The distribution pattern of two juvenile hormone related compounds in Fraser fir and the induced response to a balsam woolly adelgid infestation

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Abstract: Two juvenile hormone related compounds (JRCs), juvabione and dehydrojuvabione, were isolated from Fraser fir, *Abies fraseri* (Pursh) Poirit. Both juvabione and dehydrojuvabione concentrations had large variations across the tested trees and within trees. Juvabione, but not dehydrojuvabione, was much higher in stemwood then in branchwood. There was no significant difference in either chemical in relation to high, low, or middle branch position. To determine if juvabione concentration was influenced by an infestation of balsam woolly adelgid, *Adelges piceae* Ratz., branches from infested and uninfested 11-year-old *Abies fraseri* with were sampled at the bottom, middle, and top branches of each tree. While mean juvabione concentrations for each infested fir were higher than their uninfested counterparts, only the infested middle tree sections had mean juvabione values that were substantially higher ($p = 0.078$) than the corresponding uninfested tree section of all the infested firs. The two infested *Abies fraseri* that maintained apical dominance demonstrated the highest upper branch levels of juvabione of all the infested. These results indicate that juvabione may be induced in small *Abies fraseri* in response to adelgid attack. Trees that produce large quantities of this compound may possess tolerance to the adelgid. Further research is needed to elucidate this possibility.

Résumé : Deux composés analogues à l'hormone juvénile, la juvabione et la déhydrojuvabione, ont été isolés chez le sapin de Fraser, *Abies fraseri* (Pursh) Poirit. Les concentrations de ces deux composés présentaient de fortes variations intra et inter-arbres. Contrairement à celle de la déhydrojuvabione, la concentration en juvabione était beaucoup plus élevée dans le bois des tiges que dans celui des branches. Aucun des deux composés ne montrait de différence significative en relation avec la position inférieure, médiane ou supérieure des branches dans la cime. Pour déterminer si la concentration en juvabione était influencée par une infestation de pucerons lanigères du sapin, *Adelges piceae* Ratz., des branches ont été prélevées dans les sections inférieure, médiane et supérieure de la cime d'*Abies fraseri* âgés de 11 ans, infestés et non infestés. Alors que les concentrations moyennes en juvabione de chacun des sapins infestés étaient plus élevées que celles de leurs homologues non infestés, seules les sections médianes infestées exhibaient des valeurs moyennes de juvabione substantiellement supérieures ($p = 0{,}078$) à celles des sections correspondantes, non infestées, de tous les sapins infestés. Parmi tous les arbres infestés, les deux *Abies fraseri* qui avaient conservé une dominance apicale présentaient les niveaux les plus élevés de juvabione dans leurs branches supérieures. Ces résultats indiquent que la juvabione pourrait être induite par une attaque de pucerons chez les jeunes *Abies fraseri*. Les arbres produisant ce composé en grande quantité pourraient être tolérants aux pucerons. La vérification de cette hypothèse nécessite davantage de travaux de recherche.

[Traduit par la Rédaction]

Introduction

The balsam woolly adelgid, *Adelges piceae* (Ratzeberg) (BWA), is an introduced pest of true firs (*Abies* spp.) in North America. The insect is native to the silver fir, *Abies alba* Mill., forest of central Europe where the host tree is not seriously affected. BWA was accidentally introduced into Maine in 1908, and its first appearance in southern Appalachia occurred in 1955 on Mount Mitchell, North Carolina (Hain et al. 1991). BWA continued to threaten the Fraser fir, *Abies fraseri* (Pursh) Poirit, populations in North Carolina, Virginia, and Tennessee (Amman and Speers 1965). In North Carolina, tree mortality ranges from 44% on Roan Mountain, which has 10% of the total spruce–fir type in southern Appalachia, to 91% in the Great Smoky Mountains National Park, which has 74% of southern Appalachia's spruce–fir type (Dull et al. 1988). Mount Rogers in Virginia has experienced much less mortality from BWA. Specifically fir mortality at Mount Rogers was not different from background mortality in uninfested stands. This suggests that there may be site related (seed source) differences in susceptibility to BWA.

BWA will infest the stem and branches of *Abies fraseri*. Infestation of *Abies fraseri* by BWA can cause abnormal growth as a result of salivary secretions (Amman and Speers

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1965; Hain et al. 1991). Effects of BWA attack include rotholz wood formation (red compressionlike wood in the xylem), reduced tracheids, increased outer bark, hyperresinosis, the increased production of monoterpenes, loss of apical dominance, gout (swelling of the shoots), and the production of juvenile hormone related compounds (JRCs) including juvabione (Hain et al. 1991). The combination of the above abnormalities results in reduced water flow, tree dehydration, inhibited photosynthesis, and often tree death in as few as 3 years of infestation (Forbes 1977; Hain et al. 1991; Mitchell 1967).

Recent research indicates that, in some cases, juvabione levels in *Abies fraseri* increase with increasing BWA infestation levels (Zhang 1994). The JRCs, such as juvabione and dehydrojuvabione, are a group of sesquiterpenoids which are found in fir (*Abies*) wood (Barrero et al. 1989; Hain et al. 1991; Kawai et al. 1993; Manville 1975, 1976; Manville and Kriz 1977; Manville and Tracey 1989; Manville et al. 1977; Tuithasi and Hanazawa 1940) and Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco (Rogers and Manville 1972). JRCs were first isolated from balsam fir and were shown to impair the development of *Pyrrhocoris apterus* (L.) by producing adults that maintained immature structures (Slama and Williams 1965). Since then JRCs have been isolated from a number of fir species and have been shown to be effective in altering insect development across a wide spectrum of orders (Numata et al. 1990; Ono 1993; Retnakaran et al. 1985; Riddiford and Truman 1978).

JRCs can also affect the reproductive physiology of insects. In *Pyrrhocoris apterus*, juvabione inhibits embryogenesis and eclosion from the egg when applied early in oocyte development (Retnakaran 1980; Riddiford and Williams 1967; Riddiford 1972; Slama and Williams 1966). In addition, high levels of exogenous JRCs can cause sterility in certain insects such as *Trogoderma granarium* Everts and *Pyrrhocoris apterus* (Manser et al. 1968; Metawally and Landa 1972). JRCs have been used to induce sterility as well as morphogenic deformities in some aphids and whiteflies (Bindra and Singh 1976; Benskin and Perron 1973; Bonnemaison 1976; Fisher and Shanks 1979; LeClant et al. 1976).

Extensive research on the occurrence and identification of JRCs in *Pseudotsuga menziesii*, white fir, *Abies concolor* (Gord. & Glend.) Lindl., and subalpine fir, *Abies lasiocarpa* (Hook.) Nutt., has been conducted (Manville 1975, 1976; Manville et al. 1977; Manville and Kriz 1977; Manville and Rogers 1977). Chemical composition of JRCs among trees within the same species is both qualitatively and quantitatively different in firs (Kawai et al. 1993; Manville and Kriz 1977; Manville et al. 1977, 1975; Manville and Tracey 1989). On the other hand the JRC within-tree distribution pattern was far more homogeneous in *Pseudotsuga menziesii* (Manville and Rogers 1977) with similar JRC concentrations in branchwood samples and their corresponding bolewood samples.

Where intraspecies chemical differences do exist between individuals, they could be the result of inherited genetic and (or) individual physiological differences. For example, the acid forms of two JRCs, todomatuic acid and dehydrotodomatuic acid, were only present in the wood adjacent to where BWA feeding took place in grand fir, *Abies grandis* (Dougl.) Lindl., and Pacific silver fir, *Abies amabilis* (Dougl.) Forbes (Puritch and Nijholt 1974). In *Pseudotsuga menziesii*, which is not attacked by BWA, a more consistent presence of JRC was found (Manville and Rogers 1977). The fact that Fraser fir generates juvabione in response to BWA infestation (see above) suggests that, in some cases, juvabione may contribute to the host resistance of BWA (Puritch and Nijholt 1974).

The overall objective of our research has been to identify potential resistance mechanisms within the *Abies fraseri* populations to BWA attack. Earlier work (Hollingsworth and Hain 1992) has shown that mature fir trees infested with BWA were induced to form an outer bark layer that could lead to tree recovery. While outer bark formation may be a mechanism for resistance in mature trees, it probably does not play a role in younger trees such as those grown as Christmas trees. Thus, the role of JRCs in young *Abies fraseri* was investigated in young trees.

The current study examined JRC (dehydrojuvabione and juvabione) concentration and distribution in young uninfested and infested *Abies fraseri*. Our first objective was to determine the JRC distribution pattern in young uninfested *Abies fraseri* both among trees and within trees. Our second objective was to investigate the distribution of JRCs in branch wood and in the corresponding bole wood. The third objective was to compare endogenous juvabione distribution and quantity between uninfested and infested *Abies fraseri*. Juvabione was examined exclusively for the third objective, since its effects on insect development and reproduction have been extensively documented. This will help determine if attack by the BWA causes *Abies fraseri* to increase its production of juvabione and where in the tree this increase occurs.

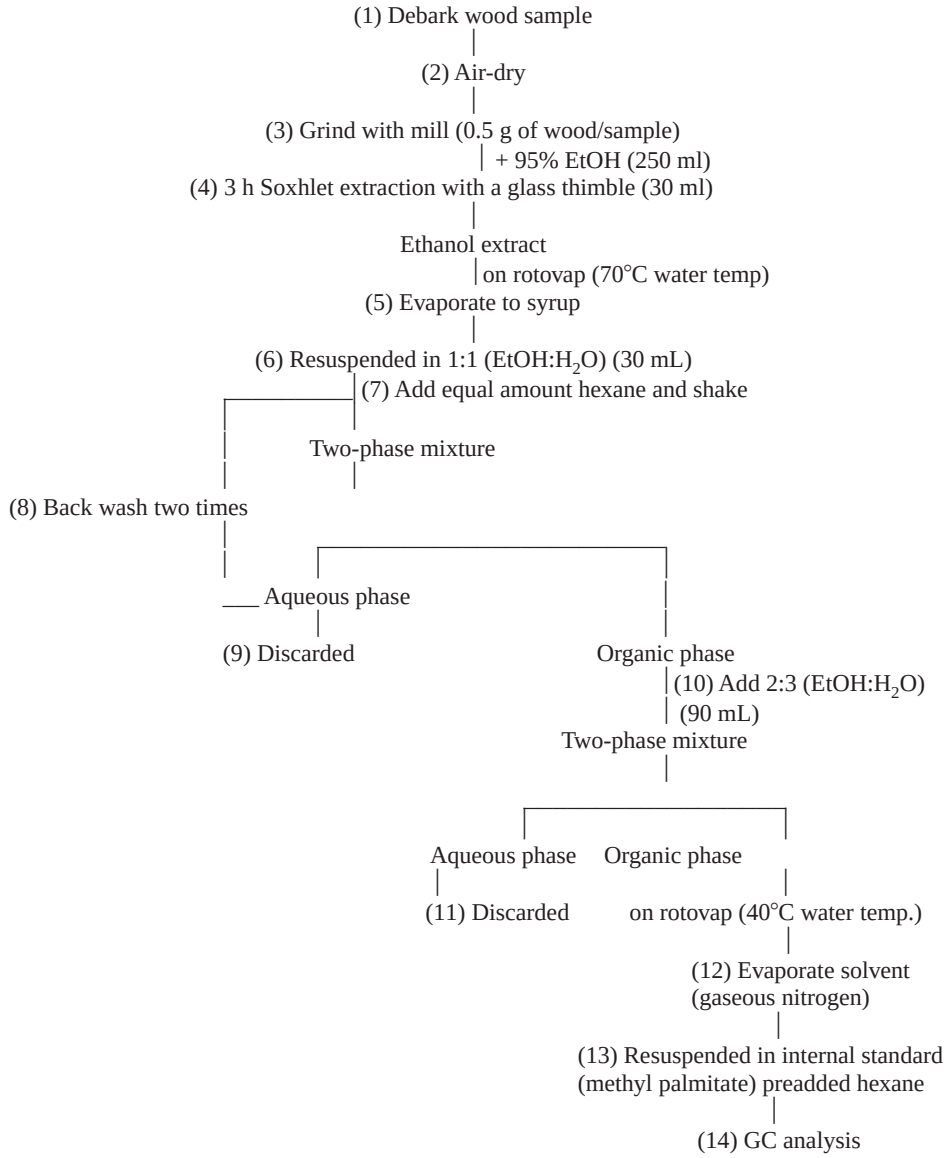
Materials and methods

JRCs in uninfested trees

Observations were carried out in 1993 on 8-year-old *Abies fraseri* in a plantation at the Upper Mountain Research Station near Laurel Springs, N.C. All trees in the plot were from one mother tree and were approximately 7 ft (1 ft = 0.305 m) tall. The study was split into two parts. First, we examined the tree-to-tree variation as well as the variation due to sampling position of JRCs in branch samples and the upper stem. We used seven trees and each tree was sampled at four sampling positions (the upper stem and high, middle, and low branches). The upper stem samples were cut just below the branch whorl from last year's growth.

Next, we examined the relationship between the JRC concentrations of branch samples and stem samples. To further investigate the distribution of JRCs in *Abies fraseri*, two of the seven trees were additionally analyzed for stem and branch JRC concentrations at the middle and low levels. The two trees were cut, and middle and low tree sections of the stem were sampled corresponding to the middle and low branches, respectively. The middle stem sample was taken at the medium height position of the sample tree and the low stem sample was cut just above the ground level. Corresponding branch samples were taken at or close to where the stem samples were collected. After the branch samples were collected, all the secondary branches were cut off and discarded. Each stem sample was between 5 and 10 cm long. There was a total of seven top, two middle, and two low stem samples; seven high, seven middle, and seven low branch samples used for the first two parts of the experiment.

Fig. 1. Procedures for preparing wood samples for gas chromatography analysis.



After the samples were collected and taken to the laboratory, they were debarked, air-dried, and powdered with a Cyclone mill. Approximately 0.5 g of powder from each sample was then weighed out for JRC extraction. Then, the samples were prepared according to the procedures outlined in Fig. 1.

JRC standards were obtained from Dr. John Manville at the Canadian Forest Service. The JRCs analyzed in this study were juvabione and dehydrojuvabione, and the purities of each were approximately 99%. The standards were obtained from *Abies balsamea* (L.) Mill. and *Abies lasiocarpa* (Hooker). Their identities were confirmed with gas chromatography (GC) – mass spectrometry and GC – Fourier transform infrared spectroscopy. The internal standard, methyl palmitate, was obtained from the Aldrich Chemical Company and was 97% pure.

The GC analysis method was adapted from Manville and Tracey (1989) using a HP5890A gas chromatograph. A Hewlett Packard 25-m Ultra-2 capillary column was used with an internal diameter of 0.20 mm. The solid phase was 5% phenyl methyl silicone with a 0.33-μm thickness. Helium was used as the carrier gas (0.42 mL/min flow rate) and splitless injection mode was used. Injector and flame ionization detector (FID) temperatures were maintained at 250 and 295°C, respectively. The oven temperature was programmed at 200°C for 1 min, 10°C/min for 7.5 min to a final temperature of 275°C, and held at 275°C for 5 min. The purge was programmed to be on at 1.40 and 13.0 min. The flow rates of make-up gas, H_2, and air through the FID detector were 30.5, 32.3, and 375 mL/min respectively. A NEC APC-H431 (286) computer equipped with a Maxima 820 chromatography workstation (Waters) collected data from the HP5890A. The Maxima data acquisition time was set for 10 min. All data acquisition and processing, as well as the initial calibration curve, were done on this system.

Two separate 0.5-g quantities of powder from each stem and branch sample were extracted and each extract was injected twice to ensure the reproducibility of the gas chromatograph. Respective injection readings were required to be within 10% of one another. Therefore, each stem or branch had four data points. The data used in the analysis, to estimate JRC concentration, were the means of the four data points. There were a total of 32 samples (11 stem plus 12 branch samples), 64 extractions, and 128 data points.

The SAS® software package (SAS Institute Inc. 1988) was used in the statistical analysis for this study. An *F* test determined among tree variation and variation due to sample position. A com-

Table 1. The concentrations (μg/g) of juvabione and dehydrojuvabione in the upper stem and branch wood at three heights of seven uninfested 8-year-old *Abies fraseri*.

Tree No.	Sample position	Juvabione		Dehydrojuvabione	
		Stem	Branch	Stem	Branch
1†	Low	12.8	nd*	516.6	366.5
	Middle	27.2	nd	169.8	342.0
	High	20.2	nd	358.9	400.9
2†	Low	77.1	16.6	280.1	240.3
	Middle	96.7	23.0	217.2	205.5
	High	100.8	46.2	294.0	178.6
3	Low		25.7		256.5
	Middle		13.8		273.0
	High	86.2	35.0	253.9	209.2
4	Low		37.5		181.9
	Middle		41.3		832.2
	High	147.1	80.4	928.8	1125.2
5	Low		78.0		755.5
	Middle		19.9		470.6
	High	241.0	49.6	553.6	742.3
6	Low		100.0		275.3
	Middle		46.4		449.8
	High	134.1	92.7	194.2	450.0
7	Low		18.6		106.1
	Middle		22.04		190.8
	High	30.9	14.9	99.6	105.8

*nd, not detected; trace levels were detected but were below the lower limit of the calibration curve.

†Trees 1 and 2 were cut down for middle and lower stem analyses.

parison was made for the following position pairs: upper stem versus others, high branch versus middle–low branch, and middle branch versus low branch. Then an all-way correlation analysis examined the relationship of JRC concentrations between the high stem and three branch levels.

Juvabione in infested and uninfested trees

Within the same research site, study trees were selected, and all but three trees were from known seed sources (Richland Balsam, Roan Mountain, Great Smokey Mountains, and Mount Rogers). This experiment was performed in 1995. None of the trees had ever been chemically treated. Ten infested and 10 uninfested trees were used in this study. The 10 infested trees had been intentionally infested in 1993 (Zhang 1994). Infestations were induced right after the first branch sampling and continued to the middle of August 1993. Infestations were induced by transferring three to five pieces of BWA-infested bark, 3–6 cm^2, to the base section of assigned trees with the phloem side away from the stem. The bark was fixed on the stem using steel pins. To achieve maximum infestations, a total of six consecutive infestations were induced through the summer. Trees were sampled at the lower (approximately 0.3 m aboveground), middle (approximately equidistant between the lower and upper samples), and upper tree sections (approximately 0.3 m below the tree top) by removing two branches from opposite sides of the bole at each respective tree section (60 branches total). Juvabione extraction and quantification was performed using the methods described above and in Fig. 1.

To compare juvabione concentrations between infested and uninfested trees statistical analysis was performed using nonparametric Wilcoxon rank-sum tests (Hollander and Wolfe 1973).

Results and discussion

JRCs in uninfested trees

Juvabione and dehydrojuvabione were the predominant JRCs detected in the uninfested trees examined in this study. Epijuvabiol was detected in inconsistent trace amounts and was not included in the analysis. Dehydrojuvabione was present in larger amounts than juvabione in all trees and tree parts (Table 1). The results indicate that JRCs are endogenous in *Abies fraseri* and not solely a response to BWA infestation. Table 1 shows that large variations in JRC concentrations were present across the tested trees and that position was an important factor in determining juvabione concentration ($F = 14.5$, $P = 0.0001$, df = 3). The uneven distribution of JRCs among trees was similar to that found in *Abies sachalinensis* (Schmidt) (Kawai et al. 1993), *Abies lasiocarpa* (Manville and Kriz 1977), *Abies balsamea* (Manville 1975), *Abies alba* (L.) (Manville et al. 1977), and *Abies bifolia* (L.) (Manville and Tracey 1989). The upper stem sample consistently had the highest amount of juvabione compared with other stem tree sections, but this was not the case for dehydrojuvabione (Table 1). The correlation analysis showed that the juvabione concentrations from upper stem and upper branch were not highly correlated (Table 2), but the dehydrojuvabione concentrations from upper stem and upper branch were highly correlated ($\alpha = 0.01$) (Table 2).

Significant correlations were also present for juvabione concentrations between the upper branch and mid-branch ($\alpha = 0.01$), and upper branch and low branch ($\alpha = 0.05$) sampling positions (Table 2). In addition to the significant correlation between the high stem and high branch concentrations of dehydrojuvabione, significant correlations were also found between high stem and mid-branch ($\alpha = 0.01$) and high branch and mid-branch ($\alpha = 0.01$).

The correlation analysis of the data from the two cut trees shows that there are three significantly correlated pairs: stem juvabione vs. branch juvabione ($\alpha = 0.05$), stem juvabione vs. branch dehydrojuvabione ($\alpha = 0.01$), and branch juvabione vs. branch dehydrojuvabione ($\alpha = 0.01$). For dehydrojuvabione, however, the results did not show a strong correlation. The significant correlations between stem juvabione and branch dehydrojuvabione and between branch juvabione and branch dehydrojuvabione are not clearly understood, since both pairs are inversely correlated. Manville and Tracey (1989) postulated a biosynthesis pathway for JRCs in coastal *Abies lasiocarpa*. According to their biosynthetic pathway, dehydrojuvabione was the precursor of juvabione. This may explain why dehydrojuvabione measurements were consistently higher than juvabione levels (Table 1). It would be interesting to measure how dehydrojuvabione and juvabione levels change throughout the year.

Juvabione in infested and uninfested trees

The infested trees had higher mean levels of juvabione than uninfested trees at each sampled tree section (upper, middle, bottom) (Tables 2 and 3). The rank-sum test revealed a significant difference between the middle tree sections at $p = 0.026$. However, since three rank-sum analyses were performed, the calculated p values should be multiplied

Table 2. Correlation analysis for the significant pairs of variables listed in Table 1 (Pearson's correlation coefficients $p > R$ under H_0: $\rho = 0$, $N = 7$).

		Juvabione			Dehydrojuvabione			
Variable		High branch	Middle branch	Low branch	High stem	High branch	Middle branch	Low branch
Juvabione								
High stem	*R*	0.6489	0.4477	0.7437	0.5268	0.6284	0.5286	0.6873
	p	0.1148	0.3137	0.0553	0.2244	0.1307	0.2225	0.0880
High branch	*R*		0.9232	0.7650	0.3939	0.5504	0.6330	–0.0030
	p		0.0030	0.0451	0.3820	0.2004	0.1270	0.9949
Middle branch	*R*			0.6736	0.2658	0.4365	0.5502	–0.2572
	p			0.0971	0.5646	0.3275	0.2007	0.5776
Low branch	*R*				0.0785	0.3620	0.3683	0.4286
	p				0.8672	0.4249	0.4163	0.3374
Dehydrojuvabione								
High stem	*R*					0.9392	0.8956	0.2317
	p					0.0017	0.0064	0.6172
High branch	*R*						0.9733	0.3093
	p						0.0002	0.4996
Middle branch	*R*							0.1211
	p							0.7959

by 3 using the Bonferroni adjustment (J.F. Monahan, Department of Statistics, North Carolina State University, Raleigh, personal communication). This removes the observed significant difference (at $\alpha = 0.05$) in the middle tree section ($p = 0.078$) but still indicates that juvabione may be an induced response because of the low p value. No significant differences were found between the top and bottom of the infested and uninfested trees, which had p values of 0.342 and 0.427, respectively (Table 2).

Branches of infested fir trees had higher mean juvabione levels than branches from uninfested trees. While this difference was not statistically significant ($p = 0.078$), we believe that future experiments with larger sample sizes will confirm that BWA infestation results in higher levels of juvabione.

Our tests included fir trees from several different provinces but were not designed to formally test for seed source effects. There were no obvious differences. Earlier experiments demonstrated that firs from Mount Rogers, Virginia, produced significantly higher levels of juvabione than controls when infested with BWA, but firs from Roan Mountain and the Great Smokey Mountain National Park did not. As in this study, the highest juvabione levels were present in the top of the fir (Zhang 1994).

If juvabione is important in the resistance of young trees to BWA attack, this resistance mechanism is probably only expressed in a small proportion of the *Abies fraseri* population. The high rate of tree mortality that has occurred in natural stands (Dull et al. 1988) demonstrates that most *Abies fraseri* are highly susceptible. Levels of BWA infestation were not measured quantitatively for the infested trees, and hence, we cannot directly relate juvabione levels to tree resistance. We did observe that two of the infested firs maintained their apical dominance, while eight trees did not (Table 4). Most infested trees lose their apical dominance in the early stages of a BWA infestation. Interestingly, the two trees that maintained their apical dominance also had the highest juvabione levels in the upper tree section. Notably, tree No. 5 had a juvabione level of 770 μg/g compared with a mean of 58.6 μg/g for the eight infested trees that lost apical dominance. High levels of juvabione were also observed in one uninfested tree (Table 3); thus, it is not clear if the high levels were induced or are constitutive. Infested trees, such as No. 5 (Table 4), with exceptionally high levels of juvabione are of particular interest. Additional research could verify the resistance of trees with abnormally high levels of juvabione by screening to identify such trees and then infesting them with BWA.

Table 3. Juvabione levels (μg/g) for uninfested trees based on tree section of sampling (two branch samples/data point).

Tree No.	Seed source*	Lower	Middle	Upper
1	GSM	76.2	54.3	111.0
2	GSM	39.9	3.5	47.5
3	GSM	98.4	68.4	76.7
4	MR	134.6	59.3	28.3
5	MR	69.9	37.4	56.7
6	na	163.7	88.6	162.8
7	RB	26.2	33.4	105.2
8	RB	65.5	66.3	115.6
9	RB	30.3	49.0	35.1
10	RB	30.4	86.2	29.6
	Mean (SD)	73.5 (46.7)	54.6 (25.6)	76.9 (45.3)

Note: All uninfested trees maintained their apical dominance.
*Seed source abbreviations are as follows: MR, Mount Rogers; GSM, Great Smokey Mountains; RB, Richland Balsam; na, not available.

There was considerable variation in juvabione levels among branches taken from each side of the tree within the same tree section. It appears that juvabione may be produced by the vascular cambium, and the vascular cambium may be stimulated to produce more juvabione when infested by BWA. We have observed comparable variation in BWA infestations among even-aged *Abies fraseri* in the Smokey

Table 4. Juvabione levels (μg/g) for infested trees based on tree section of sampling (two branch samples/data point).

Tree No.	Seed source*	Lower	Middle	Upper
1	MR	247.7	88.8	118.8
2	na	57.7	35.8	33.9
3	na	112.6	161.5	41.9
4†	RB	10.3	103.0	152.4
5†	RB	107.1	91.4	770.8
6	RB	79.2	35.1	42.8
7	RN	52.0	89.0	61.0
8	RN	78.5	79.6	66.6
9	RN	57.6	106.4	45.3
10	RN	40.4	40.4	53.9
	Mean (SD)	84.3 (64.9)	83.1 (38.9)	138.7 (225.3)

*Seed source abbreviations are as follows: MR, Mount Rogers; RB, Richland Balsam; RN, Roan Mountain; na, not available.
†Trees that maintained their apical dominance; otherwise, apical dominance was lost.

Mountains. It would be interesting to compare tree section juvabione levels with corresponding BWA infestation levels.

In conclusion, JRCs are present in uninfested *Abies fraseri*. The JRC distribution patterns in uninfested young *Abies fraseri* can be outlined as follows: (*i*) JRCs (juvabione and dehydrojuvabione) concentrations are quite different from one tree to another; (*ii*) dehydrojuvabione concentrations exceed juvabione concentrations, indicating that dehydrojuvabione may be a precursor in the biosynthetic pathway; (*iii*) juvabione has a higher concentration at higher sampling positions for both branch and stem samples; and (*iv*) higher concentrations of juvabione are present in stems than in the branches.

Infested firs had higher mean juvabione levels than uninfested trees. If a BWA infestation does induce an increased concentration of juvabione, then trees such as No. 5 (Table 4) that have unusually high levels of juvabione and are infested may possess some degree of resistance to the adelgid. Further studies investigating the interaction between juvabione and the BWA will be reported in subsequent papers.

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