

Effects of exogenous methyl jasmonate-induced resistance in *Populus × euramericana* ‘Nanlin895’ on the performance and metabolic enzyme activities of *Clostera anachoreta*

Gu Tianzi^{1,2} · Zhang Congcong^{1,2} · Chen Changyu^{1,2} · Li hui^{1,2} · Huang kairu^{1,2} · Tian Shuo^{1,2} · Zhao Xudong^{1,2} · Hao Dejun^{1,2} 

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Abstract Methyl jasmonate (MeJA) is a plant chemical elicitor that has been used to artificially induce chemical defense responses and trigger induced resistance against a broad range of arthropod herbivores. This study assessed the effects of exogenous MeJA on the growth performance, chemical detoxification, and antioxidant enzyme activities of *Clostera anachoreta*. After feeding *C. anachoreta* with 10^{-5} mol/L MeJA solution-treated *Populus × euramericana* ‘Nanlin895’ leaves, we measured the larval and pupal development time, pupal weight, eclosion rate, fecundity, and nutritional physiology of the adults. We also measured superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activities, which are reactive oxygen species (ROS) scavengers, and glutathione *S*-transferase (GST) and carboxylesterase (CarE) activities, which are probably involved in the metabolism of induced plant allelochemicals. Methyl jasmonate (MeJA) treatment reduced larval performance in terms of prolonged developmental time of larvae and pupae and decreased growth rates, but had little effect on larval nutrition physiology. The activities of the SOD and POD antioxidant enzymes increased, but CAT activity declined at 36 and 48 h after *C. anachoreta* had fed

on MeJA-treated leaves. The GST and CarE detoxification enzymes both were induced after the larvae had fed on MeJA-treated leaves. These results suggest that exogenous application of MeJA elicited induced resistance in *Populus × euramericana* ‘Nanlin895’ against *C. anachoreta*.

Keywords *Populus × euramericana* ‘Nanlin895’ · *Clostera anachoreta* · Induced resistance · Nutritional index · Development · Antioxidant enzymes · Detoxification enzymes

Introduction

Plants have developed complex direct and indirect defensive mechanisms against biotic and abiotic environmental stresses (Walters and Heil 2007; Champigny and Cameron 2009; Feng et al. 2012; Martin et al. 2012). Direct plant defense involves altering the palatability and/or toxicity of leaf tissues to reduce herbivore feeding on plants (Agrawal 1999; Graves et al. 2008; Simons et al. 2008). In contrast, indirect plant defense involves attracting predators and parasitoids of the herbivores by emitting special volatile signal substances immediately after an attack (Kessler and Baldwin 2001; Degenhardt et al. 2003; Turlings et al. 1995; Williams et al. 2008). Both strategies are inducible in the presence of potent elicitors or signaling agents.

Plant defense responses are governed by complex signaling networks, which are regulated by phytohormones. Jasmonate (jasmonic acid-JA) is one of the endogenous signals that can elicit plant-induced responses (Farmer and Ryan 1990; Wasternack and Parthier 1997; Rohwer and Erwin 2008; Schaller and Stintzi 2008). JA-mediated signaling pathways are generally activated by multiple biotic and abiotic factors (Thaler et al. 1996; Stout et al. 1998;

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✉ Hao Dejun
djhao@njfu.edu.cn

¹ Co-Innovation Center for the Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing 210037, China

² College of Forestry, Nanjing Forestry University, Nanjing, China

Walling 2000; Rodriguez-Saona et al. 2005; Schaller and Stintzi 2008). When plants are subject to a pathogen infection, wounding, or insect attack, a phosphorylation cascade is triggered leading to JA biosynthesis (Carvalhais et al. 2013). The increased endogenous levels of JA are detected by receptors that stimulate the expression of the JA-responsive genes (Reinbothe et al. 1994; Thaler et al. 1996; Schaller and Stintzi 2008), which lead to the production of metabolites involved in defense (Ballaré 2011). These defenses can affect the invaders directly (Karban and Baldwin 1997) through chemical–molecular changes that alter tissue palatability, thereby hindering herbivore digestion, development, and reproduction (Omer et al. 2000; Poelman et al. 2008).

To meet their nutritional requirements, insect can usually adjust their feeding habits, digestive physiology, and gene expression to optimally adapt to dietary challenge and successfully cope with various plant defensive substances and antimetabolites (Zhu-Salzman and Zeng 2015). A major adaptive response involves physiological adaptations via detoxification and antioxidant enzymes mainly to counter host plant defense mechanisms (Ahn et al. 2004; Li et al. 2007; Krishnan and Kodrik 2006; Rigsby et al. 2015).

Methyl jasmonate (MeJA), the volatile form of JA, has been widely used to study jasmonate signaling pathways and plant defense mechanisms. Recently, there has been an increased interest in using exogenous methyl jasmonate to induce natural host defense mechanisms against pathogens and herbivorous insects (Carvalhais et al. 2013; Fedderwitz et al. 2016; Feng et al. 2012; Liu et al. 2015; Lundborg et al. 2016; Yang et al. 2015; Zhang et al. 2015). Although most of the above research focused on the induced defensive responses caused by spraying exogenous MeJA on crop plants, there have been only few studies on deciduous trees and their herbivorous insects.

Poplar trees have been widely planted around the world due to their high productivity and adaptability. *Populus* spp. and its hybrids dominate forestry plantations in areas along the middle and lower reaches of the Yangtze River in China. However, severe defoliation by insects not only reduces the growth rate of the trees, but also renders them susceptible to other insects and diseases (Wan et al. 2015). This study investigated whether phytohormones could induce a defensive response in poplar trees and how the responses affect trade-offs in poplar. Liu et al. (2015) found that exogenous methyl jasmonate not only affected the concentrations of essential nutrients and secondary metabolites, but also increased the activities of polyphenol oxidase (PPO), lipoxygenase (LOX), trypsin inhibitor (TI), and chymotrypsin inhibitor (CI). Tang et al. (2015) reported that four defense-related enzyme activities increased when exogenous methyl salicylate was sprayed on *Populus × euramericana* ‘Nanlin 895.’ However, the

effect of these induced responses on subsequent herbivore performance needs further investigation.

Clostera anachoreta (Fabr.) (Lepidoptera: Notodontidae) is one of the most destructive herbivorous insects of poplar trees in China and is widely distributed in Heilongjiang, Jilin, Inner Mongolia, Hebei, Jiangsu, Shanghai, Guangdong, Hunan, Hubei, Sichuan, and Yunnan (Liang et al. 2006). It also occurs in other Asian countries, such as India, Indonesia, Sri Lanka, Japan, Korea, and Mongolia, and parts of Europe (Liang et al. 2006). There have been few studies on the effects of jasmonates on poplar tree defense mechanisms and there has been little information about induced defense against the performance and digestive physiology of herbivorous insects in trees, including *Populus × euramericana* ‘Nanlin895.’ Based on our former studies on exogenous MeJA-induced foliar chemistry changes in *Populus × euramericana* ‘Nanlin 895’ (Liu et al. 2015), we examined whether the defensive responses elicited by exogenous MeJA could be effective against *C. anachoreta*. We also measured larval and pupal developmental time, pupal weight, rate of survival to the adult stage, and nutritional indices, as well as effects on insect detoxification and antioxidant enzyme activities.

Materials and methods

Plant materials and insects

Populus × euramericana ‘Nanlin895’ seedlings (1 year old) were obtained from Jurong Forest Farm, Zhenjiang City (31°57′N, 119°10′E), Jiangsu Province, China. The young trees were cut into 25- to 30-cm-long stems and then soaked in water for 2–3 days. The stems were moved to a greenhouse and planted individually into plastic pots (28 cm in diameter, 40 cm in height) containing orchard soil. The greenhouse growth conditions were 26 ± 0.5 °C, RH = $60 \pm 5\%$, and a 16-h L:8-h D photoperiod. The seedlings were watered every 2 days and weeded when necessary.

C. anachoreta eggs were collected from the leaves of 7-year-old *Populus × euramericana* cv. I-72 that had been planted in an agricultural afforestation area, Pukou District, Nanjing (32°18′N, 118°28′E), Jiangsu Province, China. The eggs were kept in an incubator at the Laboratory of Entomology in Nanjing Forestry University. The incubator conditions were 26 ± 0.5 °C, RH = $70 \pm 5\%$, and a 12-h L:12-h D photoperiod. Once the larva had hatched, they were transferred to 15-cm-diameter sterilized Petri dishes and reared with fresh *Populus deltoides* leaves under the same laboratory conditions. Once they had pupated, they were kept individually in 5-cm-diameter Petri dishes. After emergence, the adults were supplied with 10% honey

solution before bioassay. The second or third instar larvae of the next generation were used for the bioassay.

MeJA

The MeJA (Sigma, St. Louis, MO, USA) was dissolved in double-distilled water to obtain a 10^{-5} mol/L solution. Once the poplar cuttings had reached a height of 50 cm, 100 mL of 10^{-5} mol/L MeJA solution was sprayed twice on the leaves, with the second spray occurring just after the initial spray had been totally absorbed. The same volume of distilled water was applied to the control leaves, and the control poplars and the treated plants were placed in two separate rooms under the same conditions to prevent signal eavesdropping. After 48 h, the MeJA-treated leaves and non-MeJA-treated leaves were collected and used to feed the *C. anachoreta* larvae.

Development time and adult fecundity

Ten newly hatched larvae were fed with MeJA-treated leaves or non-MeJA-treated leaves separately and the leaves were changed every day. Larval development time, pupal weight, and survival to the adult stage were recorded daily. Development time was defined as the average duration of the larval stage from eclosion of the egg to pupae molting. The molting of the larvae into pupae was checked daily, and pupae were weighed 24 h after molting and kept separately until they emerged as adults.

When adults emerged, one-day-old male and female were single-paired in another container (12 cm × 10 cm × 9 cm) and provided with 10% honey solution. Fifty couples and five replicates were used per treatment. Fresh leaves with petioles wrapped in water-soaked cotton were placed in the container for ovipositing for 7 days. All eggs laid by each female during the 7-day reproductive period were counted and the number of non-fertile couples was also noted.

Nutritional indices

Newly molted third instar larvae that had been reared on untreated fresh leaves were randomly chosen and starved for 24 h before the experimental diets were provided; after measuring the weight of starved larvae, the larvae were assigned to a leaf with or without MeJA treatment. The Petri dishes were cleaned and the leaves were replaced every day until the completion of the third instar. There were five replicates per treatment and ten larvae per replicate. The nutritional index values were calculated based on dry weights as described in Table 1. The weight gain of larvae (*B*) was calculated by subtracting the initial dry weight estimate of a larva from the final dry weight.

We estimated the dry weight of the larvae at the onset of the trials based on dry weight proportion calculated from newly molted fourth instars reared on the two kinds of leaves. The final dry weight was directly obtained by freezing the larvae at -20 °C for 30 min and then drying them in the oven for 2 days. The dry weight of ingested leaves (*I*) by each larva was calculated by subtracting the weight of the remaining leaf from the weight initial leaf. The dry weight of feces (*F*) remaining in the Petri dishes was measured by direct drying in the oven.

Enzyme activities

Enzyme extracts were made from insects reared on leaves with and without MeJA treatment until the end of the third instar to determine whether MeJA application can influence detoxification and antioxidant enzyme activities. When they had molted into the fourth instar, ten newly molted larvae were starved for 18 h and the midguts of the larvae were removed and washed several times with ice-cold physiological saline (0.7% NaCl). After removing the contents, the midgut was rinsed with physiological saline, homogenized in elution buffer (0.25 M sucrose, 0.05 M Tris-HCL, 1 mM EDTA, pH 7.4), and sonicated. The homogenates were centrifuged at 5000 g and 4 °C for 10 min. The protein amounts present were determined by Coomassie Blue G dye assay (Bradford 1976).

Antioxidant enzyme determination

The superoxide (SOD) activity was determined using Superoxide Dismutase (SOD) Assay Kit (Jiancheng, Nanjing, China), according to the method of Beauchamp and Fridovich (1971) with slight modification. The assay mixture consisted of 100 mM KPO_4 buffer pH 7.8, 0.01 μ M EDTA, 65 mM L-methionine, 750 μ M NBT, 2 mM riboflavin, and 0.05 mL of enzyme extract in a total volume of 3 mL. Riboflavin was added at the end and the tubes were mixed by shaking. One set of tubes was illuminated under light (20 W) for 30 min at a distance of 1 cm and another set of tubes was kept in the dark for 30 min. Control mixtures without the enzyme extract were similarly kept under light or in the dark. Absorbance was measured at 560 nm. One unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition in the NBT reduction rate.

Peroxidase (POD) activity was determined using Peroxidase Assay Kit (Jiancheng, Nanjing, China), according to the procedure reported by Mitrovic et al. (2004) with slight modification. The 1.0 mL reaction mixture consisted of 0.05 mL of enzyme extract, 30 mM guaiacol, 26 mM H_2O_2 , and 0.1 M sodium phosphate buffer (pH 6.0). The incubation, at 30 °C, lasted 5 min.

Table 1 Nutritional indices calculated for *C. anachoreta*

Abbrev.	Nutritional index	Formula	Explanation
RGR	Relative growth rate	$B/(B_m \times T)$	Rate of body weight increase per mean body weight per day
RCR	Relative consumption rate	$I/(B_m \times T)$	Rate of food consumed per mean body weight per day (=RCR–ECI)
AD	Approximate digestibility	$(I-F)/I$	Assimilated portion out of food ingested (%)
ECD	Efficiency of conversion of digested food	$B/(I-F)$	Net growth efficiency (%)
ECI	Efficiency of conversion of ingested food	B/I	Gross growth efficiency (%) (=RGR/RCR)

I dry weight of ingested leave (mg), *B* dry weight gain of larva during the feeding period (mg), B_m mean dry weight of the initial and final larva during the feeding period (mg), *T* duration of feeding period (days), *F* dry weight of feces produced (mg) (Waldbauer 1968)

The enzyme activity was represented by the extinction coefficient at 470 nm and was measured using a spectrophotometer (721G, Inesa Analytical Instrument Limited Company, Shanghai, China).

The catalase (CAT) activity was determined using Catalase (CAT) Assay Kit (Jiancheng, Nanjing, China), according to the procedure reported by Chance and Maehly (1955) and Havir and McHale (1987) with modifications. The 5.0 mL reaction mixture consisted of 0.05 mL of enzyme extract, 13 mM methionine, 50 M sodium phosphate buffer (pH 7.0), 75 μ M NBT, 0.1 mM EDTA, and 4 μ M riboflavin. After 1-min incubation at 30 °C, 2.0 mL H_2SO_4 was added to terminate the reaction. The linear portion of the curve and the extinction coefficient at 43.6 $M^{-1} cm^{-1}$ were used to express activity as mmols H_2O_2 decomposed per min per mg protein (mmols/min/mg). The enzyme activity was represented by the decrease in absorbance at 240 nm, which was measured using a spectrophotometer (721G, Inesa Analytical Instrument Limited Company, Shanghai, China).

Detoxifying enzyme determination

Carboxylesterase (CarE) activity was determined using Carboxylesterase (CarE) test kit (Jiancheng, Nanjing, China), according to the method of Asperen (1962) with modifications. The reaction mixture consisted of 0.1 mL of enzyme extract and 0.3 mM α -naphthyl acetate (containing 10^{-5} M eserine) in 40 mM sodium phosphate buffer (pH 7.0). A freshly prepared 1.0-mL aliquot of diazoblue SDS reagent (prepared with 1% fast blue B salt solution and 5% sodium dodecyl sulfate at a ratio of 2:5) was added after 30-min incubation at 30 °C. The incubation was continued for a further 15 min. Then the solution was centrifuged at about 1000 g. After centrifugation, the color developed as a result of α -naphthol formation and was measured at 600 nm. The enzyme activity was calculated from an α -naphthol standard curve.

Glutathione *S*-transferase (GST) activity was determined using Reduced Glutathione (GSH) Assay Kit

(Jiancheng, Nanjing, China), according to the method of Kao et al. (1989) with slight modification. The 3.0 mL assay mixture consisted of 0.1 mL of 30 mM CDNB, 0.1 mL of 20 mM GSH, and 0.1 mL of enzyme extract in 2.7 mL of 50 mM phosphate buffer (pH 7.5). The change in absorbance was measured at 340 nm for up to 5 min and the enzyme activity, in terms of mmol of CDNB conjugated/min/mg of enzyme protein, was calculated using an extinction coefficient of 9.6 mM/cm.

Data analysis

Statistical tests and graphical presentations were performed by SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). When necessary, data in our study were transformed to normalize the error variances using arcsine square root, log, or square root transformation. A one-sample *t* test was used to examine the treatment means of the five replications, one-way ANOVA was used to assess the treatment effect, and significant differences among means were detected by Duncan's test ($P < 0.05$).

Results

Effects of MeJA treatment on *C. anachoreta* performance

The *C. anachoreta* larvae development time on MeJA-treated leaves was 7.45% longer than that on non-MeJA-treated leaves ($t = -4.933$, $P = 0.001$) (Table 2). Furthermore, the pupation rates of the larvae fed on MeJA-treated leaves and non-MeJA-treated leaves were 78.00 and 82.00%, respectively. The average pupal weight of *C. anachoreta* fed on MeJA-treated leaves was 0.15 g which was 13.53% less than that of the Non-MeJA-treated pupae 0.17 g ($t = 3.310$, $P = 0.011$). Pupal development time was significantly delayed by 17.29% after MeJA treatment compared to the non-MeJA treatment ($t = -24.303$, $P = 0.000$). The average number of eggs produced per

Table 2 *C. anachoreta* performance on *Populus × euramericana* ‘Nanlin895’ leaves exposed to MeJA

Treatment	Duration of the larval stage (days)	Pupation rate (%)	Pupal weight (mg)	Duration of the pupal stage (days)	Eclosion rate (%)	Fecundity
Control	17.89 ± 0.18a	82.00 ± 2.00a	0.17 ± 0.01a	6.09 ± 0.15a	78.09 ± 1.31a	340.10 ± 12.83a
MeJA-treated	19.22 ± 0.20b	78.00 ± 1.22a	0.15 ± 0.03b	7.15 ± 0.41b	79.50 ± 1.16a	296.10 ± 9.22a

Data were shown as mean ± SE, $n = 50$; means between treatments and controls were not significantly different unless followed by different letters

female was not significantly affected by MeJA treatment ($t = 1.139$, $P = 0.270$).

Effects of MeJA treatment on the nutritional physiology of *C. anachoreta* larvae

The nutritional physiology of *C. anachoreta* third instar larvae was not affected when they were fed on leaves treated with MeJA (Table 3). Relative growth rate (RGR) had a difference of 5.71% between the two groups (feeding on MeJA-treated leaves versus non-MeJA-treated leaves), relative consumption rate (RCR) had a difference of 12.10% when the larvae were fed on MeJA-treated leaves, and approximate digestibility (AD) had a difference of 2.79%; all these changes were not statistically different, and the efficiency of conversion of digested food (ECD) and efficiency of ingested food (ECI) remained almost unchanged when the larvae were fed on MeJA-treated leaves.

Activities of antioxidant enzymes in *C. anachoreta* larvae feeding on leaves treated with MeJA

The SOD activities were approximately 1.04, 1.09, 1.06, 1.285, and 2.13 times higher in larvae fed on MeJA-treated leaves than in larvae fed on untreated leaves at 4, 12, 24, 36, and 48 h, respectively. The larvae fed on MeJA-treated leaves showed significantly higher SOD activities than larvae fed on untreated leaves at 36 h ($t = -3.138$, $P = 0.035$) and 48 h ($t = -10.023$, $P = 0.001$).

After the larvae had fed on MeJA-treated leaves for 4 h, the CAT activity in the larvae was 5.77% lower, but

increased to 34.8% higher than the control at 12 h ($t = -2.942$, $P = 0.042$). However, the CAT activity was 30.5 and 28.4% lower than the control at 36 h ($t = 14.368$, $P = 0.000$) and 48 h ($t = 4.378$, $P = 0.012$), respectively, which was significant.

POD activity in the larvae varied with time after MeJA treatment (Fig. 1). It increased from 4 to 48 h and was 1.6, 1.1, 1.6, 2.1, and 1.4 times higher than untreated control at 4, 12, 24, 36, and 48 h, respectively. The differences were significant at 4 h ($t = -3.834$, $P = 0.019$), 24 h ($t = -4.878$, $P = 0.008$), 36 h ($t = -11.780$, $P = 0.000$), and 48 h ($t = -4.390$, $P = 0.012$), but not at 12 h.

CarE and GST activities in *C. anachoreta* larvae after treatment with MeJA

After feeding on the MeJA-treated leaves, the CarE activity in the larvae increased between 4 h and 48 h, with larvae fed on MeJA-treated leaves showing 1.49, 1.13, 1.48, 1.54, and 1.23 times higher activity than larvae fed on untreated leaves at 4, 12, 24, 36, and 48 h, respectively. There were significant differences between the two treatments at 4 h ($t = -6.121$, $P = 0.004$), 24 h ($t = -5.114$, $P = 0.007$), and 36 h ($t = -2.040$, $P = 0.111$).

The GST activity was approximately 1.3, 1.7, 1.1, 1.1, and 1.1 times higher in larvae fed on MeJA-treated leaves than in larvae fed on untreated leaves at 4, 12, 24, 36, and 48 h, respectively, but the difference between the two treatments was only significant at 12 h ($t = -21.694$, $P = 0.000$).

Table 3 Nutritional indices for *C. anachoreta* larvae feeding on *Populus × euramericana* ‘Nanlin895’ leaves exposed to MeJA

Treatment	RGR (mg/mg/day)	RCR (mg/mg/day)	AD (%)	ECD (%)	ECI (%)
Control	0.35 ± 0.02a	4.38 ± 0.63a	62.82 ± 3.81a	19.45 ± 3.94a	10.60 ± 0.86a
MeJA-treated	0.33 ± 0.01a	3.85 ± 0.74a	64.57 ± 3.98a	19.38 ± 3.85a	10.06 ± 1.10a

Data were shown as mean ± SE, $n = 50$; means between treatments and controls were not significantly different unless followed by different letters

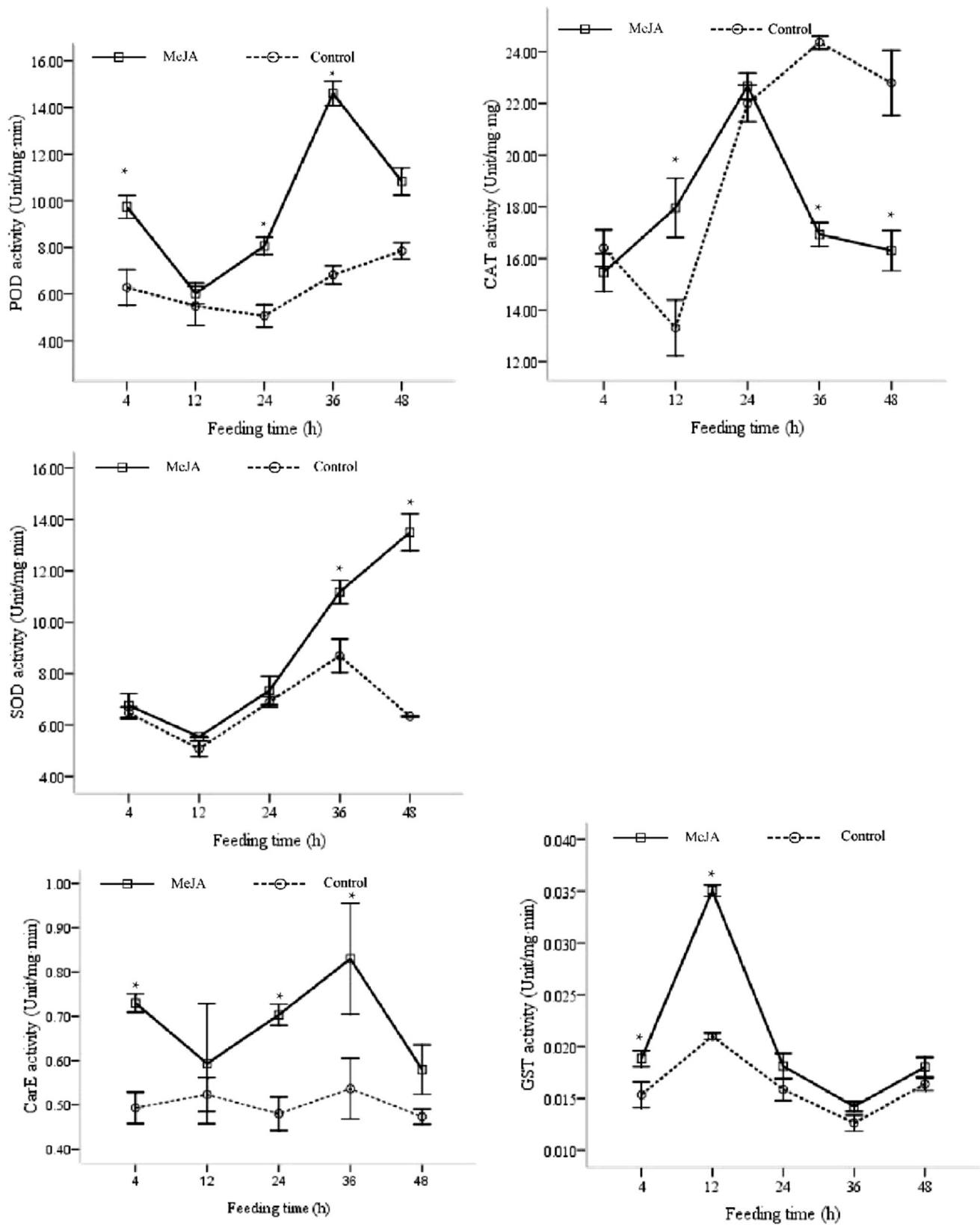


Fig. 1 Effects of MeJA-treated leaves on the midgut enzyme activities of *C. anchoreta* larvae. SOD superoxide dismutase, CAT catalase peroxidase, POD peroxidase, GST glutathione S-transferase, CarE carboxylesterase; Asterisk represents statistically significant difference

Discussion

This study demonstrates that the exogenous application of methyl jasmonate can induce a defense response in *Populus × euramericana* ‘Nanlin895,’ which affected the performance of herbivore *C. anachoreta*. The growth and development time of the larvae and pupae, and the detoxification and antioxidant enzyme activities of *C. anachoreta* were substantially affected by methyl jasmonate application. This result is consistent with the role of jasmonates in the defense responses of a number of plants (Thaler et al. 1996, 2001; Omer et al. 2000; Délano-Frier et al. 2004; Tan et al. 2011).

The larvae had a longer development time and a lower pupation rate on leaves treated with exogenous jasmonates compared to the untreated control leaves. The poor performance of the larvae may be due to a low nutritional value or the presence of deterrent components caused by the induced jasmonate response (Scott et al. 2010). Pupal weight can be used as a measure of insect dietary quality (Chapman 1998). The pupal weight was lower on MeJA-treated leaves. This is a further indication that MeJA-treated leaves are low in nutritional quality compared to the control leaves. However, the MeJA-treated leaves did not have a detrimental effect on the subsequent eclosion of adults and the fecundity of female *C. anachoreta* adults.

MeJA-induced defense has been shown to elicit chemical–molecular changes that can alter tissue palatability, which hinders herbivore digestion, development, and reproduction (Omer et al. 2000; Poelman et al. 2008). Nutritional value is also influenced by secondary plant compounds, which in *Populus* species consist primarily of phenolic glycosides and tannins. Recent studies with phenolic glycosides suggest that they may play a role in the differential defoliation of aspen by herbivorous insects (Lindroth and Bloomer 1991; Hemming and Lindroth 2000).

A second objective of this study was to show the biochemical responses of *C. anachoreta* larvae to host resistance mechanisms in *Populus × euramericana* ‘Nanlin895’ and to characterize the detoxification and antioxidant enzyme activities of *C. anachoreta* larvae when feeding on MeJA-treated leaves. This study will improve our understanding of the induced resistance mechanisms against *C. anachoreta* that are induced by MeJA treatment and the relative importance of larval physiological adaptations to these defenses.

The general role of antioxidant enzymes is to prevent oxidative stresses by scavenging reactive oxygen species (ROS). The antioxidant system comprises several enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Barbehenn 2002). Superoxide radicals

are converted to H_2O_2 by SOD, and the accumulation of H_2O_2 is prevented in the cell by CAT and POD. POD is efficient at removing low concentrations of hydrogen peroxide that is not normally scavenged by CAT because of its high K_m (Ahmad and Pardini 1990; Felton and Duffey 1991; Ahmad 1992). It has been demonstrated that SOD, CAT, and POD activities are induced in lepidopteran larvae (Krishnan and Kodrik 2006). The gut-based antioxidant enzymes are upregulated in larvae in order to protect against endogenous and exogenous oxidative radicals generated by ingested pro-oxidant allelochemicals. Alteration of the diet can thus cause changes in the regulation of antioxidant enzyme systems and the insects can develop specific adaptive mechanisms to combat pro-oxidant allelochemicals (Ahmad and Pardini 1990; Felton and Summers 1995).

The activities of all three antioxidant enzymes assayed in this study were enhanced to different degrees after MeJA treatment, which indicated that larvae feeding on MeJA-treated leaves may be subject to higher levels of oxidative stress than those feeding on untreated leaves. The substantially higher SOD activity observed in larvae feeding on MeJA-treated leaves suggested that superoxide radical accumulation or production was greater after they had fed on MeJA-treated leaves. The elevated CAT and POD activities indicated that H_2O_2 may also be more abundant in MeJA-treated leaves. We observed that CAT activity was significantly reduced after 36 h, which was probably caused by plant-derived ROS scavengers, such as ascorbate, carotenoids, and plant CAT enzymes (Krishnan and Kodrik 2006).

Glutathione *S*-transferases are major detoxification enzymes that are found mainly in the cytosol. In addition to their role in catalyzing the conjugation of electrophile substrates to glutathione, these enzymes also exhibit peroxidase and isomerase activities (Krishnan and Kodrik 2006; Weinhold et al. 1990). The carboxylesterases represent a multigene family and the genes are localized in the endoplasmic reticulum of many tissues. These enzymes efficiently catalyze the hydrolysis of ester- and amide-containing chemicals and are classified as the serine hydrolase superfamily. They are responsible for the detoxification or metabolic activation of various xenobiotics and also play an important physiological role in lipid metabolism. GSTs and CarEs have been shown to play a role in the dietary tolerance of allelochemicals (Li et al. 2007; Bass and Field 2011). Both their activities were differentially upregulated in larvae fed on MeJA-treated leaves, which might suggest that there would be increased detoxification. Prior research on MeJA-induced expression of secondary metabolites in *Populus × euramericana* ‘Nanlin895’ indicated that flavone content significantly increases at 12 h (Liu et al. 2015). Flavones are one type of

xenobiotics detoxified by GST (Lv et al. 2012). The GST activity upregulation suggests its functional role in accordance with the dynamics of xenobiotics. The CarE activities of larvae fed on MeJA-treated leaves were significantly higher than those of control at 4, 24, and 36 h, which suggested that increased levels of xenobiotics targeted for CarE detoxification.

Previous investigations have shown that exogenous methyl salicylate and MeJA sprayed on *Populus × euramericana* ‘Nanlin895’ increased peroxidase (POD), polyphenol oxidase (PPO), lipoxygenase (LOX), and Protease inhibitors (PIs) (Tang et al. 2015; Liu et al. 2015). *C. anachoreta* larval attack could elevate the production of hydrogen peroxide (H₂O₂) and enhance the activities of these three H₂O₂ scavenging enzymes, i.e., peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT), in herbivore-wounded poplar leaves (Hu et al. 2009). These comparative studies identified the potential mechanisms responsible for the induced *Populus × euramericana* ‘Nanlin895’ resistance to herbivores. However, there have been no further investigations into the effect of induced resistance in host plants on herbivore insects. Our findings suggest that there is an effect on the larvae when fed with MeJA-treated leaves. Further detailed experiments are needed to explore defense gene expressions in *Populus × euramericana* ‘Nanlin895’ that are induced by MeJA and to investigate the interaction between MeJA and other signaling molecules, and how the interaction influences induced defenses against *C. anachoreta*.

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Compliance with ethical standards

Conflict of interest We declare that there is no conflict of interest. All of the authors agree to submit this paper. The manuscript has not been previously published in any language anywhere and it is not under simultaneous consideration or in press by another journal.

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