in identical ratios of saturated: unsaturated FAs between low and high NAR genotypes in September.

3.4. Genotype and Sampling Date Main Effects

Genotype had a significant main effect on coniferonic and sciadonic acids throughout the experiment (Figure 4). Both coniferonic and sciadonic acids were lower in high NAR genotypes in the September baseline analysis and remained significantly lower throughout the experiment. Coniferonic acid and sciadonic acids were an average of 6% lower and 12% lower, respectively, in high NAR genotypes.

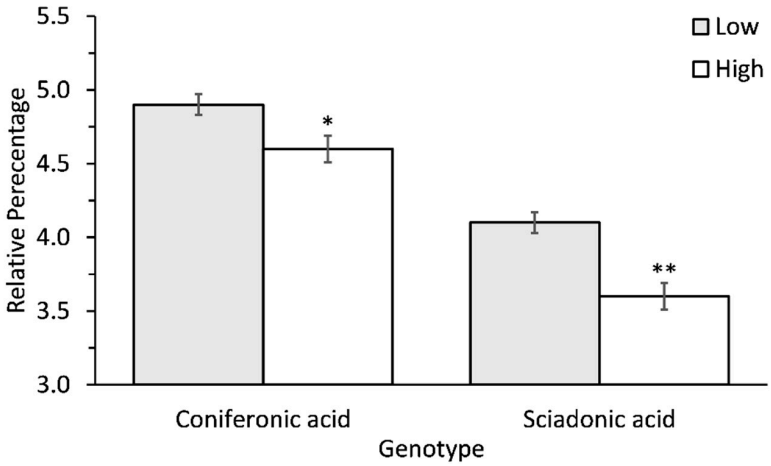


Figure 4. Fatty acids in balsam fir branches that only had significant differences between low and high needle abscission resistance regardless of sampling time. Each mean was calculated using 50 branches. Error bars represent standard error. An * indicates 1 to 5% significance while ** indicates less than 1% significance when high NAR genotypes are compared to low NAR genotypes.

Sampling date had a significant main effect on stearic, icosadienoic, sciadonic, and behenic acids across both genotypes (Table 2). Stearic acid did not increase from September to October, but then increased by 15% and remained higher until January. Icosadienoic acid increased by 50% after September then remained constant until January. Sciadonic acid remained constant in September and October, then decreased by approximately 12% until January. Finally, behenic acid increased 14% from September to October, then another 19% in November before holding constant.

Table 2. Fatty acid relative percentages in balsam fir needles from low and high needle abscission resistance genotypes of balsam fir collected initially in September to establish a baseline fatty acid profile at the beginning of the experiment. Data is presented as mean ± standard error as calculated from 20 branches. An * indicates 1–5% significance while ** indicates less than 1% significance due to sampling month.

Fatty Acid	Sept	Oct	Nov	Dec	Jan	Sig.
Stearic acid (18:0)	2.7 ± 0.1	2.6 ± 0.1	3.0 ± 0.1	3.2 ± 0.1	3.0 ± 0.1	**
Icosadienoic acid (11,14-*cis*-20:2)	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	*
Sciadonic acid (5,11,14-*cis*-20:3)	4.3 ± 0.1	4.3 ± 0.2	3.8 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	*
Behenic acid (22:0)	1.4 ± 0.1	1.6 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	**

3.5. Interaction Effects between Genotypes and Sampling Date

Linoleic, oleic, pinolenic, and arachidic acid generally increased throughout the experiment, though there were significant interactions between sampling time and genotype. Linoleic acid increased by 38% in high NAR genotypes from September to November and then remained high, while linoleic acid increased only 13% in low NAR genotypes before decreasing back to its September concentration (Figure 5a). Oleic acid increased in both low and high NAR genotypes at a similar rate until December, before decreasing 9% in low NAR genotypes and increasing by 35% in high NAR genotypes (Figure 5b). Pinolenic acid remained at its initial concentration in low NAR genotypes until December before decreasing by 9% in January. Conversely, pinolenic acid increased by 41% from September to January in high NAR genotypes (Figure 5c). Finally, arachidic acid increased by 60% in low NAR genotypes but 225% in high NAR genotypes from September to December. Arachidic acid continued to increase in high NAR genotypes, but significantly decreased in low NAR genotypes in January (Figure 5d).

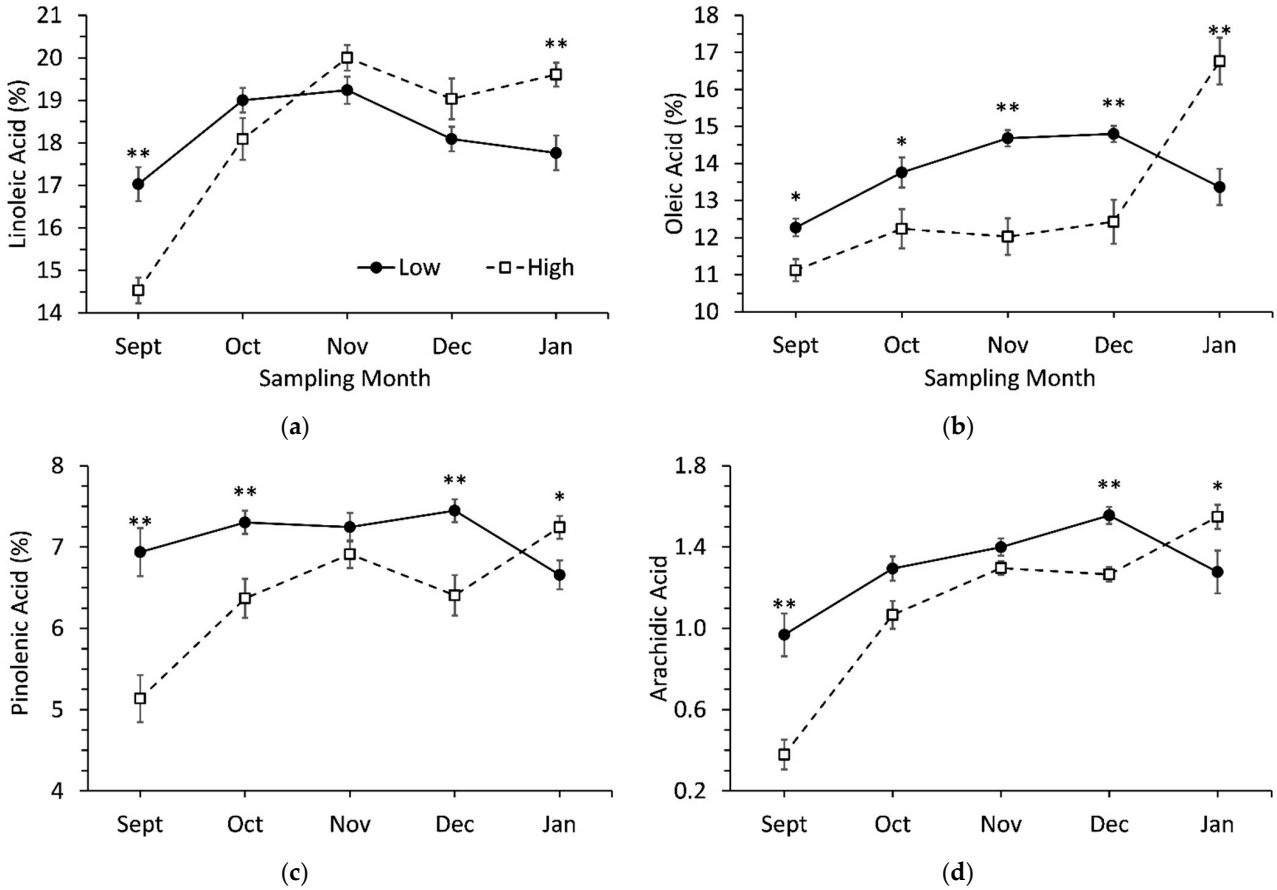


Figure 5. Interactive effects between balsam fir genotype (low and high needle abscission resistance) and sample collection date for (**a**) linoleic acid, (**b**) oleic acid, (**c**) pinolenic acid, and (**d**) and arachidic acid. Each data point represents a mean ± standard error as calculated from of 10 branches. The * indicated 1% to 5% significance and the ** indicates less than 1% significance between genotypes at any month.

Palmitic, linolenic, and palmitoleic generally decreased throughout the experiment, though there were significant interactions between sampling time and genotype. Palmitic acid decreased by 30% in high NAR genotypes from September to January but decreased by 14% in low NAR genotypes until December before returning to initial concentrations (Figure 6a). Linolenic acid decreased by 35% in high NAR genotypes from September to January but decreased by 13% in low NAR genotypes until December and then did not significantly change (Figure 6b). Finally, palmitoleic acid decreased by 66% in high NAR

genotypes from September to January but decreased by 29%in low NAR genotypes, then did not significantly change (Figure 6c).

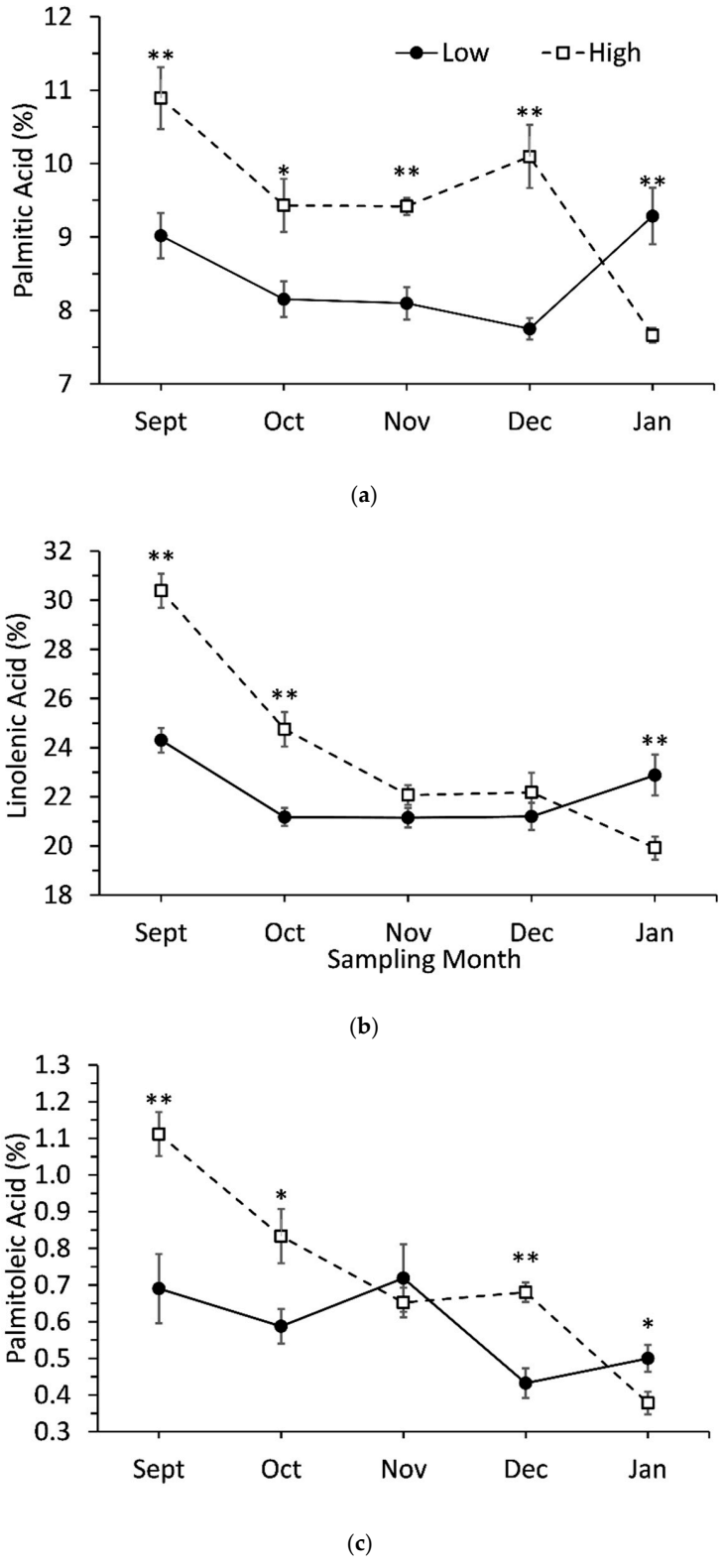


Figure 6. Interactive effects between balsam fir genotype (low and high needle abscission resistance) and sample collection date for (**a**) palmitic acid, (**b**) linolenic acid, and, (**c**) and palmitoleic acid. Each data point represents a mean ± standard error as calculated from of 10 branches. The * indicated 1% to 5% significance and the ** indicates less than 1% significance between genotypes at any month.

3.6. Relationships between FAs and NAR

High needle abscission resistance was related to high concentrations of palmitic, palmitoleic, linolenic, dihomolinolenic, and juniperonic acids and lower concentrations of isopalmitic, oleic, pinolenic, archidic, coniferonic, and sciadonic acids (Figure 7). Needle abscission resistance tended to be lower when there were higher concentrations of sciadonic, coniferonic, archidic, pinolenic, oleic, and isopalmitic acids (Figure 7). The FAs most correlated with NAR were palmitic acid and pinolenic acid (in opposite directions), but individually each only explain approximately 21% of the variation in NAR. The optimum regression equation, which accounted for 57% of the variation in NAR, was created by stepwise regression at $\alpha = 0.15$ to enter and remove variables:

$$NAR = -49.9 + 11.4 \times L - 11.8 \times P - 43.3 \times A - 103.0 \times I \quad (1)$$

L represents linolenic acid, P represents pinolenic acid, A represents arachidic acid, and I represents icosadienoic acid in equation 1 above.

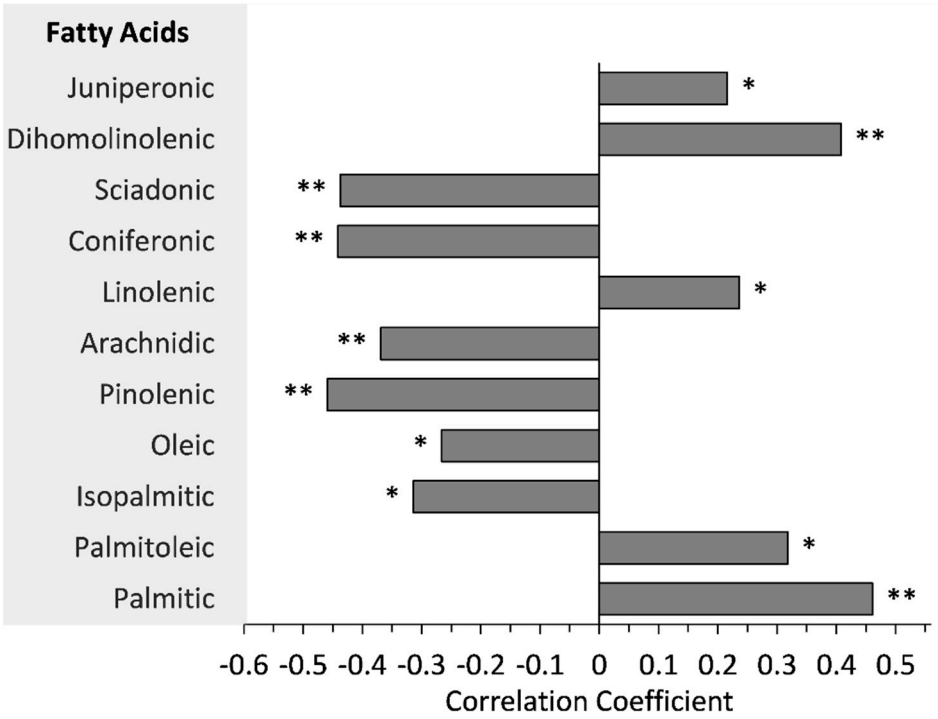


Figure 7. Significant correlation coefficients of fatty acids compared to needle retention in balsam fir. An * indicates 1−5% significance and ** indicates less than 1% significance. $n = 100$ for each.

4. Discussion

4.1. Genotypic Changes

Both genotypes contained the same 20 FAs as past studies for balsam fir needles [12]. This profile is unique to balsam fir, as FAs can be used as taxonomic markers for species. One study compared the fatty acids in needles of four conifer species: Siberian larch (*Larix sibirica*), Scots Pine (Pinus sylvestris), Korean pine (*Pinus koraiensis*), and white spruce (*Picea pungens*) [22]. The FA spectrum from [22] for these species included 24–25 acids between C14–C22 as compared to 20 in our balsam fir. A second study examined FAs in Dahurian larch (*Larix gmelia*), Japanese larch (*Larix kaempferi*), Ezo spruce (*Picea jezoensis*), Sakhalin spruce (*Picea glehnii*), and Sakhalin fir (*Abies sachalinensis*) [23]. The FA spectrum from [23] had a similar number of fatty acids as balsam fir, though at much different concentrations. A major characteristic that was unique to our study was that saturated FAs were notably lower in balsam fir (~24%) opposed to 30–38% reported by [22] and 44–52% reported by [23].

There was also considerably more oleic and pinolenic acids in the balsam fir than most other conifer species described above.

It is difficult to compare profiles, as they are the result of adaptation to the environment [21]. However, any difference between high and low NAR genotypes in September was of interest and the adaptation of these genotypes could possibly be linked to their postharvest quality. There is more palmitic and palmitoleic acid in high NAR versus low NAR genotypes, which could be important for oxidative stability of the membranes as oil manufacturers will often try to enhance palmitic acid to increase shelf life [24]. Conversely, longer chain unsaturated FAs are less stable, extremely prone to oxidation, and therefore more likely to produce reactive oxidative species that could damage cellular membranes [25]. Such long chain unsaturated FAs are not common in membrane lipids, but it is noteworthy that sciadonic acid was significantly higher in low NAR balsam fir genotypes.

Linolenic acid was present at higher concentrations than any other FA in each balsam fir genotype, but it was 25% higher in high NAR genotypes. Linolenic acid can be released from membranes of organelles, such as chloroplast, and is perhaps most noteworthy for being a precursor to jasmonic acid [26]. Jasmonic acid helps mediate several forms of abiotic stress, including cold and dessication [27]. Since jasmonic acid biosynthesis operates on a positive feedback loop [28], it is conceivable that balsam fir with significantly more linolenic acid would have opportunity for increased jasmonic acid synthesis, which may contribute towards superior postharvest needle retention.

It may be that ABA is affecting FA concentrations. Though not directly measured in our study, previous studies have confirmed that ABA increases during natural cold acclimation in balsam fir [5,29]. Further, ABA has been associated with specific FA changes in other species. Exogenous ABA increased oleic and linoleic acid accumulation in Siberian apricot [30], increased linoleic and elaidic acid in grapes [31], and linoleic acid in oil palm [32]. The interaction between ABA and FAs in balsam fir requires study.

Delta5-unsaturated polymethylene-interupted fatty acids (Δ5-UPFIAs) were previously thought of as rare but are common in the oil and needles of conifers [33]. The predominant Δ5-UPIFA in balsam fir were pinolenic, coniferonic and sciadonic acid, which supports previous research [12]. Those three Δ5-UPIFAs were also found in significantly lower concentrations in high NAR genotypes. These Δ5-UPIFA are much more commonly found in seed lipids with much of their potential role in needles unknown [21,34].

4.2. Changes in FAs and Sampling Date

Icosadienoic acid increased between September and October. Icosadienoic acid is not commonly associated with plants but has been identified in pine nuts [35]. Usually if a fatty acid accumulates, then it is being produced at a faster rate than it is metabolized. Icosadienoic acid results from elongation of linoleic acid, 18:2n6, and is metabolized in animals into dihomolinolenic acid [36].

Sciadonic acid decreased throughout the experiment, which is a little surprising. Sciadonic acid is a trenoic fatty acid, which often are crucial for adaptation to temperature stress [37]. As noted above, longer chain unsaturated FAs can become more vulnerable to oxidation. Perhaps reactive oxygen species generated through cold stress contributed to the decrease of sciadonic acid.

4.3. Interactive Effects Due to Cold Acclimation

Many of the FAs that exhibited interactions between genotype and sampling time were either 16C or 18C FAs that are very closely linked in FA biosynthesis. Further, the interactive effect was often due to a differential response from December to January, which means that there were some consistent general trends that occurred throughout autumn. Palmitic acid (and consequently palmitoleic acid) tended to decrease while stearic acid increased through autumn, which suggests a potential shift to longer chained FAs through autumn at the onset of FA synthesis. Stearic acid is unsaturated via reductase to yield 18:1

FAs (i.e., oleic acid) then 18:2 FAs (i.e., linoleic acid) [11]. Oleic and linoleic acids increased throughout autumn. The 18:2 FAs are further reduced to 18:3 FAs, such as linolenic acid [11]. However, linolenic acid decreased through autumn despite increased concentrations of its precursor. It is suggested that signals and enzymes associated with mobilization of linolenic acid towards the lypoxygenase pathway are induced during autumn to favor jasmonic acid synthesis and mediate cold acclimation.

5. Conclusions

NAR genotypes behaved as expected with respect to needle abscission; low NAR genotypes lost needles earlier than high NAR genotypes. The FA profile was significantly different between the two NAR genotypes with palmitic, palmitoleic, and linolenic acid significantly higher and arachidic, oleic, linoleic, pinolenic, coniferonic, icosadienoic, and sciadonic acid significantly lower in high versus low NAR genotypes. Though this experiment could not ascertain whether any specific FA alone caused differences in NAR, a combination of linoleic, pinolenic, arachidic, and icosadienoic acid did account for 57% of the observed NAR variation in balsam fir. Further efforts would be needed to determine any causative relationship between FAs and NAR.

Our results could be useful as a screening tool to identify trees with superior needle retention. Most producers export a portion of their trees, but also sell a portion to local consumers. Producers could identify trees with higher needle retention to be harvested for export, as exported trees are often harvested earliest so they can arrive in their markets by a specific time. Producers could then use poorer needle retaining trees for local markets since those trees are often harvested closer to Christmas and do not need as high a postharvest shelf life.

Understanding the relationship between FAs and NAR could be useful in selectively breeding balsam fir trees with superior NAR. NAR was highest in balsam fir with high concentrations of linolenic acid and low concentrations of pinolenic, arachidic, and icosadienoic acids (Equation (1)). FA content is typically a quantitative trait that is controlled by multiple genes [38] and selectively breeding to modify FAs has been effective in other species [39] Selectively breeding for high linolenic acid and/or low pinolenic, arachidic, and icosadienoic acids could potentially increase NAR in balsam fir, which could have practical commercial application.

It is possible that this research could be used towards the development of metabolic engineering of balsam fir. A commonly used approach is infection with *Agrobacterium tumefaciens*, which can transfer a portion of its DNA from plasmid to plant cells [40]. The T-DNA genes of *Agrobacterium* are replaced with genes of interest, such as those that code for production of different fatty acids. This method has been used successfully in a variety of crops, such as monocots, cereals, and legumes [41]. By inserting genes coding for palmitic, pamitoleic, or linolenic acid, it may be possible to use metabolic engineering to improve needle retention.

Author Contributions: Conceptualization, all authors; methodology, all authors; software, G.E.M.; validation, all authors; formal analysis, G.E.M., R.R.L. and M.T.M.; investigation, G.E.M. and M.T.M.; resources, R.R.L.; data curation, G.E.M., R.R.L. and M.T.M.; writing—original draft preparation, G.E.M. and M.T.M.; writing—review and editing, all authors; visualization, M.T.M.; supervision, R.R.L., C.D.C. and C.C.U.; project administration, R.R.L.; funding acquisition, R.R.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by NSERC, grant number CRDPJ-364061-07/09 and ACOA, grant number 195310.

Data Availability Statement: Not applicable.

Acknowledgments: The authors gratefully acknowledge the Nova Scotia Department of Natural resources for access to the clonal orchard. The authors also acknowledge Ruth Welti of the Kansas Lipidomic Research Center at Kansas State University for their technical expertise.

Conflicts of Interest: The authors declare no conflict of interest.

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