Chemoecology (2012) 22:131–138
DOI 10.1007/s00049-012-0105-y

CHEMOECOLOGY

RESEARCH PAPER

o-Coumaric acid from invasive *Eupatorium adenophorum* is a potent phytotoxin

Guowei Zheng · Yanxia jia · Xu Zhao · Fujuan Zhang · Shihong Luo · Shenghong Li · Weiqi Li

Received: 18 October 2011 / Accepted: 8 February 2012 / Published online: 21 February 2012

Abstract A phytochemical study of the invasive *Eupatorium adenophorum* indicated that the plant was rich in a phenolic compound *o*-coumaric acid (or 2-hydroxycoumaric acid). Biological investigations with the model plant *Arabidopsis thaliana* and crop plants showed that *o*-coumaric acid strongly inhibited seed germination, plant growth and root elongation, reduced the photosynthesis in old leaves, and induced the root cell death and the expression of genes related to senescence, oxidative stress, and systemic acquired resistance. The phytotoxic effects of *o*-coumaric acid exhibit selectivity between under- and above-ground parts of test plants and between *E. adenophorum* and other plants. These results indicate that *o*-coumaric acid is a potent toxin that might play an important role in the competition of *E. adenophorum* with its neighboring plants during its invasion and establishment.

Keywords Phytotoxin · *Arabidopsis thaliana* · Germination inhibition · Growth inhibition · Root cell death · Leaf senescence

Electronic supplementary material The online version of this article (doi:10.1007/s00049-012-0105-y) contains supplementary material, which is available to authorized users.

G. Zheng · Y. jia · F. Zhang · W. Li (✉)
The Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
e-mail: weiqili@mail.kib.ac.cn

X. Zhao · S. Luo · S. Li (✉)
State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
e-mail: shli@mail.kib.ac.cn

Introduction

Eupatorium adenophorum Spreng. (or *Ageratina adenophora* (Spreng.) King and Robinson) is a native of Mexico and is a notorious weed worldwide (Trounce and Dyason 2003). Since it was first introduced into Yunnan province in Southwest China in the 1940s, *E. adenophorum* has spread very rapidly in China, and is one of the most economically important destructive weeds (Sang et al. 2010).

A large number of reports have indicated that the secondary metabolites synthesized and accumulated in *E. adenophorum* have wide biological activities. For example, aqueous extracts of *E. adenophorum* leaves inhibited the seed germination and seedling growth of ten plant species investigated (Zheng and Feng 2005); aqueous leachates from roots, stems, and leaves of *E. adenophorum* were phytotoxic on the growth of five species of the family Gesneriaceae (Li et al. 2007) and inhibited spore germination and gametophyte development of *Macrothelypteris torresiana* (Zhang et al. 2008). Many studies have been carried out to identify the bioactive compounds such as phytotoxins from *E. adenophorum*. Baruah et al. (1994) isolated three cadinene-type sesquiterpenes, (8β–hydroxy-ageraphorone, 7-epi-8-oxoageraphorone and 8-oxoageraphorone) and found that they had inhibitory activity in seed germination and seedling growth of onion, radish, and cucumber. Yang et al. (2006, 2008) isolated and identified two sesquiterpenes, (4,7-dimethyl-1-(propan-2-ylidene)-1,4,4a, 8a-tetrahydronaphthalene-2, 6 (1H, 7H)-dione and 6-hydroxyl-5-isopropyl-3, 8-dimethyl-4a, 5, 6, 7, 8, 8a-hexahydronaphthalen-2 (1H)-one), from the leachates of *E. adenophorum*, and found that they could cause membrane lipid peroxidation, damage the cell membrane, and influence the content of endogenous hormones (abscisic acid, indole-3-acetic acid, and zeatin riboside) of roots in upland rice.

In our investigation on the secondary metabolites of *E. adenophorum* and their possible ecological functions, we isolated and identified 23 compounds from the aerial parts of the plant, and found that only a few sesquiterpenes showed weak phytotoxic effects on *Arabidopsis* seed germination, while most other compounds, including the above-mentioned sesquiterpenes, were not active (Zhao et al. 2009; Zhao 2009). However, the most abundant phenolic compound (1 g/10 kg fresh weight), *o*-coumaric acid (or 2-hydroxycoumaric acid), showed strong inhibitory effect on *Arabidopsis* seed germination. We therefore examined the physiological and biochemical effects of *o*-coumaric acid on *Arabidopsis* plants, *E. adenophorum* itself, and on three crop plants. We propose that *o*-coumaric acid could be the major phytotoxic compound of *E. adenophorum*.

Materials and methods

Plant material and chemicals

The aerial parts of *E. adenophorum* were collected from the Botanic Garden of Kunming Institute of Botany, Chinese Academy of Sciences, in November 2007. Seeds of *Arabidopsis thaliana* were of the Columbia ecotype. Crop seeds (*Brassica napus*, *Raphanus sativus*, and *Brassica pekinensis*) were purchased from Jingdian Seed Company (Kunming, China). Authentic *o*-coumaric acid, *p*-coumaric acid, *m*-coumaric acid, and fluorescein diacetate (FDA) were purchased from Sigma-Aldrich (h22809, c9008, h23007, and f7378, respectively).

Isolation and identification of *o*-coumaric acid

Fresh aerial parts of *E. adenophorum* (10 kg) were chopped and extracted with methanol as described previously (Zhao et al. 2009). The methanol extract showed a strong inhibitory effect on *Arabidopsis* seed germination. It was concentrated subsequently and partitioned between water and ethyl acetate (AcOEt) (3 × 2.5 l). The AcOEt part which contained bioactive substances was dried to give 20 g of residue. The residue was subjected to column chromatography using silica gel as the stationary phase and eluted with an increasing gradient of methanol in chloroform (0–100%) to provide seven fractions Frs. A1–A7. Approximately 1.5 g of crude *o*-coumaric acid was crystallized directly from Frs. A3–A5. Further recrystallization of *o*-coumaric acid with acetone and methanol yielded 1 g of pure sample. For structure identification, the compound was subjected to ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy in deuterated methanol (CD_3OD) using a 400-MHz NMR spectrometer (Bruker AM-400; Germany).

Surface sterilization of seeds and plant growth

All seeds were sterilized with ethanol (70% v/v) for 2 min and sodium hypochlorite (5% v/v) for 2 min, and then rinsed three times with sterile distilled water. Surface-sterilized seeds were germinated on MS medium (Murashige and Skoog 1962) that contained 0.2% gellan gum (G1910; Sigma-Aldrich) and 1% sucrose. Hydroponic seedlings were grown in 1/4 Hoagland solution as described by Tocquin et al. (2003). The conditions of the growth chamber were 23/18°C, a 12/12 h light/dark cycle, and 150 μmol m^{-2} s^{-1} photosynthetic photon flux density.

Seed germination bioassay

A filter paper assay was used to test the phytotoxic effect of the methanol extract, *o*-coumaric acid, and its two isomers on the germination of *Arabidopsis* seeds. Test solutions of *o*-/*m*-/*p*-coumaric acid were prepared using methanol as the initial solvent carrier (100 mM), and then diluted with MS liquid medium to a range of concentrations from 0.05 to 1.0 mM. For each test solution, 5 ml were added to a 6-cm sterile glass Petri dish lined with three layers of filter paper. To assess the toxic effects of methanol, MS medium with methanol was added to additional Petri dishes as control. There were three replicates for each treatment. We placed 40 surface-sterilized seeds into each Petri dish and allowed them to germinate in growth chambers. The germination levels were recorded after 7 days of incubation. To study the phytotoxic effects of *o*-coumaric acid on the germination of crop seeds (*Brassica napus*, *Raphanus sativus*, and *Brassica pekinensis*), the crop seeds were soaked first in MS liquid medium that contained 0.4 mM *o*-coumaric acid for 4 h, because these seeds are larger than those of *Arabidopsis*, and then transferred onto filter paper to measure the germination as described above.

Seedling growth and biomass

To test the allelopathic effects of *o*-coumaric acid on *Arabidopsis* seedling growth, seeds were allowed to germinate on MS agar plates. After 5 days of growth, 20 seedlings were transferred to agar plates that contained different concentrations of *o*-coumaric acid. After growth on the treated plates for 7 days, the number of dead seedlings was recorded. Twenty plants were dried overnight in an oven at 105°C, and their dry weights were measured. We used hydroponic seedlings to investigate the different phytotoxic effects of *o*-coumaric acid on aerial parts and roots. Seedlings aged approximately 20 days were treated with different concentrations of *o*-coumaric acid in the hydroponic medium and, after 7 days, the fresh weights of roots and aerial parts were recorded. We also sprayed

0.4 mM *o*-coumaric acid on the leaves of hydroponic *Arabidopsis* seedlings every day, and after 7 days, the photos of the plants were taken.

Root growth and detection of root death

To assess the inhibitory effects of *o*-coumaric acid on *Arabidopsis* root growth, *o*-coumaric acid was added to MS agar plates at different concentrations. Surface-sterilized seeds were cold stratified for 2 days at 4°C, and subsequently sown in the Petri dishes that contained different concentrations of *o*-coumaric acid. Petri dishes with seeds were placed vertically in growth chambers. After incubation for 7 days, the root lengths of eight seedlings per plate were recorded. The experiments were repeated three times, and each replicate consisted of three agar plates. Hydroponic seedlings were used to evaluate root biomass. Twenty-day-old seedlings were treated with different concentrations of *o*-coumaric acid, and the fresh weights of the roots were recorded at 7 days after treatment.

To detect root death, seedlings of *Arabidopsis* were grown on vertical plates for 5 days. To measure viability, roots were stained for 5 min with 5 μg ml^{-1} FDA, and then rinsed three times with MS liquid medium. After staining and rinsing, the roots were treated with 0.6 mM *o*-coumaric acid and observed under a confocal laser scanning microscope (FV-1000; Olympus, Japan) at 5-min intervals. FDA fluorescence decreased as the dye leaked from dead cells. Images were processed with Adobe Photoshop CS.

Measurement of photosynthetic parameters

Chlorophyll fluorescence was measured using an IMAGIN-PAM chlorophyll fluorometer and ImagingWin software application (Walz, Effeltrich, Germany). Hydroponic seedlings were grown in 1/4 Hoagland solution that contained different concentrations of *o*-coumaric acid. After treatment for 7 days, seedlings were allowed to adapt in the dark for 20 min prior to the induction of fluorescence measurements. Chlorophyll fluorescence parameters were measured as described by Woo et al. (2008).

Real-time quantitative RT-PCR

Hydroponic *Arabidopsis* were treated with 0.6 mM *o*-coumaric acid for indicated number of days, and total RNA was extracted from treated leaves using RNAiso Plus (D9108A; Takara, Japan). Potential contaminating genomic DNA was digested with RNase-free DNase I (D2215; Takara). To produce cDNA, 1.2 μg of total RNA were reverse transcribed using an oligo(dT)18 primer in a 10-μl reaction mixture with Quant Reverse Transcriptase (KR103-04; Tiangen, Beijing, China). When the reaction was completed, the reaction mixture was diluted to 30, and 2 μl of the synthesized cDNA was used for quantitative RT-PCR. Quantitative RT-PCR was performed on an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, USA) using Faststart Universal SYBR Green Master (Rox) (11602200; Roche, Switzerland). Specific primers (Sangon Biotech, Shanghai, China) (Supplementary Table S1) were designed. ACT2 (AT3G18780) was used as an endogenous control to normalize the relative expression level. The data were analyzed with the ABI 7500 System SDS software. Three independently performed experiments that used independent tissue samples were analyzed.

Results

o-Coumaric acid isolated from *E. adenophorum* shows strong phytotoxic activity on seed germination

Twenty-three compounds (Supplementary Table S2) were isolated from the aerial parts of *E. adenophorum*. These include a major phenolic compound, with an abundance of approximately 1 g per 10 kg of fresh plant material. The ^{1}H NMR spectrum of this compound showed signals at δ_H 8.03 (1H, *d*, $J = 16.1$ Hz), 7.60 (1H, *d*, $J = 7.6$ Hz), 7.24 (1H, *t*, $J = 8.2$ Hz), 6.96 (1H, *d*, $J = 8.2$ Hz), 6.89 (1H, *t*, $J = 7.6$ Hz), and 6.61 (1H, *d*, $J = 16.1$ Hz), which indicated the existence of a 1,2-disubstituted phenyl ring and a *trans* double bond. The ^{13}C NMR and DEPT spectra of *o*-coumaric acid exhibited six olefinic methines (δ_C 141.3d, 132.3d, 129.7d, 120.8d, 118.6d, and 116.9d) which were in agreement with the signals observed in the ^{1}H NMR spectrum, a conjugated carboxylic carbonyl carbon (δ_C 168.9 s), and two olefinic quaternary carbons (δ_C 157.3 and 122.2 s), with the downfield one oxygenated. These data established the structure of this phenolic compound as *o*-coumaric acid or *o*-hydroxyl cinnamic acid, which was further confirmed by direct HPLC comparison with an authentic standard from Sigma-Aldrich.

We tested the phytotoxic effects of all aforementioned 23 compounds on *Arabidopsis* seed germination. Among those major terpenes isolated (2 monoterpenes and 9 sesquiterpenes) only two sesquiterpenes retarded seed germination (Zhao et al. 2009), while most of other compounds were not active (Zhao 2009). We showed here that *o*-coumaric acid was highly active (Fig. 1). *o*-Coumaric acid inhibited germination of *A. thaliana* completely at a concentration of 0.5 mM, with an EC_{50} of 0.1 mM (Fig. 1), which was about 25-fold lower than that for lettuce (Li et al. 1993). To test whether the phytotoxic activity was structurally specific, we treated *A. thaliana* seeds with three

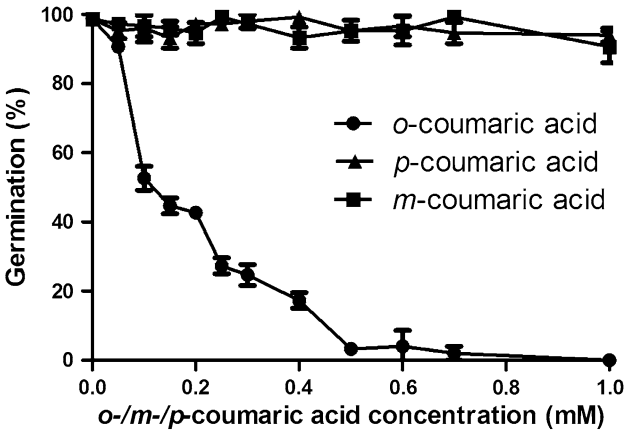

Fig. 1 Effects of the three coumaric acid isomers on *Arabidopsis* seed germination. Surface-sterilized *Arabidopsis* seeds were sown on MS agar plates that contained different concentrations of coumaric acid. Forty seeds were sown on each plate. There were three replicates of each plate, and the experiments were repeated at least three times. Values are means ± SD, $n = 3$

isomers of coumaric acid (*o*-, *p*-, and *m*-coumaric acids) at the same time, and examined the seed germination. There were no observable effects on seed germination by *p*- and *m*-coumaric acids, even at concentrations as high as 1.0 mM (Fig. 1). We also tested the phytotoxic effect of *o*-coumaric acid on crop seeds and found that it inhibited *Brassica napus* and *Raphanus sativus* seed germination completely and significantly reduced *Brassica pekinensis* seed germination at a concentration of 0.4 mM (Supplementary Table S3). In addition, *o*-coumaric acid also had a strong phytotoxic effect on *E. adenophorum* seed germination (Supplementary Fig. S1). All these results suggest that *o*-coumaric acid in *E. adenophorum* is a potent phytotoxin, and its phytotoxic activity is highly structure specific.

o-Coumaric acid inhibits growth of *Arabidopsis,* and roots are more sensitive than leaves

To further investigate the phytotoxic effect of *o*-coumaric acid, we examined the growth of plate- and hydroponically grown *Arabidopsis* plants. When plate-grown plants were treated for 7 days with *o*-coumaric acid, their biomass decreased dramatically as the concentration of *o*-coumaric acid increased (Fig. 2a). Some of the plants treated with 0.8 and 1.0 mM *o*-coumaric acid died after 7 days, as determined by leaf bleaching (Fig. 2b and Supplementary Fig. S2). We also investigated the effects of *o*-coumaric acid on the aerial parts and roots of *Arabidopsis* by adding it to hydroponic medium. The fresh weight of the aerial parts was not affected at lower concentrations (0.1 and 0.2 mM) of *o*-coumaric acid, but decreased significantly at ≥0.4 mM (Fig. 2c). In contrast, the fresh weight of the roots decreased rapidly in plants grown with *o*-coumaric acid, with a reduction of 30% by 0.1 mM (Fig. 2d). We sprayed 0.4 mM *o*-coumaric acid solution daily onto the aerial parts of hydroponically grown *Arabidopsis* seedlings for 7 days, and found no difference between control and treated plants (Supplementary Fig. S3), meaning that *o*-coumaric acid had no direct effect on the aerial parts of *Arabidopsis*. When *Arabidopsis* and *E. adenophorum* plants were parallel treated with *o*-coumaric acid in the hydroponic medium for 7 days, we found that the *Arabidopsis* seedlings withered and the older leaves died, but *E. adenophorum* looked normal morphologically by visual inspection (Supplementary Fig. S4). These results indicated that *o*-coumaric acid inhibited the growth of *Arabidopsis* through its harmful effects on the plant roots. *o*-Coumaric acid had indirect effects on the aerial parts and older leaves were more sensitive. *E. adenophorum* was resistant to *o*-coumaric acid-induced growth inhibition even at 0.8 mM.

o-Coumaric acid inhibits root elongation and induces root cell death in *Arabidopsis*

To investigate how *o*-coumaric acid affects the roots of *Arabidopsis*, we first examined root elongation and root viability in *Arabidopsis* treated with *o*-coumaric acid. In the presence of 0.1 mM of *o*-coumaric acid, root length was only 50% of that observed under control conditions, and elongation was inhibited completely by 0.3 mM *o*-coumaric acid (Fig. 3). We determined whether *o*-coumaric acid induced cell death at the root tips using the vital stain FDA, and found that 5 min after treatment with 0.6 mM *o*-coumaric acid, the fluorescence of all root tip cells had faded dramatically. Cells of the meristematic zone were affected first and lost their viability within 15 min, as shown by loss of FDA fluorescence. By 30 min of *o*-coumaric acid treatment, almost all cells in the central elongation zone (CEZ) had died (Fig. 4a). We also monitored cell death at the roots with a confocal laser scanning microscope and found that cells in the CEZ grew darker as the duration of *o*-coumaric acid treatment increased (Fig. 4b), which confirmed the pattern of cell death detected by fluorescence microscopy. These results suggested that *o*-coumaric acid inhibited root elongation by inducing cell death at the root tips.

o-Coumaric acid has different effects on photosynthetic activity in old and young *Arabidopsis* leaves

Photosynthesis has been known to be sensitive to environmental stresses. The maximum quantum efficiency of photosystem II (Fv/Fm) usually declines under stress. To investigate the long-term effects of *o*-coumaric acid on plant growth, we examined the F_v/F_m of leaves of

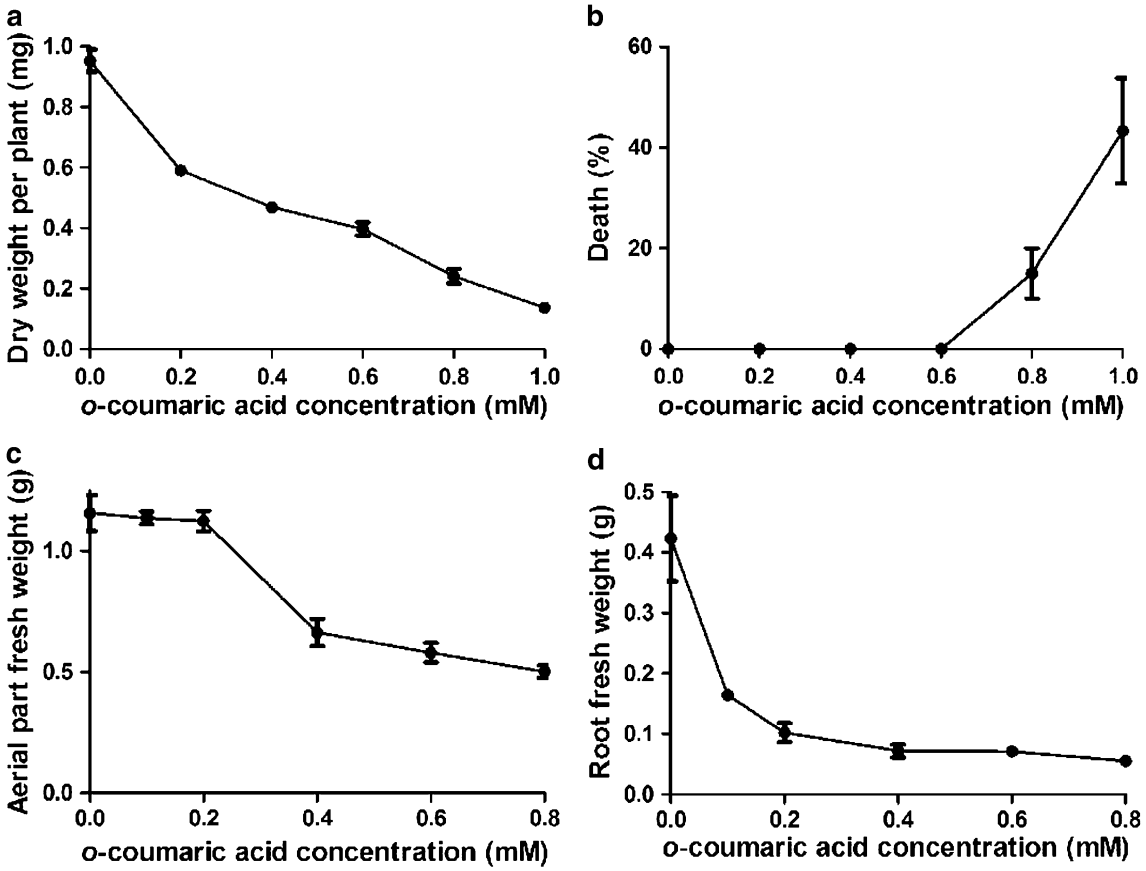

Fig. 2 Growth of *Arabidopsis* seedlings exposed to different concentrations of *o*-coumaric acid. Values are means ± SD, $n = 3$. **a**, Dry weight per plant after treatment with *o*-coumaric acid for 7 days. **b** Death rate after treatment with *o*-coumaric acid for 7 days. Seeds were germinated on MS agar plates for 5 days, and 20 seedlings were transferred to each treatment plate. **c** Fresh weight of aerial parts of hydroponic seedlings after treatment with *o*-coumaric acid for 7 days. **d** Fresh weight of roots of hydroponic seedlings after treatment with *o*-coumaric acid for 7 days. Twenty-day-old hydroponic seedlings were transferred to hydroponic solutions that contained different concentrations of *o*-coumaric acid

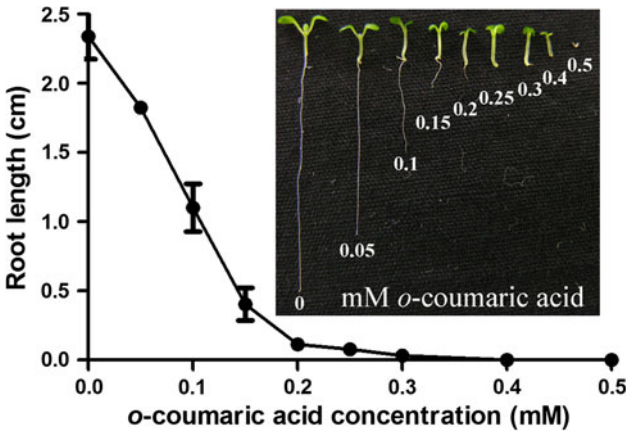

Fig. 3 Effect of *o*-coumaric acid on *Arabidopsis* roots. Inhibition by *o*-coumaric acid of *Arabidopsis* seedling root elongation. Values are means ± SD, $n = 24$. *Inset*, inhibition of root length. Surface-sterilized *Arabidopsis* seeds were sown on MS agar plates that contained different concentrations of *o*-coumaric acid, and after growth for 7 days, photographs were taken and the root lengths were measured

Arabidopsis under *o*-coumaric acid treatment. After 7 days of treatment, we found that the F_v/F_m of the older leaves had dropped dramatically in the presence of 0.2 and 0.4 mM of *o*-coumaric acid and was nearly lost completely in the presence of 0.8 mM of *o*-coumaric acid, which meant that the older leaves were dying rapidly. In contrast, the younger central leaves were hardly affected (Fig. 5). These results indicated that *o*-coumaric acid had an indirect effect on the development of the aerial parts and that the major effects were on older leaves. Given that older leaves were sensitive to *o*-coumaric acid, the results indicated that *o*-coumaric acid could promote leaf senescence.

o-Coumaric acid increases expression of genes related to senescence, oxidative stress, and systemic acquired resistance

Plant responses to environmental stimuli usually include crosstalk among the different response mechanisms. To investigate the possible cellular responses induced by

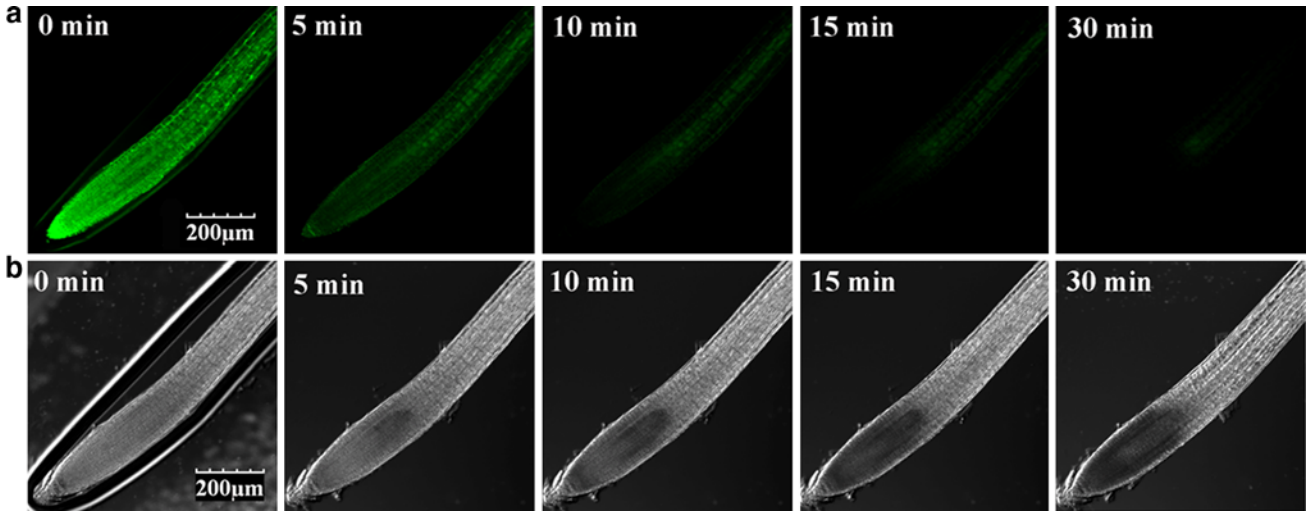

Fig. 4 Induction of cell death by 0.6 mM *o*-coumaric acid in meristematic and CEZ cells of *Arabidopsis*. **a** Cell death proceeds with sequential loss of FDA fluorescence. **b** Effect of *o*-coumaric acid on *Arabidopsis* roots

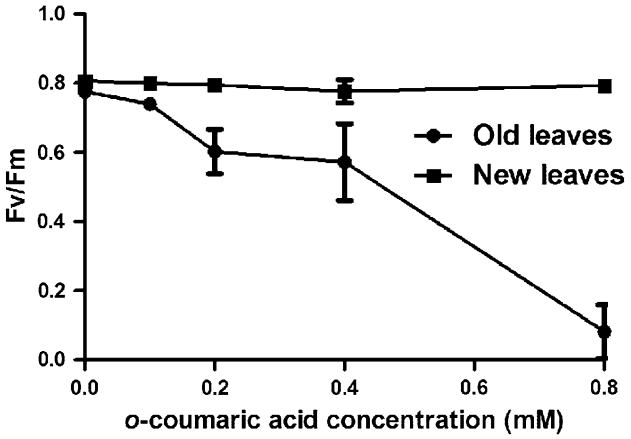

Fig. 5 Effect of *o*-coumaric acid on F_v/F_m of older and younger leaves. Leaf chlorophyll fluorescence was measured after treatment for 7 days with different *o*-coumaric acid concentrations. Five seedlings were used to measure F_v/F_m. Values are means ± SD, $n = 12$

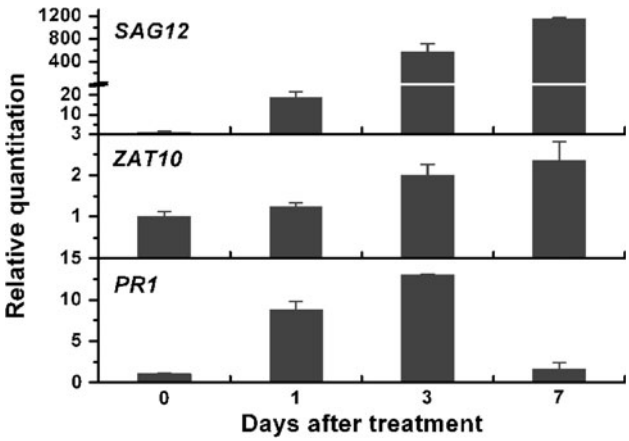

Fig. 6 Gene expression in leaves of hydroponic *Arabidopsis* treated with 0.6 mM of *o*-coumaric acid. Ten seedlings of *Arabidopsis* were transferred to solutions that contained different concentrations of *o*-coumaric acid, and 7 days after treatment, the expression of three different genes in the leaves of hydroponic *Arabidopsis* treated with 0.6 mM *o*-coumaric acid was measured

o-coumaric acid, we examined the expression of three genes, *SAG12*, *ZAT10*, and *PR1*, which are typically induced in senescence, oxidative stress, and systemic acquired resistance, respectively (Fig. 6). In the presence of 0.6 mM *o*-coumaric acid, we found that expression of *SAG12* and *ZAT10* increased dramatically throughout the 7 days of treatment, and expression of *PR1* increased during the first 3 days of treatment and then decreased to background levels after 7 days. The high induction of *SAG12* expression supported the observation that *o*-coumaric acid promoted senescence of older leaves. The high induction of *ZAT10* suggested that oxidative stress occurred as a result of *o*-coumaric acid treatment. These results indicated that multiple stress responses could be induced by *o*-coumaric acid.

Discussion

It is widely believed that the allelopathic effects of invasive plants are a major factor in their successful invasion, particularly in *E. adenophorum*. Phytotoxins in *E. adenophorum* have attracted much attention and have been investigated extensively. Several sesquiterpenes isolated from *E. adenophorum* were reported to be the major phytotoxins (Baruah

et al. 1994; Yang et al. 2006, 2008). However, our previous work showed that these sesquiterpenes did not affect *Arabidopsis* seed germination, whilst another two sesquiterpenes exhibited only a weak phytotoxic effect by retarding seed germination (Zhao et al. 2009). Therefore, we proposed that cadinene-type sesquiterpenes are only part of the phytotoxic weaponry of *E. adenophorum* and that non-terpene substances may have stronger phytotoxic effects worthy of detailed investigation (Zhao et al. 2009). The isolation of *o*-coumaric acid from *E. adenophorum* and discovery of its potent phytotoxicity in the present study corroborated our previous speculation.

o-Coumaric acid is a common chemical constituent in the plant kingdom (Habib and Abdul Rahman 1988; Vega et al. 2008; Ngoc et al. 2009; Sellami et al. 2009; Canuto et al. 2010). Its phytotoxic effects have also been previous described (Chou and Patrick 1976; Aliotta et al. 1993; Li et al. 1993; Chon et al. 2002; Chon and Kim 2002). However, its unusual high content in plants is first reported. The unusually high levels of phytotoxin in this invasive plant strongly imply that this compound may have a functional role in the plant's invasion. Consequently, *E. adenophorum* may use *o*-coumaric acid as an allelochemical for its successful invasion. Based on our observation, *o*-coumaric acid showed two types of harmful effects on test plants: an inhibition of seed germination; and the induction of cell death in the root tips, which can promote senescence in mature leaves, thus leading to the death of the plant.

Our physiological data also indicate that the phytotoxic effects of *o*-coumaric acid are quite selective and might provide clues to explain its possible ecological function. First, its harmful effects differ between under- and aboveground parts of test plants as roots rather than leaves are affected. Thus, *o*-coumaric acid probably acts on plants through solution in the soil. Second, its harmful effects occur on both seed germination and growth in *Arabidopsis* but only on seed germination in *E. adenophorum*, showing selectivity between *E. adenophorum* and other plants. This selective property may benefit the invasion ability of *E. adenophorum*. Seeds of *E. adenophorum* have pappus and are able to be transported long distances by wind, which could help *E. adenophorum* to avoid self-toxicity by *o*-coumaric acid. Moreover, *E. adenophorum* seedlings can propagate vegetatively and thus can take advantage of neighboring plants which are growing under toxic stress by *o*-coumaric acid.

Structure-specific phytotoxic effects have been found in other cases of phytotoxins. With tyrosine for example, only *m*-tyrosine has toxic activity (Bertin et al. 2007). For the three isomers of coumaric acids, *m*-, *p*-, and *o*-coumaric acid, their phytotoxic effects are different. Phytotoxic effects of *m*- and *p*-coumaric acids were less than that of *o*-coumaric acid in alfalfa and lettuce (Chon and Kim 2002; Chou and Patrick 1976). In our case, *m*- and *p*-coumaric acids were not active at all over the concentration used. It should be noted that *m*- and *p*-coumaric acids were not isolated and even not detected, whereas *o*-coumaric acid content was unusually high in *E. adenophorum*. Thus, the different distribution of three coumaric acid isomers might biologically correlate to their phytotoxic activities between species. The high *o*-coumaric acid content might be necessary for the ecological functions of *E. adenophorum*.

In summary, we isolated *o*-coumaric acid from the invasive plant *E. adenophorum* and showed that this compound has potent phytotoxic effects on seed germination and root growth and promotes leaf senescence. Although there is no direct evidence to demonstrate that *o*-coumaric acid is an allelochemical of *E. adenophorum*, its contents, selectivity of harmful effects, and structure-specific phytotoxic effects suggest that its ecological functions could assist *E. adenophorum* to invade.

Acknowledgments The study was supported by grants from National Natural Science Foundation of China (NSFC 30670474, 30870571, 31070262 and 30870404), Fund of State Key Laboratory of Phytochemistry and Plant Resources in West China (0807B01211 and 097C1211Z1), Germplasm Bank of Wild Species and CAS Innovation Program of Kunming Institute (540806321211), 100-Talents Program of CAS, and XiBuZhiGuang Project. The authors would like to thank Dr. Hugh Pritchard for his critical reading of the paper.

References

Aliotta G, Cafiero G, Fiorentino A, Strumia S (1993) Inhibition of radish germination and root growth by coumarin and phenylpropanoids. J Chem Ecol 19:175–183

Baruah NC, Sarma JC, Sarma S, Sharma RP (1994) Seed germination and growth inhibitory cadinenes from *Eupatorium adenophorum* Spreng. J Chem Ecol 20:1885–1892

Bertin C, Weston LA, Huang T, Jander G, Owens T, Meinwald J, Schroeder FC (2007) Grass roots chemistry: *meta*-Tyrosine, an herbicidal nonprotein amino acid. Proc Natl Acad Sci USA 104:16964–16969

Canuto KM, Lima MA, Silveira ER (2010) Amburosides C-H and 6-*o*-protocatechuoyl coumarin from *Amburana cearensis*. J Braz Chem Soc 21:1746–1753

Chon S-U, Kim J-D (2002) Biological activity and quantification of suspected allelochemicals from alfalfa plant parts. J Agron Crop Sci 188:281–285

Chon S-U, Choi S-K, Jung S, Jang H-G, Pyo B-S, Kim S-M (2002) Effects of alfalfa leaf extracts and phenolic allelochemicals on early seedling growth and root morphology of alfalfa and barnyard grass. Crop Prot 21:1077–1082

Chou CH, Patrick ZA (1976) Identification and phytotoxic activity of compounds produced during decomposition of corn and rye residues in soil. J Chem Ecol 2:369–387

Habib SA, Abdul Rahman AA (1988) Evaluation of some weed extracts against field dodder on alfalfa (*Medicago sativa*). J Chem Ecol 14:443–453

Li HH, Inoue M, Nishimura H, Mizutani J, Tsuzuki E (1993) Interactions of *trans*-cinnamic acid, its related phenolic allelochemicals, and abscisic acid in seedling growth and seed germination of lettuce. J Chem Ecol 19:1775–1787

Li YB, Xu H, Shi L, Li Z (2007) Allelopathic effects of *Eupatorium adenophorum* on five species of the family Gesneriaceae. Biodivers Sci 15:486–491

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Ngoc TM, Lee I, Ha DT, Kim HJ, Min BS, Bae K (2009) Tyrosinase-inhibitory constituents from the twigs of *Cinnamomum cassia*. J Nat Prod 72:1205–1208

Sang WG, Zhu L, Axmacher JC (2010) Invasion pattern of *Eupatorium adenophorum* Spreng in Southern China. Biol Invasions 12:1721–1730

Sellami IH, Maamouri E, Chahed T, Wannes WA, Kchouk ME, Marzouk B (2009) Effect of growth stage on the content and composition of the essential oil and phenolic fraction of sweet marjoram (*Origanum majorana* L.). Ind Crop Prod 30:395–402

Tocquin P, Corbesier L, Havelange A, Pieltain A, Kurtem E, Bernier G, Perilleux C (2003) A novel high efficiency, low maintenance, hydroponic system for synchronous growth and flowering of *Arabidopsis thaliana*. BMC Plant Biol 3:2

Trounce B, Dyason R (2003) Agfacts—crofton weed. NSW Agriculture http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0010/155962/crofton-weed.pdf. Accessed 12 June 2010

Vega M, De Carvalho M, Vieira I, Braz-filho R (2008) Chemical constituents from the Paraguayan medicinal plant, Eupatorium macrocephalum Less. J Nat Med 62:122–123

Woo N, Badger M, Pogson B (2008) A rapid, non-invasive procedure for quantitative assessment of drought survival using chlorophyll fluorescence. Plant Methods 4:27

Yang GQ, Wan FH, Liu WX, Zhang XW (2006) Physiological effects of allelochemicals from leachates of *Ageratina adenophora* (Spreng.) on rice seedlings. Allelopathy J 18:237–246

Yang GQ, Wan FH, Liu WX, Guo JY (2008) Influence of two allelochemicals from *Ageratina adenophora* Sprengel on ABA, IAA and ZR contents in roots of upland rice seedlings. Allelopathy J 21:253–262

Zhang KM, Shi L, Jiang CD, Li ZY (2008) Inhibition of *Ageratina adenophora* on spore germination and gametophyte development of *Macrothelypteris toresiana*. J Integr Plant Biol 50:559–564

Zhao X (2009) Chemical constituents from *Eupatorium adenophorum* and their allelopathic effects. Master's thesis

Zhao X, Zheng GW, Niu XM, Li WQ, Wang FS, Li SH (2009) Terpenes from *Eupatorium adenophorum* and their allelopathic effects on *Arabidopsis* seeds germination. J Agric Food Chem 57:478–482

Zheng L, Feng YL (2005) Allelopathic effects of *Eupatorium adenophorum* Spreng. on seed germination and seedling growth in ten herbaceous species. Acta Ecol Sin 25:2782–2787