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Full Length Research Paper

Effects of exogenous jasmonates on free amino acid contents in needles of *Larix olgensis* seedlings

Zhao Jun Meng, Shan Chun Yan*, Dan Liu and Chuan Ping Yang

Key Laboratory of Forestry Tree Genetic Improvement and Biotechnology of Ministry of Education, College of Forestry, Northeast Forestry University, Harbin 150040, China.

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Jasmonates (JAs) are plant signal compounds and can induce many biochemical responses. In the current study, the effects of three concentrations of exogenous JAs plus control on the free amino acid (FAA) contents in needles of *Larix olgensis* Herry seedlings were investigated. Our results showed that the proportions of nine essential FAAs detected/identified in all treatments varied with concentrations of JAs. Total FAA contents in control needles fluctuated in an increasing-decreasing-increasing pattern within a 20-day period; however, a decreasing-increasing-decreasing pattern was observed in JAs-treated needles compared to their controls. They were significantly affected by the interactions of time, JAs type and JAs concentration. Furthermore, methyl jasmonate (MeJA) showed stronger treatment effect on total FAA contents than did *cis*-jasmone (*cis*J) or jasmonic acid (JA). While total FAA contents were significantly influenced by 0.01 mM JA, 0.1 mM *cis*J and 0.1 and 1 mM MeJA treatments compared to their corresponding controls. The results indicated that exogenous JAs might induce significant changes of the individual FAA ratios and total FAA contents in larch needles. This could be a potential factor influencing the preference and performance of pest insects that utilize *L. olgensis* as a host.

Key words: *Larix olgensis*, jasmonic acid, methyl jasmonate, *cis*-jasmone, induce function.

INTRODUCTION

Free amino acids (FAA, nonprotein-bound amino acids) play an especially important role in plant adaption to unfavorable environmental conditions (Kishor et al., 2005), and have previously been involved in the resistance of cereals to aphids (Corcuera, 1993; Eleftherianos et al., 2006). Then, the changes of FAAs content in plants could be induced by various factors. The UV irradiance, increasing temperature and $ZnSO_4$ increased the content of FAAs in needles of saplings (Cánovas et al., 2007). Soil-grown tea plants supplied with different levels of potassium (K) exhibited increased concentrations of FAA with K_2SO_4 supply and decreased concentrations of FAA with the KCl supply in young shoots (Ruan et al., 2007). Saltzmann et al. (2008) reported that the hessian fly (*Mayetiola destructor* Say)-infested susceptible wheat plants (*Triticum aestivum* L.) were more responses than resistant plants (*T. aestivum*) or uninfested controls, showing higher concentrations of alanine, glutamic acid, glycine, phenylalanine, proline, serine and the aromatic amino acids phenylalanine and tyrosine. Some species of aphids could alter the nutritional quality of host wheat plants by increasing the abundance of plant FAAs in phloem (Telang et al., 1999; Sandström et al., 2000; Eleftherianos et al., 2006). FAA levels could be also affected by following inducible factors. Jasmonates (JAs) are important signaling molecules in plants (Halitschke and Baldwin, 2005; Howe, 2005), which can induce many biochemical responses (Thaler et al., 2002) and activate host defense responses against a broad spectrum of herbivores (Thaler, 1999; Gols et al., 2003; Chen et al., 2005). Jasmonic acid (JA) and its amino acid conjugates,

*Corresponding author. E-mail: yanshanchun@126.com.

Abbreviations: Jas, Jasmonates; **JA,** jasmonic acid; **MeJA,** methyl jasmonate; ***cis*J,** *cis*-jasmone; **FAA,** free amino acid; **AA,** amino acid; **Ala,** alanine; **Arg,** arginine; **Asp,** asparagine; **Cys,** cysteine; Glu, glutamine; **Gly,** glycine; His, histidine; **Ile,** isoleucine; **Leu,** leucine; Lys, lysine; **Met,** methionine; **Phe,** phenylalanine; **Pro,** proline; **Ser,** serine; **Thr,** threonine; **Tyr,** tyrosine; **Val,** valine.

Table 1. Concentrations and amounts of the exogenous JAs tested.

JAs	Received dosage (ml) /per seedling	Concentration mmol/L	Received dosage (mg)/per seedling	Abbreviation	Note
Water		0.00	0	control	
cis-jasmone		0.01	0.01	*cis*J0.01	
		0.10	0.08	*cis*J0.1	
		1.00	0.82	*cis*J1	
Methyl jasmonate	5	0.01	0.01	MeJA0.01	All solution contained 0.1% acetone (v/v)
		0.10	0.11	MeJA0.1	
		1.00	1.12	MeJA1	
Jasmonic acid		0.01	0.01	JA0.01	
		0.10	0.11	JA0.1	
		1.00	1.05	JA1	

particularly JA-isoleucine (JA-Ile), play important roles in plant-herbivore interactions (Wang et al., 2008). JA-inducible proteins (JIPs), arginase and threonine deaminase (TD), strongly influence the midgut protein content of phytophagous insects, and might have a potential in plant protection against herbivores (Chen et al., 2005).

Larix olgensis Herry is one of the major tree species of natural and plantation forests in northeast of China, with characteristics of cold hardiness, drought resistance and fast growth. However, *Larix* spp. in Northern China is often seriously damaged by various insect pests, such as *Dendrolimus superans* Butler (Lepidoptera: Lasiocampidae). *D. superans* is a major pest in the northeastern forests of China, feeding mainly on larches and Korean pine, *Pinus koraiensis* Sieb. and Zucc. Mass outbreaks of the moth can degrade large stands of larch by slowing the growth of affected trees and may further result in significant tree mortality via sequential infestations of trunk borers (Xiao, 1992). Up to now, chemical insecticides were the main control agent for *D. superans*, however, sustained use of insecticides has probably harmed non-target organisms, thereby threaten current forest ecosystems (Xiao, 1992; Gitahi et al., 2002). Our overall objective of this paper was to study the potential effects of exogenous JAs on the FAA contents in the needles of *L. olgensis* Herry, and to discuss whether the changes of FAA contents in the larch needles affected on host preference and feeding performance of a major larch defoliator, *D. superans*.

The specific goals include answering the following questions: (1) after the larch seedlings are sprayed with the different concentrations of jasmonate (JA, MeJA and *cis*J) solutions, how do the FAA levels in larch seedling needles fluctuate? (2) are there any different treatment effect on the FAA levels among the JAs? (3) which concentrations of JAs significantly affect the FAA levels in the larch needles?

MATERIALS AND METHODS

Larix olgensis seedlings

At the end of April in 2007, 2000 two-year-old larch seedlings were planted in the plastic barrels (high: 40 cm, diameter: 35 cm) of the seedling-raising net chamber at the Laoshan station of Mao'ershan Forestry Centre of Northeast Forestry University (45° 45′ N, 126° 38′ E), Heilongjiang province, China, respectively, and grown under natural light. Each seedling was planted in per barrel. The seedlings were watered and fertilized with nitrogenous, potassium, phosphorus fertilizer and micronutrients on June 4th, July 3rd and August 1st 2007, respectively.

Treatment of larch seedlings

On August 5th 2007, 900 healthy seedlings with similar size were selected and stochastically divided into 10 groups, with 90 seedlings in each group. Each solution of different concentrations of three jasmonate compounds (*cis*J, MeJA and JA, Sigma Company, USA) including control with 0.1% acetone and distilled water was evenly sprayed onto 90 seedlings of the designated group with a 1 L hand sprayer, respectively (Table 1). Each seedling received about 5 ml of the corresponding treatment solution [that is, 0.01~0.82 mg *cis*J, 0.01~1.12 mg MeJA, 0.01~1.05 mg JA, or 0 mg *cis*J, MeJA, and JA (control) per corresponding treatment seedling, respectively; such dosage ranges had not shown significant negative effects on the normal growth of larch seedlings], respectively. In order to get the same amount of solution on each seedling uniformly, before spraying, the 5 ml of treatment solution was further diluted to 30 ml with 0.1% acetone and distilled water (Lu et al., 2004; Heijari et al., 2005).

Sampling of the seedling needles

About 1 g of larch needles from all branches of each seedling and the fifteen seedlings from each treatment/date in each group were sampled at the 1st, 3rd, 5th, 10th, 15th and 20th day after the spray application, respectively. The seedlings of each group were sampled for six times/dates. The seedlings from each treatment/date were consisted of three replicates and 5 seedlings were regarded as a replicate. The needles of each seedling were sampled only once

Table 2. HPLC operation procedures for FAA analysis.

Time (min)	Velocity of flow (ml/min)	Mobile phase (%)			Analysis curve
		A	B	C	
Initial	1.0	100	0	0	6
0.5	1.0	99	1	0	11
18.0	1.0	95	5	0	11
19.0	1.0	91	9	0	11
29.5	1.0	83	17	0	6
33.0	1.0	0	60	40	11
36.0	1.0	100	0	0	11
47.0	1.0	100	0	0	11

and then the sampled seedlings were taken away for avoiding the effects of the mechanical damage to FAAs content in the needles. These sampled needles were ground in liquid nitrogen and kept frozen in order to determine the content of FAAs (Neves et al., 2002).

Extraction and derivatization of FAAs

Approximately, 1 g of larch needles of each sampling unit was homogenized by mortar and pestle in the presence of 3 ml of water : chloroform : methanol (3 : 5 : 12, v / v) and were further diluted with another 3 ml of the same mixture and kept at 4°C for 24 h. After centrifugation (15 min, 12000 rpm, 4°C), the supernatant was collected. Chloroform (1.5 ml) and water (1 ml) were added, and the resulting mixture was centrifuged again. The upper water-methanol phase was collected, evaporated and dissolved in 1 ml of Milli-Q water. Then, 1 ml of supernatant filtered through a 0.45 μm filter (Millipore) was transferred to a clean autosampler vial for derivatization (Neves et al., 2002; Chen et al., 2005). 20 μL of FAA extract of each sample was derivatized using Waters AccQ · Fluor Reagent Kit (according to Waters AccQ · Tag Chemistry Package Instruction Manual).

Analysis of the FAA content

The derivative samples were analyzed and identified by high performance liquid chromatography (HPLC, Waters 2695 Separations Module, Waters 2996 PDA Detector, Waters AccQ · Tag Amino Acid Analysis Column, American Waters Company). Mobile phase A was composed of the mixed-solution with Waters AccQ · Tag Eluent A Concentrate and Milli-Q water in 1:10 (V/V), and mobile phase B was 100% acetonitrile (Honeywell international Inc., Burdick and Jackson, USA), and then mobile phase C was Milli-Q water. All the mobile phases were filtered through a 0.45 μm filter and degasified. The on-line degasification with the high-purity helium gas was conducted when analyzing the samples (according to Waters AccQ · Tag Chemistry Package Instruction Manual). Some of the HPLC operation procedures/parameters for FAA analysis are shown in Table 2. The column temperature was 30°C, and the wavelength of the ultraviolet detection ranged from 200 ~ 400 nm. The flow speed was 1 ml min^{-1}, and then the sampling volume was 10 μL per time. The components and contents of FAA samples were identified and quantified by comparison of retention times and peak areas with those of authentic standards (Waters Amino Acid Hydrolysate Standard, according to Waters AccQ · Tag Chemistry Package Instruction Manual).

Data analysis

The Microsoft Excel was used for data presentations in both table and figure formats. One-way ANOVA on the different concentrations of *cis*J (MeJA or JA) and control, Univariate ANOVA on time, JAs types and concentrations of JAs were analyzed by SPSS13.0 followed by multiple comparisons of Tukey HSD.

RESULTS

Chromatogram of the 17 amino acid standards and the FAAs of four larch needle samples

The representative chromatogram of the 17 AA standards is shown in Figure 1, in which the amount of cystine was 0.25 mM, whereas, the others were 0.5 mM each. Their retention time ranged from 18.281 ~ 35.358 min. The representative chromatograms of the FAAs from four larch needle samples taken at the 3rd day after the following treatments: water control, 0.1 mM *cis*J, 0.1 mM MeJA and 0.1 mM JA are shown in Figure 2.

Temporal variation of total FAA contents

According to Figure 3a, total FAA contents in the water control *Larix* needles fluctuated in an increasing-decreasing-increasing pattern, however, an opposite variation pattern, that is, decreasing-increasing-decreasing fluctuation was observed in needles *cis*J treated. At 1st, 5th and 10th day after the treatment, the total needle FAA contents in the *cis*J treatments were significantly higher than those of controls. While, at the 3rd, 15th and 20th day, all treatments were significantly lower than controls in their FAA contents except for the *cis*J0.1-d15 and *cis*J0.01-d20. At the 5th day after the treatment, there were no differences in FAA contents among the *cis*J treatments. As shown in Figure 3b and 3c, the variations of total FAA contents in the larch needles treated with MeJA and JA were similar to those of *cis*J treatments. At the 15th day after the treatment, total FAA contents in the needle samples treated with MeJA did not differ from that of controls, but were somewhat different among the MeJA treatments (Figure 3b). However, there were no

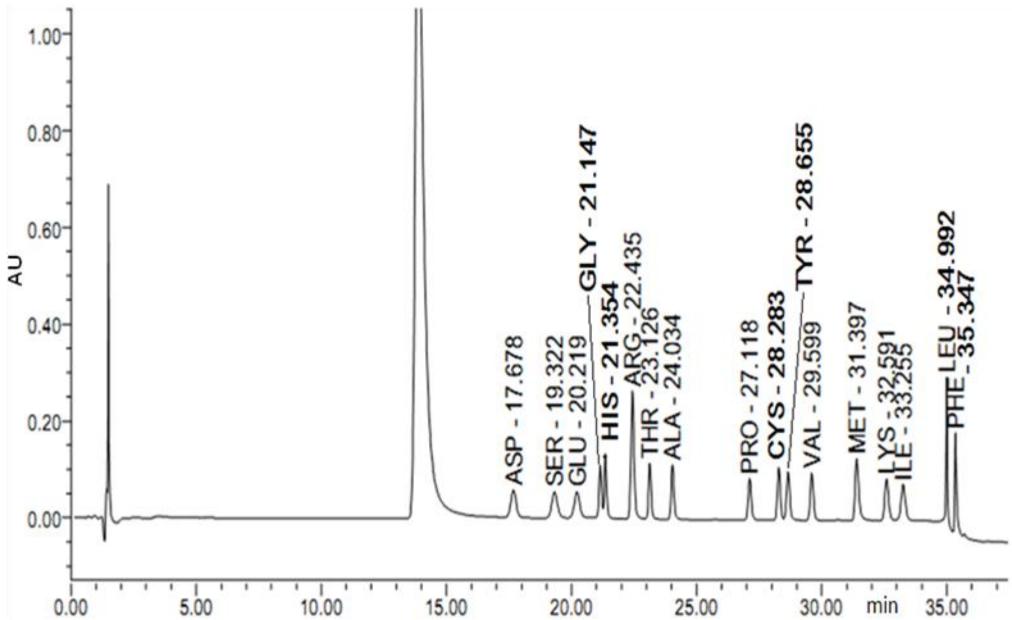

Figure 1. Representative HPLC chromatogram of the 17 amino acid standards.

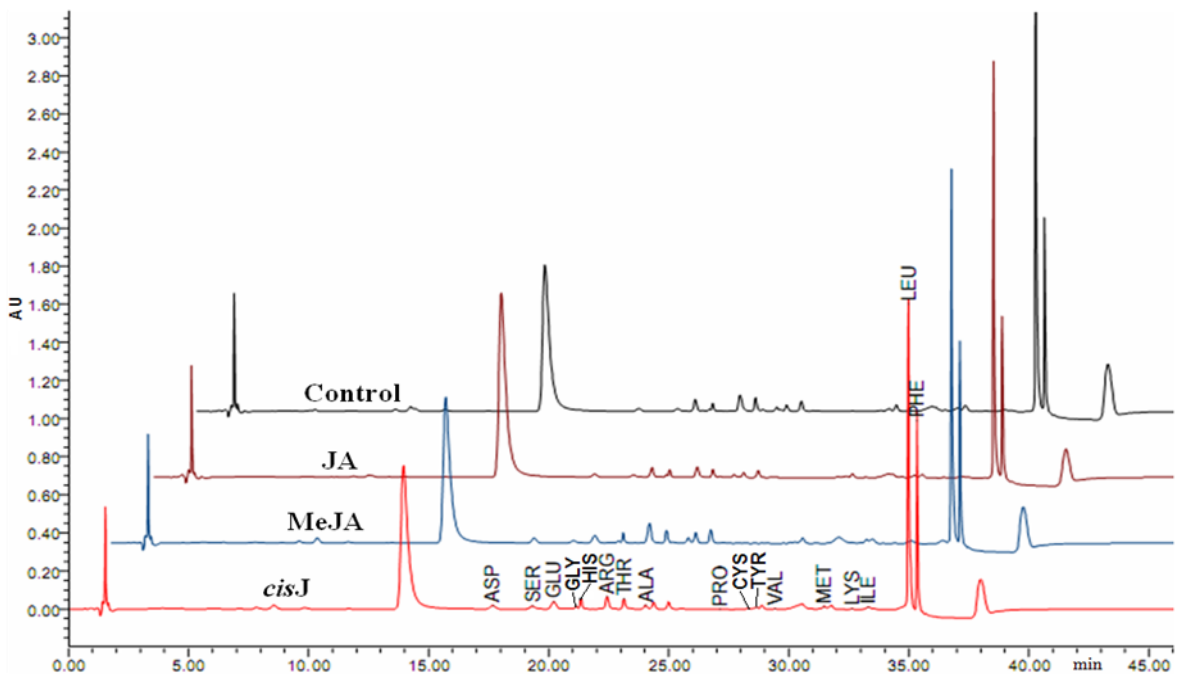

Figure 2. Representative HPLC chromatograms of the FAAs in the control, 0.1 mM *cis*J, 0.1 mM MeJA and 0.1 mM JA treated larch needle samples taken on the 3rd day after treatment (Note: color only on-line).

differences in total FAA contents among the JA treatments, but they were all significantly lower than control (Figure3c).

Univariate analysis of variance of total FAA contents

Based on the Multiple Comparisons of Tukey HSD of Univariate Analysis of Variance (n = 3), 0.1 mM *cis*J, 0.1 and 1 mM MeJA, and 0.01 mM JA significantly increased total FAA contents in larch needles compared to other treatments ($P < 0.05$) (Figure 4a to c). MeJA significantly increased total FAA contents in *L. olgensis* compared with JA and *cis*J ($P < 0.001$) (Figure 5). While, the interactions between the different time and the different concentrations

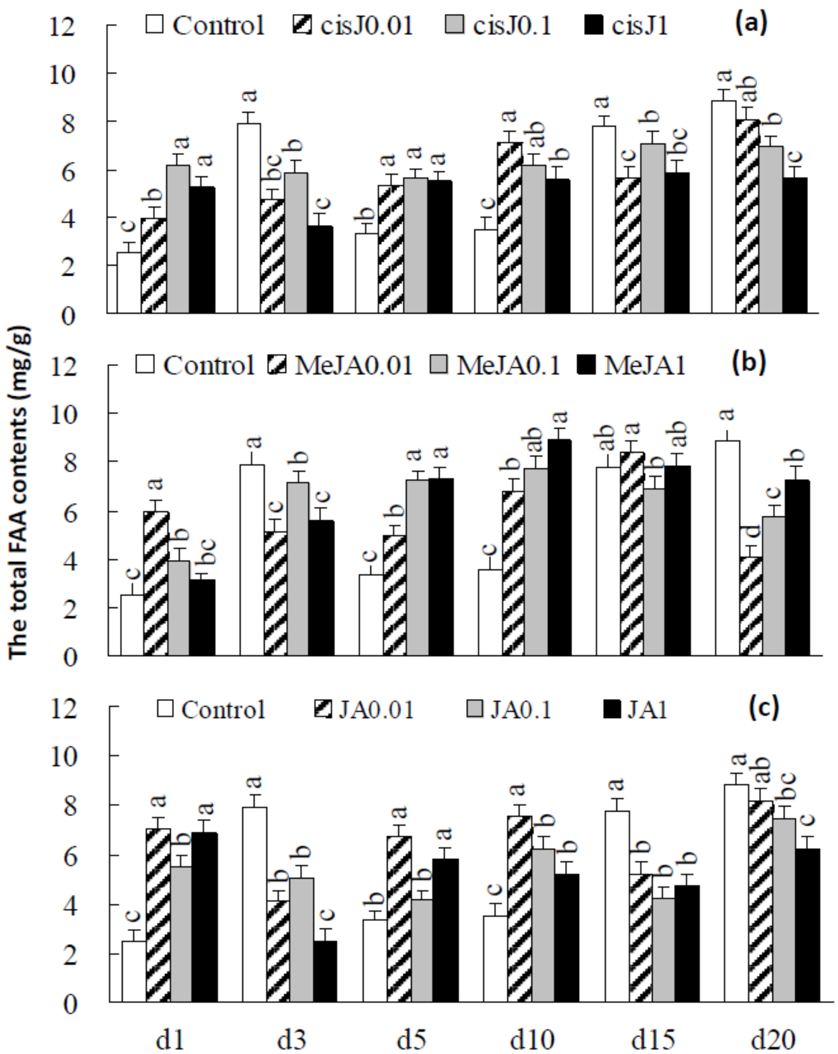

Figure 3. Total FAA contents (mean ± S.E) in the needles of *L. olgensis* seedlings at the 1st, 3rd, 5th, 10th, 15th and 20th day after the *cis*J (a), MeJA (b) and JA (c) treatments. Within the same sampling date, bars with different letters are significantly different (ANOVA followed by Multiple Comparisons of Tukey HSD, n = 3, $P < 0.05$).

of *cis*J, MeJA or JA significantly influenced total FAA contents in larch needles ($F_{Time \times cisJ\ contents} = 30.798$, $P < 0.001$; $F_{Time \times MeJA\ contents} = 43.577$, $P < 0.001$; $F_{Time \times JA\ contents} = 45.959$, $P < 0.001$) (Figure 4a to c). The interaction between the different time and the different JAs significantly affected total FAA contents in larch needles (F = 15.123, $P < 0.001$) (Figure 5).

Comparative analysis of 9 essential FAA ratios

17 FAA components were detected/identified in the larch needles. Compared to the controls, the relative contents (that is, each FAA content accounts for the percentage of total FAAs contents) of each FAA in the treatment needles at different sampling dates and different concentrations were significantly different. However, they did not show the distinct tendencies in the changes in 9 FAAs according to the time and the concentrations of all treatment. Among these FAAs, relative contents of 9 essential FAAs, Arg, His, Ile, Leu, Lys, Met, Phe, Thr and Val ranged from 0.70 ~ 5.14%, 0.18 ~ 2.18%, 1.58 ~ 11.31%, 39.44 ~ 63.71%, 1.38 ~ 4.41%, 0.65 ~ 3.45%, 8.74 ~ 40.11%, 0.30 ~ 2.73% and 0.19 ~ 1.81%, respectively. Leu, Phe and Ile were three dominant essential FAAs, accounting for 78.14 ~ 91.08% of total FAA contents (Table 3 to 5). After the *cis*J treatments, relative contents of Arg, Thr at the 1st and 10th day, Val, Met at the 5th and 15th day, Leu at the 5th day and Ile at the 15th day were significantly lower than those of their

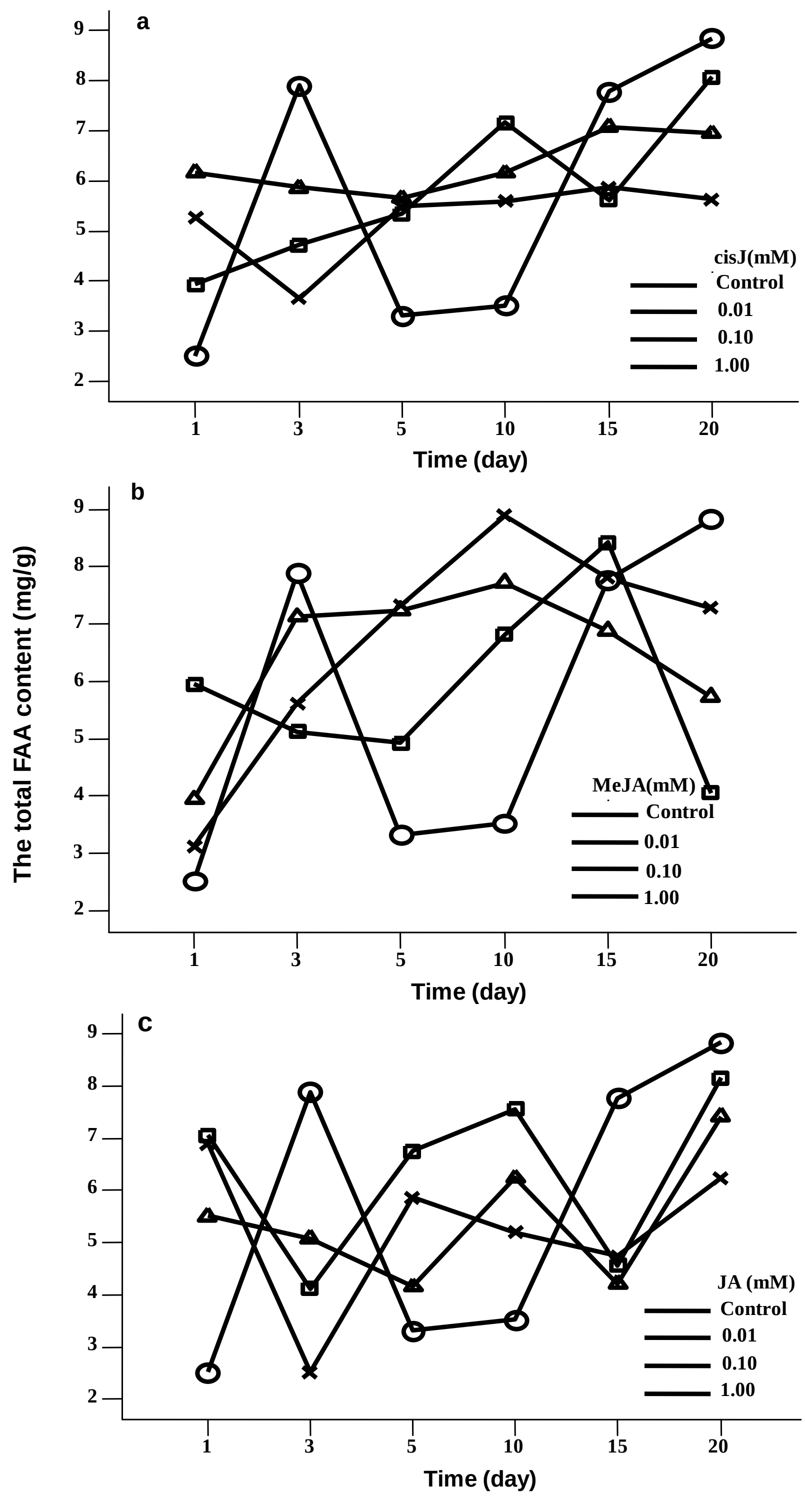

Figure 4. Effect of the interaction of time and *cis*J, MeJA or JA contents on the total FAA content in *L. olgensis* needles after the JAs treatment at univariate analysis of variance (n = 3).

controls. However, relative contents of Ile, Met at the 1st, 3rd and 10th day, Lys at the 1st and 10th day, Val at the 1st and 3rd day, Phe at the 5th day and Leu at the 15th day were significantly higher than those of their controls (Table 3). Among the three concentrations of the *cis*J treatments, relative contents of Ile, Lys, Val in 0.01 mM

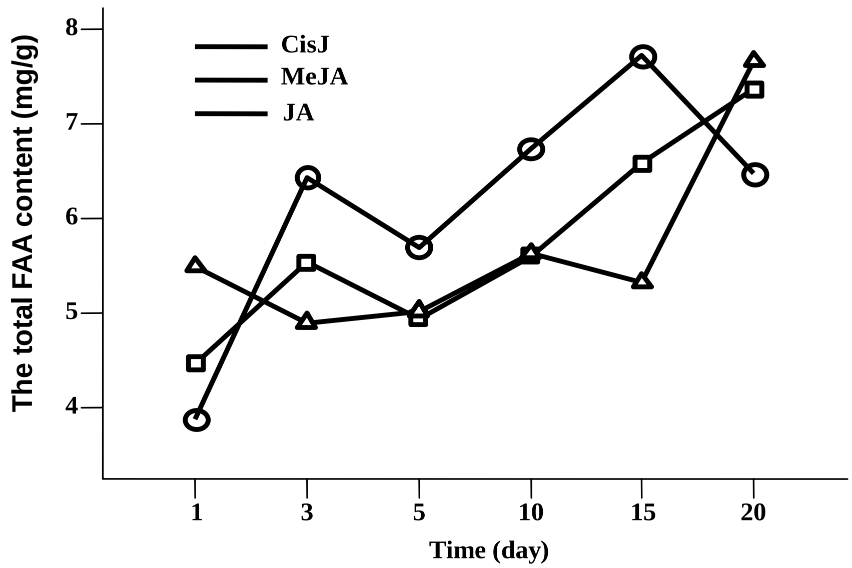

Figure 5. Effect of the interaction of time and JAs types on the total FAA content in *L. olgensis* needles after the JAs treatment at univariate analysis of variance (n = 3).

*cis*J treatment at the 1st and 5th day, Met, Thr at the 1st, 3rd and 15th day, Arg at the 5th and 15th day, and then relative contents of His, Lys in 1 mM *cis*J treatment at the 3rd, 10th and 20th day, Met, Thr, Val at the 3rd and 20th day, Arg at the 1st, 3rd, 10th and 20th day, Phe at the 1st, 15th and 20th day were significantly higher than those of their corresponding others (Table 3).

After the MeJA treatments, relative contents of Arg at the 1st, 5th and 10th day, Leu at the 5th, 10th and 20th day, Thr at the 3rd and 10th day, His at the 3rd and 5th day, Met, Val at the 5th day and Ile at the 15th day were significantly lower than those of their controls. However, relative contents of Ile, Lys, Val at the 1st, 3rd and 10th day, Met at the 1st and 15th day, Phe at the 5th and 15th day, Leu at the 15th day and Lys, Thr, Val at the 20th day were significantly higher than those of their controls (Table 4). Among the three concentrations of the MeJA treatments, relative contents of Arg, His, Leu, Met, Phe, Val in 0.01 mM MeJA treatment at the 3rd day, Arg, Ile, Lys at the 5th day, Lys, Met at the 10th day, Met, Val at the 15th day, Ile, Leu at the 20th day, and then relative contents of Thr in 0.1 mM MeJA treatment at the 5th, 15th and 20th day, Ile at the 15th day, Arg, Val at the 20th day, and relative contents of Ile, Lys, Val in 1 mM MeJA treatment at the 1st day, His, Thr, Val at 10th day were significantly higher than those of their corresponding others (Table 4). After the JA treatments, relative contents of Arg, Thr at the 1st and 10th day, Leu, His at the 1st, 5th day, Phe at the 3rd, 15th day, Leu at the 20th day and Ile at the 15th day were significantly lower than those of their controls. However, relative contents of Met at the 1st, 15th day, Phe at the 1st, 5th day, Thr at the 15th, 20th day, Arg, Leu, Lys at the 15th day and His at the 20th day were significantly higher than those of their controls (Table 5).

Among the three concentrations of the JA treatments, relative contents of Arg, Val in 0.01 mM JA treatment at the 1st and 5th day, His, Ile at the 1st day, Ile, Met, Val at the 3rd day, and then relative contents of Lys, Thr in 0.1 mM JA treatment at the 1st and 5th day, His, Ile, Phe at the 5th day, and relative contents of His, Lys in 1 mM JA treatment at the 3rd, 10th and 20th day, Ile, Met at the 10th and 20th day, Arg at the 3rd and 20th day, Thr, Val at the 15th and 20th day and Thr at the 10th day were significantly higher than those of their corresponding others (Table 5).

DISCUSSION

Effects of application of JAs on total FAA contents in needles of larch seedlings and on host preference and feeding performance of *D. superans*

Our results showed that the variations of total FAA contents in the needles of JAs treated larch seedlings were significantly different from those of their controls. Total FAA contents of controls tended to fluctuate in an increasing-decreasing-increasing pattern during testing period. However, total FAA contents of the JAs treated seedlings showed opposite fluctuation patterns compared to their controls. Studies of Kim and Glerum (1995) showed that the concentrations of FAAs in the red pine (P. resinosa Ait.) and the white spruce [Picea glauca (Moench) Voss] needles exhibited seasonal fluctuations. Lee et al. (2011) found that the levels of FAAs were significantly increased only in August by girdling woody stems of Japanese larch tree (L. leptolepis) (42-year-old) potential role in the glassy-winged sharpshooter [GWSS, compared

Table 3. Relative contents (%) of the 9 essential FAAs identified in the larch needles after *cis*J treatments.

Treatment	Time/day	FAA relative contents (%)								
		Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Control	d1	3.78±0.37^{a}	0.95±0.00^{b}	4.87±0.09^{c}	59.72±2.94^{a}	2.14±0.02^{d}	1.29±0.09^{c}	19.08±2.88^{bc}	1.84±0.06^{a}	0.44±0.09^{d}
*cis*J0.01		1.89±0.01^{c}	0.42±0.07^{d}	8.26±0.33^{a}	54.34±1.13^{bc}	3.35±0.05^{a}	2.92±0.17^{a}	17.84±1.72^{c}	1.71±0.01^{b}	1.17±0.02^{a}
*cis*J0.1		1.27±0.02^{d}	1.19±0.03^{a}	6.17±0.07^{b}	57.86±1.11^{ab}	2.34±0.01^{c}	2.04±0.05^{b}	22.49±1.02^{b}	1.47±0.02^{c}	0.94±0.00^{b}
*cis*J1		2.10±0.00^{b}	0.62±0.00^{c}	6.41±0.07^{b}	51.78±0.44^{c}	2.48±0.02^{b}	2.04±0.04^{b}	27.38±0.34^{a}	1.47±0.01^{c}	0.81±0.03^{c}
Control	d3	1.52±0.08^{c}	0.96±0.01^{a}	2.91±0.03^{c}	53.37±0.83^{a}	1.95±0.06^{c}	1.58±0.02^{c}	32.22±0.63^{a}	1.46±0.01^{b}	0.72±0.00^{c}
*cis*J0.01		2.12±0.11^{b}	0.48±0.07^{c}	6.70±0.31^{b}	49.98±0.94^{b}	3.25±0.23^{b}	2.17±0.09^{b}	28.49±1.20^{a}	1.39±0.03^{b}	0.89±0.01^{b}
*cis*J0.1		1.33±0.11^{c}	0.63±0.04^{b}	6.05±0.19^{b}	53.07±0.97^{ab}	2.38±0.13^{c}	2.07±0.04^{b}	27.38±1.14^{a}	0.30±0.12^{c}	0.83±0.02^{b}
*cis*J1		2.45±0.09^{a}	1.03±0.04^{a}	9.59±0.82^{a}	53.79±1.77^{a}	4.11±0.42^{a}	3.20±0.25^{a}	16.21±3.34^{b}	2.12±0.07^{a}	1.22±0.05^{a}
Control	d5	2.20±0.03^{a}	0.74±0.06^{b}	6.16±0.03^{b}	63.41±3.22^{a}	2.26±0.01^{b}	2.67±0.02^{a}	11.63±3.20^{d}	1.70±0.09^{b}	1.10±0.03^{a}
*cis*J0.01		2.41±0.06^{a}	0.60±0.08^{c}	6.86±0.11^{a}	53.29±1.53^{b}	2.56±0.07^{a}	2.28±0.02^{b}	22.16±1.43^{c}	2.57±0.04^{a}	0.86±0.05^{b}
*cis*J0.1		1.54±0.08^{b}	1.19±0.02^{a}	3.65±0.17^{c}	45.92±0.59^{c}	1.38±0.05^{c}	0.65±0.19^{c}	40.11±0.39^{a}	1.47±0.10^{c}	0.34±0.07^{c}
*cis*J1		1.24±0.12^{c}	1.28±0.00^{a}	2.59±0.23^{d}	51.14±0.89^{b}	2.31±0.04^{b}	0.78±0.14^{c}	33.75±0.09^{b}	1.60±0.08^{bc}	0.27±0.07^{c}
Control	d10	3.48±0.19^{a}	0.33±0.05^{c}	3.51±0.43^{c}	58.56±4.27^{a}	2.03±0.02^{d}	0.99±0.29^{b}	21.28±2.77^{b}	2.51±0.13^{a}	0.35±0.28^{b}
*cis*J0.01		1.61±0.08^{c}	0.35±0.03^{c}	5.22±0.15^{b}	44.29±1.18^{b}	2.12±0.00^{c}	1.86±0.04^{a}	36.02±0.31^{a}	0.40±0.07^{c}	0.66±0.12^{b}
*cis*J0.1		1.30±0.09^{d}	0.44±0.03^{b}	5.89±0.03^{a}	56.56±2.19^{a}	2.32±0.01^{b}	2.17±0.01^{a}	22.63±1.40^{b}	1.49±0.04^{b}	1.16±0.10^{a}
*cis*J1		2.29±0.00^{b}	0.91±0.01^{a}	6.37±0.04^{a}	53.84±1.97^{a}	2.47±0.03^{a}	2.12±0.06^{a}	23.82±1.10^{b}	1.66±0.05^{b}	0.81±0.18^{ab}
Control	d15	1.64±0.08^{c}	0.75±0.06^{b}	5.39±0.05^{a}	50.25±0.65^{c}	2.49±0.06^{b}	1.42±0.04^{a}	29.72±0.32^{b}	0.77±0.06^{c}	1.36±0.03^{a}
*cis*J0.01		3.90±0.08^{a}	1.06±0.05^{a}	1.95±0.20^{bc}	57.43±1.22^{a}	3.39±0.15^{a}	1.12±0.09^{b}	20.46±0.95^{d}	2.73±0.07^{a}	0.45±0.13^{b}
*cis*J0.1		3.25±0.02^{b}	0.58±0.09^{c}	2.39±0.16^{b}	58.36±1.00^{a}	3.19±0.12^{a}	0.77±0.10^{c}	25.38±0.41^{c}	0.79±0.05^{c}	0.17±0.09^{b}
*cis*J1		1.00±0.17^{d}	1.15±0.06^{a}	1.58±0.29^{c}	54.00±1.40^{b}	2.55±0.04^{b}	0.67±0.16^{c}	33.56±0.09^{a}	1.07±0.08^{b}	0.19±0.18^{b}
Control	d20	1.91±0.01^{b}	0.34±0.11^{c}	4.28±0.04^{a}	57.38±0.50^{b}	2.19±0.04^{b}	2.17±0.04^{b}	26.22±0.19^{b}	0.30±0.10^{c}	0.58±0.00^{c}
*cis*J0.01		1.25±0.04^{c}	0.48±0.09^{c}	4.30±0.03^{a}	59.32±0.64^{a}	1.78±0.03^{c}	1.60±0.01^{d}	24.68±0.27^{c}	0.47±0.07^{c}	0.56±0.01^{c}
*cis*J0.1		1.09±0.03^{d}	1.12±0.08^{b}	3.49±0.03^{b}	57.23±0.63^{b}	2.07±0.03^{b}	1.73±0.02^{c}	26.17±0.22^{b}	1.34±0.05^{b}	0.77±0.00^{b}
*cis*J1		2.45±0.05^{a}	2.04±0.06^{a}	4.34±0.08^{a}	39.44±0.58^{c}	2.87±0.10^{a}	2.44±0.07^{a}	35.19±0.41^{a}	1.73±0.01^{a}	1.03±0.00^{a}

Note: Numbers (mean ± SE) in the same column with different letters within the same time of *cis*J treatments are significant different ($P < 0.05$) (ANOVA followed by Multiple Comparisons of Tukey HSD, n = 3).

to the non-girdled trees. These results indicated that the levels of FAAs could be significantly affected by the JAs, girdling and season. Changes of the amino acids might play a *Homalodisca vitripennis* (Germar)] host selection (Bi et al., 2007). Mean concentrations of the FAAs were

Table 4. Relative contents (%) of the 9 essential FAAs identified in the larch needles after MeJA treatments.

Treatment	Time/day	FAA relative contents (%)								
		Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Control	d1	3.78±0.37[a]	0.95±0.00[a]	4.87±0.09[d]	59.72±2.94[a]	2.14±0.02[d]	1.29±0.09[c]	19.08±2.88[a]	1.84±0.06[b]	0.44±0.09[d]
MeJA0.01		3.00±0.10[b]	0.69±0.04[b]	6.79±0.10[c]	57.51±0.93[ab]	3.08±0.05[c]	2.71±0.06[b]	16.03±1.21[a]	2.10±0.04[a]	1.03±0.01[c]
MeJA0.1		2.57±0.06[b]	0.72±0.02[b]	9.25±0.54[b]	50.72±0.83[b]	4.04±0.18[b]	3.17±0.21[a]	20.51±1.77[a]	1.80±0.01[b]	1.23±0.04[b]
MeJA1		1.91±0.10[c]	0.82±0.12[ab]	11.31±0.09[a]	58.88±4.47[a]	4.41±0.00[a]	3.45±0.06[a]	8.74±3.74[b]	1.53±0.12[c]	1.45±0.04[a]
Control	d3	1.52±0.08[c]	0.96±0.01[a]	2.91±0.03[c]	53.37±0.83[b]	1.95±0.06[b]	1.58±0.02[b]	32.22±0.63[b]	1.46±0.01[a]	0.72±0.00[d]
MeJA0.01		5.14±0.27[a]	0.58±0.03[b]	6.74±0.40[a]	60.62±2.07[a]	2.58±0.18[a]	2.47±0.14[a]	14.49±2.94[c]	1.22±0.06[b]	1.06±0.02[a]
MeJA0.1		2.00±0.06[b]	0.21±0.06[c]	4.82±0.15[b]	45.20±0.35[c]	2.34±0.09[a]	1.23±0.02[d]	36.95±0.31[a]	0.34±0.09[c]	0.92±0.03[b]
MeJA1		1.79±0.11[bc]	0.24±0.06[c]	6.28±0.30[a]	41.84±0.14[d]	2.34±0.10[a]	2.10±0.04[b]	38.54±0.04[a]	0.33±0.13[c]	0.85±0.00[c]
Control	d5	2.20±0.03[a]	0.74±0.06[a]	6.16±0.03[a]	63.41±3.22[a]	2.26±0.01[c]	2.67±0.02[a]	11.63±3.20[c]	1.70±0.09[ab]	1.10±0.03[a]
MeJA0.01		1.54±0.12[b]	0.23±0.11[c]	6.15±0.01[a]	51.35±1.31[bc]	3.26±0.11[a]	2.22±0.00[b]	27.49±0.91[b]	1.28±0.10[c]	0.84±0.07[b]
MeJA0.1		1.20±0.08[c]	0.34±0.05[bc]	5.53±0.04[b]	47.50±0.69[c]	2.68±0.03[b]	2.09±0.06[b]	32.67±0.24[a]	1.87±0.07[a]	0.80±0.05[b]
MeJA1		1.27±0.07[c]	0.52±0.03[b]	3.08±0.17[c]	54.55±0.91[b]	1.97±0.03[d]	1.60±0.10[c]	30.82±0.24[ab]	1.54±0.07[b]	0.77±0.05[b]
Control	d10	3.48±0.19[a]	0.33±0.05[b]	3.51±0.43[c]	58.56±4.27[a]	2.03±0.02[d]	0.99±0.29[c]	21.28±2.77[c]	2.51±0.13[a]	0.35±0.28[c]
MeJA0.01		0.70±0.14[c]	0.33±0.01[b]	5.88±0.06[a]	42.87±1.36[c]	3.11±0.06[a]	3.25±0.06[a]	32.64±0.95[ab]	0.24±0.12[c]	1.28±0.09[b]
MeJA0.1		2.05±0.03[b]	0.18±0.04[c]	4.54±0.18[b]	50.49±1.37[b]	2.68±0.00[b]	1.19±0.09[c]	30.32±0.51[b]	0.34±0.09[c]	0.92±0.10[b]
MeJA1		1.98±0.04[b]	0.51±0.02[a]	5.29±0.06[a]	41.13±0.59[c]	2.45±0.02[c]	2.04±0.02[b]	35.78±0.13[a]	0.61±0.07[b]	1.81±0.04[a]
Control	d15	1.64±0.08[a]	0.75±0.06[b]	5.39±0.05[a]	50.25±0.65[c]	2.49±0.06[a]	1.42±0.04[d]	29.72±0.32[a]	0.77±0.06[c]	1.36±0.03[a]
MeJA0.01		1.29±0.09[b]	0.66±0.05[b]	1.98±0.16[d]	56.24±0.94[a]	2.48±0.03[a]	2.07±0.01[a]	29.15±0.27[ab]	0.71±0.05[c]	1.36±0.02[a]
MeJA0.1		1.31±0.13[b]	1.49±0.02[a]	3.39±0.09[b]	53.48±1.19[b]	2.41±0.05[a]	1.83±0.05[b]	28.31±0.62[b]	1.46±0.02[a]	0.75±0.07[b]
MeJA1		1.45±0.08[ab]	1.54±0.03[a]	3.01±0.13[c]	55.09±1.06[ab]	1.92±0.00[b]	1.63±0.06[c]	28.78±0.39[ab]	1.23±0.04[b]	0.49±0.09[c]
Control	d20	1.91±0.01[a]	0.34±0.11[a]	4.28±0.04[c]	57.38±0.50[a]	2.19±0.04[c]	2.17±0.04[b]	26.22±0.19[b]	0.30±0.10[c]	0.58±0.00[c]
MeJA0.01		1.59±0.02[c]	0.36±0.28[a]	8.05±0.59[a]	55.35±0.92[b]	3.47±0.26[a]	2.88±0.14[a]	19.25±1.29[d]	1.87±0.05[b]	0.93±0.02[b]
MeJA0.1		1.96±0.04[a]	0.29±0.22[a]	4.78±0.12[c]	52.76±0.57[c]	3.20±0.17[ab]	3.07±0.11[a]	23.30±0.68[c]	2.21±0.00[a]	1.11±0.03[a]
MeJA1		1.78±0.02[b]	0.60±0.15[a]	5.66±0.14[b]	49.29±0.08[d]	3.02±0.13[b]	2.35±0.07[b]	28.58±0.00[a]	1.82±0.03[b]	0.86±0.03[b]

Note: Numbers (mean ± SE) in the same column with different letters within the same time of MeJA treatments are significant different ($P < 0.05$) (ANOVA followed by Multiple Comparisons of Tukey HSD, n = 3).

higher in the young orange trees (*Citrus sinensis* var. Valencia) than in old trees during the September to February period; similarly, higher densities of adult GWSS were observed on the young compared to the old trees (C. sinensis var.) (Bi et al., 2007).

Table 5. Relative contents (%) of the 9 essential FAAs identified in the larch needles after JA treatments.

Treatment	Time/day	FAA relative contents (%)								
		Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Control	d1	3.78±0.37^{a}	0.95±0.00^{a}	4.87±0.09^{b}	59.72±2.94^{a}	2.14±0.02^{c}	1.29±0.09^{c}	19.08±2.88^{b}	1.84±0.06^{a}	0.44±0.09^{c}
JA0.01		1.63±0.02^{b}	0.61±0.00^{b}	6.13±0.09^{a}	49.57±0.49^{c}	2.98±0.05^{b}	1.59±0.01^{b}	28.64±0.54^{a}	0.86±0.06^{c}	0.84±0.01^{a}
JA0.1		1.38±0.02^{c}	0.51±0.02^{c}	3.04±0.13^{c}	54.66±0.85^{b}	3.11±0.06^{a}	1.72±0.03^{a}	29.91±0.41^{a}	1.27±0.02^{b}	0.27±0.07^{d}
JA1		1.15±0.03^{d}	0.20±0.04^{d}	4.87±0.04^{b}	53.08±0.54^{bc}	2.07±0.01^{c}	1.69±0.01^{ab}	31.00±0.28^{a}	0.28±0.08^{d}	0.64±0.02^{b}
Control	d3	1.52±0.08^{c}	0.96±0.01^{c}	2.91±0.03^{d}	53.37±0.83^{b}	1.95±0.06^{c}	1.58±0.02^{c}	32.22±0.63^{a}	1.46±0.01^{b}	0.72±0.00^{b}
JA0.01		2.51±0.13^{b}	0.53±0.10^{d}	8.14±0.53^{a}	55.60±1.73^{b}	3.08±0.22^{b}	2.69±0.14^{a}	18.51±2.47^{b}	2.24±0.06^{a}	1.09±0.03^{a}
JA0.1		1.44±0.14^{c}	1.41±0.03^{b}	4.60±0.16^{c}	63.71±2.49^{a}	2.57±0.18^{bc}	2.18±0.10^{b}	17.20±2.71^{b}	1.44±0.04^{b}	0.68±0.02^{bc}
JA1		2.83±0.04^{a}	2.16±0.22^{a}	6.53±0.62^{b}	59.33±3.57^{ab}	4.02±0.58^{a}	1.67±0.09^{c}	14.51±4.94^{b}	2.15±0.09^{a}	0.58±0.08^{c}
Control	d5	2.20±0.03^{b}	0.74±0.06^{a}	6.16±0.03^{b}	63.41±3.22^{a}	2.26±0.01^{c}	2.67±0.02^{a}	11.63±3.20^{d}	1.70±0.09^{a}	1.10±0.03^{b}
JA0.01		3.04±0.03^{a}	0.27±0.04^{c}	6.02±0.02^{b}	44.18±0.47^{c}	2.54±0.03^{b}	2.50±0.02^{a}	31.73±0.27^{b}	0.42±0.17^{b}	1.37±0.01^{a}
JA0.1		1.96±0.08^{c}	0.53±0.05^{b}	7.91±0.16^{a}	41.58±0.44^{c}	3.57±0.16^{a}	2.51±0.11^{a}	34.45±0.12^{a}	1.35±0.13^{a}	1.00±0.04^{b}
JA1		2.14±0.05^{b}	0.25±0.07^{c}	2.44±0.20^{c}	56.45±1.33^{b}	2.16±0.04^{c}	1.09±0.14^{b}	29.26±0.49^{c}	0.35±0.16^{b}	0.28±0.09^{c}
Control	d10	3.48±0.19^{a}	0.33±0.05^{b}	3.51±0.43^{b}	58.56±4.27^{a}	2.03±0.02^{c}	0.99±0.29^{b}	21.28±2.77^{b}	2.51±0.13^{a}	0.35±0.28^{a}
JA0.01		1.22±0.08^{b}	0.31±0.03^{b}	1.84±0.33^{c}	52.27±1.61^{a}	1.98±0.05^{c}	0.75±0.14^{b}	36.04±0.27^{a}	0.31±0.08^{c}	0.14±0.13^{a}
JA0.1		1.10±0.14^{b}	0.19±0.05^{c}	2.02±0.36^{c}	54.96±2.11^{a}	2.42±0.01^{b}	0.74±0.20^{b}	34.10±0.43^{a}	0.33±0.12^{c}	0.16±0.14^{a}
JA1		1.22±0.13^{b}	0.46±0.03^{a}	7.66±0.20^{a}	56.65±0.42^{a}	3.28±0.04^{a}	2.72±0.02^{a}	18.26±1.99^{b}	1.04±0.02^{b}	0.52±0.20^{a}
Control	d15	1.64±0.08^{b}	0.75±0.06^{a}	5.39±0.05^{a}	50.25±0.65^{b}	2.49±0.06^{c}	1.42±0.04^{b}	29.72±0.32^{a}	0.77±0.06^{c}	1.36±0.03^{b}
JA0.01		2.06±0.08^{a}	0.62±0.08^{a}	4.95±0.01^{b}	55.33±2.06^{a}	3.01±0.12^{b}	2.73±0.02^{a}	19.87±1.98^{b}	2.41±0.08^{b}	1.18±0.05^{b}
JA0.1		1.99±0.07^{a}	0.45±0.19^{a}	4.93±0.02^{b}	57.57±2.32^{a}	3.21±0.17^{ab}	2.73±0.06^{a}	15.64±2.26^{b}	2.51±0.07^{b}	1.19±0.13^{b}
JA1		2.11±0.09^{a}	0.52±0.09^{a}	4.68±0.01^{c}	58.64±2.17^{a}	3.41±0.20^{a}	2.74±0.07^{a}	15.54±2.07^{b}	2.73±0.09^{a}	1.70±0.07^{a}
Control	d20	1.91±0.01^{b}	0.34±0.11^{d}	4.28±0.04^{b}	57.38±0.50^{a}	2.19±0.04^{b}	2.17±0.04^{b}	26.22±0.19^{c}	0.30±0.10^{d}	0.58±0.00^{c}
JA0.01		0.84±0.07^{d}	0.91±0.07^{c}	3.89±0.03^{b}	50.28±0.17^{d}	1.78±0.02^{c}	1.35±0.00^{d}	34.42±0.33^{a}	1.09±0.07^{c}	0.52±0.00^{d}
JA0.1		1.18±0.04^{c}	1.55±0.02^{b}	3.38±0.01^{c}	52.80±0.26^{b}	2.12±0.03^{b}	1.88±0.04^{c}	28.23±0.02^{b}	1.63±0.04^{b}	0.72±0.01^{b}
JA1		2.15±0.04^{a}	2.18±0.03^{a}	6.95±0.31^{a}	51.82±0.39^{c}	3.21±0.13^{a}	2.75±0.08^{a}	19.37±0.84^{d}	2.36±0.04^{a}	1.23±0.03^{a}

Note: Numbers (mean ± SE) in the same column with different letters within the same time of JA treatments are significant different ($P < 0.05$) (ANOVA followed by Multiple Comparisons of Tukey HSD, n = 3).

The abnormal fluctuations of FAA concentrations in JAs-induced (or treated) larch seedlings in the current study might be one of the factors influencing the preference and performance of D. superans that utilize L. olgensis as a host. D. superans is a major forest pest insect in north eastern China, feeding mainly on larches and Korean pine (*P. koraiensis* Sieb. and Zucc.) (Xiao, 1992). Numerous studies on nutritional ecology have demonstrated the importance of nitrogen nutrition, especially AAs, on survival, growth, and reproduction of phytophagous insects (Joern and Behmer, 1997; Blackmer and Byrne, 1999; Bi et al., 2003). The fluctuation of FAAs content in the needles of larch seedlings treated with JAs solution might affect the normal growth and development of the *D. superans* larvae before the cold winter and further influence their overwintering survival rates.

Effects of the different contents of JAs on the individual FAAs content in needles of larch seedlings and on growth and development of *Lymantria dispar*

Among the 17 FAAs identified, relative contents of 9 essential FAAs were significantly affected by the JAs treatments. In the needles of girdled Japanese larch tree, the dominant amino acids for nitrogen transport switched from Glu and Ala in July to Asp and Tyr in August (Lee et al., 2011). In our study, Leu, Phe and Ile were the three most dominant FAAs (accounting for 78.14 ~ 91.08% of total FAA contents, Tables 3 to 5), with Leu being the highest, followed by Phe and Ile. While JA's amino acid conjugates, JA-isoleucine is a major regulator that controls gene expression and production of secondary metabolites after (a) biotic challenges, and plays an important role in activating plant defenses (Kang et al., 2006; Svoboda and Boland, 2010). *Manduca sexta* attacks could induce Phe ammonia lyase that synthesizes many phenolic metabolites (Baldwin et al., 2001). Thus, the three major FAAs were precursors of plant secondary metabolites, and would play a key role in plant defense against insect pest attacks. The *Lymantria gmelinii* seedlings with 1 mmoL/L MeJA and *cis*-J solution treatments significantly reduced survival rate, larval weight, pupal weight and adult fecundity of *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) (Feng et al., 2010). These indicated that jasmonares induced the variation of the nutrition substance and secondary metabolites (Meng et al., 2010) and then influenced the growth and development of *L. dispar.*

The data analysis of One-way ANOVA and/or Univariate ANOVA on total FAA contents showed that 0.01 mM JA, 0.1 mM *cis*J and 0.1/1 mM MeJA showed significantly stronger impact on total FAA contents than did the other treatments, and then the effect of MeJA on total FAA contents was significantly higher than those of JA and *cis*J. These indicated that 0.1 or 1 mM MeJA further suited to being larch induced defense. In a study on the Scots pine (*Pinus sylvestris*) saplings, exclusion of UV-A and UV-B increased the content of FAAs (Krywult et al., 2008). The FAA contents were significantly higher in needles of saplings grown under UV-B/UV-A exclusion compared to the controls or UV-B exclusion (Krywult et al., 2008). Their results showed that the effects of different factors on FAA concentration varied.

In our study, the induction activity of the low-concentration JA (0.01 mM) was the strongest among the three concentrations of JA; it was followed by MeJA of the high concentration (1 mM), and *cis*J of the medium concentration (0.1 mM). Similarly, Scots pine seedlings were exposed to 0, 0.075, 0.15 and 0.3 μL/L of O_3 during 8 h/day, 5 day/week for a period of 5 weeks, and then concentrations of FAA in the needles were significantly elevated at O_3 concentrations of 0.3 μL/L (Kainulainen et al., 2000). This indicates a strong variation in the induced functionality among the different factors on the same plant, or among different plants with the same factor. Further research is surely needed to better understand the mechanisms and applied technology of plant induced defense.

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REFERENCES

Baldwin IT, Halitschke R, Kessler A, Schittko U (2001). Merging molecular and ecological approaches in plant-insect interactions. Curr. Opin. Plant Biol., 4: 351–358.

Bi JL, Castle SJ, Toscano NC (2007). Amino acid fluctuations in young and old orange trees and their influence on glassy-winged sharpshooter (*Homalodisca vitripennis*) population densities. J. Chem. Ecol., 33: 1692–1706.

Bi JL, Toscano NC, Madore MA (2003). Effect of cotton nitrogen fertilization on *Bemisia argentifolii* populations in relation to soluble proteins and free amino acids in cotton. J. Chem. Ecol., 29: 747–761.

Blackmer JL, Byrne DN (1999). Changes in amino acids in *Cucumis melo* in relation to life-history traits and flight propensity of *Bemisia tabaci*. Entomol. Exp. Appl., 93: 29–40.

Cánovas FM, Avila C, Cantón FR, Cañas RA, de la Torre F (2007). Ammonium assimilation and amino acid metabolism in conifers. J. Exp. Bot., 58: 2307–2318.

Chen H, Wilkerson CG, Kuchar JA, Phinney BS, Howe GA (2005). Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. PNAS., 102: 19237–19242.

Corcuera LJ (1993). Biochemical basis for the resistance of barley to aphids. Phytochemistry, 33: 741–747.

Eleftherianos I, Vamvatsikos P, Ward D, Gravanis F (2006). Changes in the levels of plant total phenols and free amino acids induced by two cereal aphids and effects on aphid fecundity. J. Appl. Entomol., 130: 15–19.

Feng CF, Yan SC, Mao HB, Fang W, Li SW (2010). Effect of induced

resistance of *Larix gmeliniion* growth and development of *Lymantria dispar*. J. Northeast Fore. Univer., 38: 136–137.
Gitahi SM, Harper DM, Muchiri SM, Tole MP, Ng'ang'a RN (2002). Organochlorine and oranophosphorus pesticide concentrations in water, sediment, and selected organisms in Lake Naivasha (Kenya). Hydrobiologia, 488: 123–128.
Gols R, Roosjen M, Dijkman H, Dicke M (2003). Induction of direct and indirect plant responses by jasmonic acid, low spider mite densities, or a combination of jasmonic acid treatment and spider mite infestation. J. Chem. Ecol., 29: 2651–2666.
Halitschke R, Baldwin IT (2005). Jasmonates and related compounds in plant–insect interactions. J. Plant Growth Regul., 23: 238–245.
Heijari J, Nerg A-M, Kainulainen P, Viiri H, Vuorinen M, Holopainen JK (2005). Application of methyl jasmonate reduces growth but increases chemical defence and resistance against *Hylobius abietis* in Scots pine seedlings. Entomol. Exp. Appl., 115: 117–124.
Howe GA (2005). Jasmonates as signals in the wound response. J. Plant Growth Regul., 23: 223–237.
Joern A, Behmer ST (1997). Importance of dietary nitrogen and carbohydrates to survival, growth, and reproduction in adults of the grasshopper *Ageneotettix deorum* (Orthoptera: Acrididae). Oecologia, 112: 201–208.
Kainulainen P, Holopainen JK, Holopainen T (2000). Combined effects of ozone and nitrogen on secondary compounds, amino acids, and aphid performance in Scots pine. J. Environ. Qual., 29: 334–342.
Kang JH, Wang L, Giri A, Baldwin IT (2006). Silencing threonine deaminase and JAR4 in *Nicotiana attenuata* impairs jasmonic acid-isoleucine-mediated defenses against *Manduca sexta*. Plant Cell, 18: 3303–3320.
Kim YT, Glerum C (1995). Seasonal free amino acid fluctuations in red pine and white spruce needles. Can. J. Forest Res., 25: 697–703.
Kishor PBK, Sangan S, Amrutha RN, Laxmi PS, Naidu KR, Rao KRSS, Rao S, Reddy KJ, Theriappan P, Sreenivasulu N (2005). Regulation of proline biosynthesis, degradation, uptake, and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Curr. Sci., 88: 424–438.
Krywult M, Smykla J, Kinnunen H, Martz F, Sutinen M-L, Lakkala K, Turunen M (2008). Influence of solar UV radiation on the nitrogen metabolism in needles of Scots pine (*Pinus sylvestris* L.). Environ. Pollut., 156: 1105–1111.
Lee WY, Lee JS, Lee J-H, Noh EW, Park E-J (2011). Enhanced seed production and metabolic alterations in *Larix leptolepis* by girdling. Forest Ecol. Manag., 261: 1957–1961.
Lu YB, Liu SS, Liu YQ, Furlong MJ, Zalucki MP (2004). Contrary effects of jasmonate treatment of two closely related plant species on attraction of and oviposition by a specialist herbivore. Ecol. Lett., 7: 337–345.
Meng ZJ, Zhou YQ, Yan SC, Jin H, Hu X (2010). Effects of exogenous jasmonates on tannin content in needles of two larch species. Sci. Silv. Sin., 46: 96–104.
Neves C, Santos H, Vilas-Boas L, Amâncio S (2002). Involvement of free and conjugated polyamines and free amino acids in the adventitious rooting of micropropagated cork oak and grapevine shoots. Plant Physiol. Biochem., 40: 1071–1080.
Ruan JY, Gerendás J, Haerdter R, Sattelmacher B (2007). Effect of alternative anions (Cl^- *vs*. SO_4^{2-}) on concentrations of free amino acids in young tea plants. J. Plant Nutr. Soil Sci., 170: 49–58.
Saltzmann KD, Giovanini MP, Zheng C, Williams CE (2008). Virulent hessian fly larvae manipulate the free amino acid content of host wheat plants. J. Chem. Ecol., 34: 1401 – 1410.
Sandström JP, Telang A, Moran NA (2000). Nutritional enhancement of host plants by aphids-a comparison of three aphid species on grasses. J. Insect. Physiol., 46: 33 – 40.
Svoboda J, Boland W (2010). Plant defense elicitors: Analogues of jasmonoyl–isoleucine conjugate. Phytochemistry, 71: 1445–1449.
Telang A, Sandström JP, Dyreson E, Moran NA (1999). Feeding damage by *Diuraphis noxia* results in nutritionally enhanced phloem diet. Entomol. Exp. Appl., 91: 403 – 412.
Thaler JS, Farag MA, Paré PW, Dicke M (2002). Jasmonate-deficient plants have reduced direct and indirect defences against herbivores. Ecol. Lett., 5: 764–774.
Thaler JS (1999). Jasmonate-inducible plant defences cause increased parasitism of herbivores. Nature, 399: 686– 688.
Wang L, Allmann S, Wu JS, Baldwin IT (2008). Comparisons of lipoxygenase3- and jasmonate-resistant4/6-silenced plants reveal that jasmonic acid and jasmonic acid-amino acid conjugates play different roles in herbivore resistance of Nicotiana attenuata. Plant Physiol., 146: 904–915.
Xiao GR (1992). Forest Insects of China. China Forestry Publishing House, Beijing, p. 959 –961.