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Responses of *Mikania micrantha*, an Invasive Weed to Elevated CO_2: Induction of β-Caryophyllene Synthase, Changes in Emission Capability and Allelopathic Potential of β-Caryophyllene

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Abstract To better understand the effect of predicted elevated levels of carbon dioxide (CO_2) on an invasive weed *Mikania micrantha*, we constructed a suppressive subtractive hybridization (SSH) library from the leaves of *M. micrantha* exposed to CO_2 at 350 and 750 ppm for 6 d, and isolated a novel gene named β-caryophyllene synthase. β-Caryophyllene synthase catalyses the conversion of farnesyl diphosphate to β-caryophyllene, a volatile sesquiterpene with allelopathic potential. Real-time PCR analysis revealed that gene expression of β-caryophyllene synthase in *M. micrantha* leaves was strongly induced in response to elevated CO_2. Gas chromatography-mass spectrometry (GC-MS) and gas chromatography (GC) analyses showed that emission levels of β-caryophyllene from leaves of *M. micrantha* increased when exposed to 750 ppm CO_2. Bioassays showed that phytotoxicity of β-caryophyllene against *Raphanus sativus*, *Brassica campestris*, *Lactuca sativa,* and *M. micrantha* was dose-dependent and varied with the receptor plants and concentrations of CO_2. β-Caryophyllene displayed higher phytotoxic effects at 750 ppm than those at 350 ppm CO_2, especially on *R. sativus*. These results suggest that elevated atmospheric CO_2 levels may enhance biosynthesis and phytotoxicity of allelochemicals in *M. micrantha*, one of the worst invasive weeds in the world, which in turn might enhance its potential allelopathic effect on neighboring native plants if released in bioactive concentrations. Further investigations are required to determine the adaptive responses of both invasive and native plants to a gradual increase of atmospheric CO_2 to 750 ppm predicted over a 100 year period.

Key Words Carbon dioxide · *Mikania micrantha* · Allelopathy · β-Caryophyllene synthase · β-Caryophyllene

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Introduction

Over the last two centuries, human use of fossil carbon has increased the concentration of atmospheric CO_2, which significantly contributes to global warming. It is estimated that the concentration of CO_2 in the atmosphere will mount from the current level of 380 ppm to 500–750 ppm by the end of this century (Falkowski et al., 2000). Recent work suggests that emission of volatile organic compounds (VOCs) from plants may be particularly sensitive to elevated CO_2 concentrations (Rapparini et al., 2004; Possell et al., 2005; Himanen et al., 2009). Increasing atmospheric CO_2 concentration as an environment stress (Iwasaki et al., 1998; Beuf et al., 1999; Polle et al., 2008) could increase VOC emissions from plants (Constable et al., 1999; Rapparini et al., 2004; Possell et al., 2005; Tiiva et al.,

2008; Himanen et al., 2009). Accumulated plant VOCs may volatilize into the atmosphere, depending on their concentration and physiochemical properties (Niinemets et al., 2004). Some VOCs, such as sesquiterpenes, may have allelopathic effects on neighboring plants (Fischer et al., 1989; Macías et al., 1996; Abdelgaleil and Hashinaga, 2007; Abdelgaleil et al., 2009; Wang et al., 2009).

Mikania micrantha H.B.K. (Compositae), a fast-growing vine species, is one of the world's most aggressive weeds. The plant originated from South and Central America (Maffei et al., 1999), and has led to serious ecological problems in Southeast Asia and the Pacific region (Zhang et al., 2004). In Southern China, *M. micrantha* has caused serious damage to crops and forests (Zhang et al., 2004; Wang et al., 2009). In natural ecosystems, rapid invasion of *M. micrantha* during recent years is correlated with extinction of native species (Lee and Klasing, 2004). Beside the efficient take-up of water and nutrients, *M. micrantha* releases phytotoxic compounds that inhibit germination and growth of neighboring plants (Cock et al., 2000; Ismail and Chong, 2002; Shao et al., 2005; Ni et al., 2006). Several studies indicate that allelopathy may be a phenomenon that increases fitness of invasive plants (Callaway and Aschehoug, 2000; Inderjit and Duke, 2003; Gómez-Aparicio and Canham, 2008). In general, allelopathy is more common under extreme environmental conditions, where water, light, or nutrients are limited (Anaya, 1999; Valladares et al., 2007). A similar stress situation might be true in the case of elevated CO_2 concentration.

β-Caryophyllene synthase catalyses the conversion of farnesyl diphosphate to β-caryophyllene, a common sesquiterpene of essential oils of many plants (Cai et al., 2002). β-Caryophyllene inhibits seedling growth of *Brassica campestris* and *Raphanus sativus* (Wang et al., 2009). Kil et al. (2000) reported that β-caryophyllene is a component of the essential oil of *Artemisia lavandulaefolia*, which suppresses seedling growth of *Achyranthes japonica* (Miq.). Overexpression of the sesquiterpene synthase gene namely Os*TPS3* [(*E*)-β-caryophyllene synthase)] in rice plants increases the production of the compound after methyl jasmonate (MeJA) treatment, and MeJA-treated transgenic rice plants attract more parasitoid wasps of *Anagrus nilaparvatae* than the wild-type (Cheng et al., 2007). These studies suggest that the β-caryophyllene synthase and β-caryophyllene may play a role in the indirect defense of plants.

We hypothesized that increasing atmospheric CO_2 concentrations may change allelochemical production and allelopathic potential of the invasive alien plant *M. micrantha*, which, in turn, may affect its interactions with native plants by affecting their germination and seedling growth.

Methods and Materials

Plant Materials Plants of *M. micrantha* H.B.K. (1.5 m high) were collected from a natural population in Qi Ao Island, Zhuhai (N 21°48′, E 113°3′) and maintained in a greenhouse in Guangzhou (N 23°8′, E 113°17′). They were cut into 10 cm pieces and transplanted into plastic pots (20 cm diam; 25 cm high) and allowed to climb on 1.5 m bamboo stakes. Uniform seedlings (about 1.2 m high) were selected and planted in 12 pots (one plant per pot). Transplanted pots were transferred into growth chambers at 350 or 750 ppm CO_2 concentration with 10 hr daylight 12 000 lx at 25°C; 14 hr night at 15°C and 80% relative humidity. Plants were watered with diluted Hoagland solution (25% *v*/*v*) and randomized twice a week to avoid internal chamber effects. Seeds of *R. sativus*, *B. campestris,* and *Lactuca sativa* were purchased from Guangzhou Seed Company (Guangzhou, China), and seeds of *M. micrantha* were collected from Qi Ao Island, Zhuhai. β-Caryophyllene (>98.5% purity) was purchased from Sigma-Aldrich Chemie GmbH (D-89555 Steinheim, Germany).

SSH Library Construction Suppression subtractive hybridization (SSH) was used to generate a cDNA library (Diatchenko et al., 1996). Briefly, 30-d-old *M. micrantha* plants grown at 350 ppm CO_2 were exposed to 750 ppm CO_2 for 6 d, or left at 350 ppm CO_2. Total RNA then was isolated from the 5th and 6th leaves (counted basipetally from the apex) using the standard guanidine thiocyanate method (Chomczynski and Sacchi, 2006). Double strand cDNA was synthesized using the SMART™ cDNA amplification Kit (Clontech). A subtracted cDNA library was constructed using the PCR-Select cDNA Subtraction Kit (Clontech). PCR products were purified using the QIAquick PCR purification kit (Qiagen) and ligated into the pGEM-T easy vector (Promega). The constructs were transformed into *E. coli* Top10 (Invitrogen). Positive clones were verified by PCR using T7 and SP6 primers and sequenced with an ABI 3730 sequencer (Applied Biosystems, Inc.). All obtained sequences were compared with DNA databases using the BLASTX sequence comparison software (Altschul et al., 1997) at the NCBI website (http://www.ncbi.nlm.nih.-gov/BLAST). An identified expressed sequence tag (EST) named WGJ87 (658 bp) showed high level of sequence similarity (e^{-35}) to a characterized β-caryophyllene synthase gene from *Artemisia annua* (accession number AAL79181) (Cai et al., 2002), and this inspired us to do further analysis.

Real-time Quantitative PCR Real-time PCR was performed with RNA isolated from *M. micrantha* leaves to confirm activation of β-caryophyllene synthase expression in response to increased CO_2 concentration. Twelve pots of

M. micrantha (1.2 m high) were transferred into the growth chambers at 350 or 750 ppm CO_2 for up to 12 d. The 5th and 6th leaves of each plant from three pots were harvested at 3, 6, 9, and 12 d. Total RNA was isolated from the harvested leaves and quantified based on the absorbance at 260 nm. The integrity of RNA was checked with agarose gel electrophoresis. The RNA (1 μg) was treated first with DNase I (Invitrogen) to remove any genomic DNA contamination. The RNA then was reverse transcribed using oligo (dT) primer and ThermoScript RT-PCR System (Invitrogen) according to the manufacturer's instructions.

Real-time PCR was performed on the ABI PRISM 7000 sequence detection system (Applied Biosystems) in a volume of 20 μl containing 0.2 μM of each primer, 10 μl of 2×SYBR Green I (Roche) and 1 μl of the cDNA temple. The PCR cycling conditions were: 95°C for 1 min, 40 cycles of 95°C for 15 sec, 55°C for 15 sec, 72°C for 45 sec, and 85°C for 20 sec for signal collection in each cycle. To assess the specificity of the PCR amplification, a melt-curve analysis was performed at the end of the reaction by increasing temperature from 55 to 99°C and held for 5 sec at every increment of 1°C, and a single peak was observed.

The PCR primers were WGJ87F1 (5′-TAAGAAGGAG CAAGAAAGAGTGC-3′) and WFG87R1 (5′-CTC TTTGATGTCTTCTTCCACTTC-3′) for β-caryophyllene synthase, and WGJ60 (5′-GATTCCACCAGACCAG CAAAGG-3′) WGJ61 (5′-CACCACGAAGACGAAGCA CAAG-3′) for ubiquitin. The primers were designed from the sequenced ESTs, and the ubiquitin gene was chosen as internal standard. Real-time PCR reactions were performed in triplicate. Analysis of relative gene expression data was performed with the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) using *M. micrantha* plants exposed to 350 ppm CO_2 as a reference.

GC-MS and GC Analysis Twelve pots of *M. micrantha* grown at 350 ppm CO_2 for 30 d were exposed to 750 ppm for 3, 6, 9, and 12 d. Control plants were left at 350 ppm CO_2. The 5th and 6th leaves of each plant were harvested at 9:30 in the morning 3, 6, 9, and 12 d after exposure. For analysis of the volatiles sesquiterpenes, 500 mg fresh leaves were ground in liquid N_2. After addition of 5 g Na_2SO_4, the powder was enclosed in a 22-ml glass tube, and the 65-μm solid-phase microextraction (SPME) PDMS-DVB fiber (Supelco, Bellefonte, PA, USA) was inserted into the tube to collect volatiles at 80°C for 25 min. The SPME fiber was injected into a GC-MS system for analysis. For quantification, 11.3 ng of β-caryophyllene was added as an internal standard in one of the control samples.

GC-MS analysis of volatile sesquiterpenes was performed with a Finnigan Voyager using a BPX5 column (25 m×0.22 mm×0.25 μm), He (1 $ml\,min^{-1}$ gas flow rate), a splitless injection temperature 220°C, a quadrupole-type mass selective detector with a transfer line temperature 230°C, a source temperature 200°C, an ionization potential 70 eV, and a scan range 35 to 450 amu. The initial temperature was 60°C. After 3 min, the temperature was increased to 120°C (gradient of 10°C$\,min^{-1}$); the temperature was then further increased to reach 180°C (gradient of 5°C$\,min^{-1}$) and finally to 250°C (gradient of 25°C$\,min^{-1}$). Individual sesquiterpenes were tentatively identified by a peak matching library search that used authentic standards and NIST (National Institute of Standard and Technology) and Wiley libraries.

Changes of the β-caryophyllene were analyzed by GC-FID using an HP-5MS column (30 m×0.25 mm×0.25 μm), N_2 (1 $ml\,min^{-1}$ gas flow rate) as carrier. Two ml headspace samples were analyzed using the parameters described above.

Bioassay of β-Caryophyllene Seeds of *R. sativus*, *B. campestris*, *L. sativa,* and *M. micrantha* were surface-sterilized with 0.5% $KMnO_4$ for 10 min and then washed × 3 with sterile distilled water. β-Caryophyllene was dissolved in ethyl acetate, and different volumes of this solution were added to filter paper in glass containers (6× 6×10 cm) to get 0.375, 3, and 24 $mg\,L^{-1}$ concentrations. After complete evaporation of ethyl acetate, 20 seeds of each target plant and 5 ml distilled water were placed in each glass container (Wang et al., 2009). Plants were grown in growth chambers at 350 or 750 ppm CO_2 (at 25±1°C with10 hr light, 14 hr dark and 80% relative humidity). Germination rate (3 d for *R. sativus* and *B. campestris*; 7 d for *L. sativa* and *M. micrantha*), root length, and shoot height of seedlings were recorded from each filter paper at the time of harvest (9 d for *R. sativus* and *B. campestris*; 16 d for *L. sativa* and *M. micrantha*). The magnitude of inhibition or stimulation in bioassay was used as the response index (*RI*) as per Williamson and Richardson (1988):

$$\mathrm{RI} = 1 - \mathrm{C}/\mathrm{T}(\mathrm{T} \geq \mathrm{C})$$
$$\text{or } \mathrm{RI} = \mathrm{T}/\mathrm{C} - 1(\mathrm{T} < \mathrm{C})$$

Where, C is control and T is treatment value. The absolute value of *RI* represents the phytotoxic effect. Value of *RI*>0 is considered stimulatory, while <0 is considered inhibitory.

Statistical Analysis Phytotoxicity of β-caryophyllene expressed as *RI* index was analyzed using one-way ANOVA followed by the Duncan's multiple range tests. Relative expression level of β-caryophyllene synthase gene and relative abundance of β-caryophyllene in *M. micrantha* leaves were analyzed using two-way ANOVA followed by the Duncan's *post hoc* tests using the SPSS 13.0 software package (SPSS, Inc., Chicago, IL, USA).

Results

Induction of β-Caryophyllene Synthase Real-time PCR analysis showed that elevated CO_2 significantly increased the transcript levels of β-caryophyllene synthase in the leaves of *M. micrantha* (Fig. 1). Transcript levels were increased 9.07, 18.95, 16.55, and 27.68 fold after the plants were exposed to 750 ppm CO_2 for 3, 6, 9, and 12 d, respectively. However, the gene did not significantly change its expression level when plants were grown in the chamber with CO_2 at 350 ppm.

Levels of β-Caryophyllene GC analysis indicated differences in the emission of β-caryophyllene between *M. micrantha* plants exposed to 750 ppm CO_2 and those kept at 350 ppm CO_2 (Fig. 2). Control plants grown at 350 ppm CO_2 did not significantly change during the experiment (12 d). However, plants at 750 ppm CO_2 varied in emission of β-caryophyllene during the experiment time. Maximum emission was found in plants exposed to 750 ppm CO_2 for 9 d. *M. micrantha* leaves in all plants exposed to 750 ppm CO_2 had significantly higher levels of β-caryophyllene than those exposed to 350 ppm CO_2 (Fig. 2).

Phytotoxicity of β-Caryophyllene β-Caryophyllene inhibited seed germination, root and shoot growth of *B. campestris*, *R. sativus*, *L. sativa,* and *M. micrantha*. The response indices (*RI*) of *B. campestris* grown under 350 ppm CO_2 were −0.08, −0.15, and −0.18 for the seed germination, root and shoot growth, respectively (Table 1). Root growth of *R. sativus*, *L. sativa* and *M. micrantha* was more sensitive than shoot growth. β-Caryophyllene at 24 mg L^{-1} significantly inhibited the seed germination and seedling growth of all four tested plant species (Table 1).

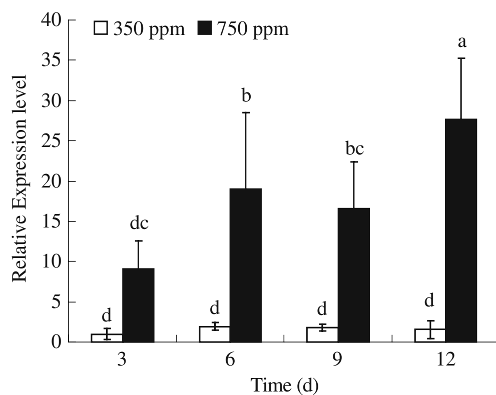

Fig. 1 Relative expression level of β-caryophyllene synthase gene in *Mikania micrantha* leaves as analyzed by real-time PCR. Plants were grown at 350 ppm CO_2 or 750 ppm CO_2 for the indicated period. Three replicates were used for treatment. Each bar represents means± SD. Different letters above the bars indicate significant differences ($P<0.05$) according to Duncan's multiple range tests. Two-way ANOVA: time effect, $F=3.82$, $df=3$, $P<0.05$ ($P=0.031$); the concentration of CO_2 effect, $F=66.62$, $df=1$, $P<0.001$; time×the concentration of CO_2 interaction, $F=3.41$, $df=3$, $P<0.05$ ($P=0.043$)

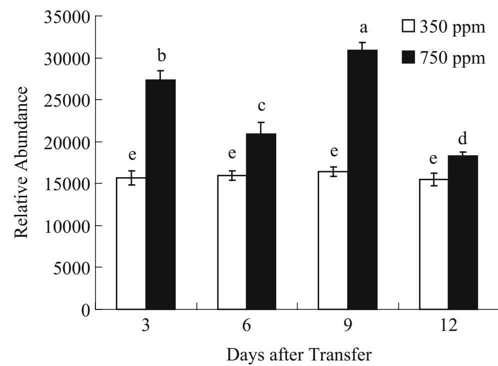

Fig. 2 Relative abundance of β-caryophyllene emission from *Mikania micrantha* leaves analyzed by GC. Plants were grown at 350 ppm CO_2 or 750 ppm CO_2 for the indicated period. Three replicates were used for treatment. Each bar represents means±SD. Different letters above the bars indicate significant differences ($P<0.05$) according to Duncan's multiple range tests Two-way ANOVA: time effect, $F=69.88$, $df=3$, $P<0.001$; the concentration of CO_2 effect, $F=542.86$, $df=1$, $P<0.001$; time×the concentration of CO_2 interaction, $F=57.18$, $df=3$, $P<0.001$

To determine the effects of elevated CO_2 on the phytotoxicity of β-caryophyllene, the seeds of the four plants were treated with β-caryophyllene in a similar way and then exposed to chambers with different CO_2 concentrations (350 and 750 ppm). β-Caryophyllene at a concentration of 24 mgL^{-1} inhibited root growth of *B. campestris*, *R. sativus*, *L. sativa,* and *M. micrantha* by 37, 38, 24, and 24% (corresponsive *RI*=−0.37, −0.38, −0.24, and −0.24), respectively, when CO_2 concentration was 350 ppm (Table 1). At elevated CO_2 concentration (750 ppm) β-caryophyllene at 24 mgL^{-1} inhibited root growth of *B. campestris*, *R. sativus*, *L. sativa,* and *M. micrantha* by 45, 58, 34, and 28% (corresponsive *RI*=−0.45, −0.58, −0.34, and −0.28), respectively (Table 1). Elevated CO_2 concentration enhanced the allelopathic potential of β-caryophyllene on all test species. Similar trends were observed with respect to seed germination and shoot growth of all the target plants.

Discussion

We identified a β-caryophyllene synthase gene sequence (GenBank FJ767894) in *M. micrantha.* Real-time PCR analysis showed that elevated CO_2 significantly increased the transcript levels of the β-caryophyllene synthase gene in the course of the experiment (Fig. 1). GC analysis confirmed that plants of *M. micrantha* did not significantly change their emission of β-caryophyllene when they were grown in the chamber with CO_2 at 350 ppm. However, plants exposed to 750 ppm CO_2 significantly increased the

Table 1 Effect of β-caryophyllene on seed germination and seedling growth expressed as Response Indices (*RI*) of four plants under 350 and 750 ppm CO_2. All data are presented as means±SD. Each value is the mean of three replicates. Different letters in the same row indicate significant differences ($P<0.05$) according to Duncan's multiple range test

Tested plant	Bioassay parameter	β-Caryophyllene concentration (mg L^{-1})					
		350 ppm CO_2			750 ppm CO_2		
		0.375	3	24	0.375	3	24
Brassica campestris	Germination	0.10±0.03 a	−0.08±0.06 b	−0.10±0.03 bc	0.03±0.05 a	−0.16±0.03 cd	−0.24±0.05 d
	Root length	0.17±0.15 a	−0.15±0.21 b	−0.37±0.14 c	0.06±0.15 a	−0.20±0.13 b	−0.45±0.08 d
	Shoot height	0.13±0.16 a	−0.18±0.24 c	−0.25±0.22 c	−0.08±0.16 b	−0.20±0.19 c	−0.32±0.16 d
Raphanus sativus	Germination	0.02±0.05 ab	−0.07±0.03 bc	−0.12±0.03 cd	0.07±0.03 a	−0.16±0.08 cd	−0.22±0.11 d
	Root length	0.05±0.15 a	−0.16±0.14 c	−0.38±0.13 e	−0.10±0.18 b	−0.25±0.14 d	−0.58±0.12 f
	Shoot height	0.09±0.14 a	−0.12±0.15 c	−0.33±0.11 e	0.02±0.13 b	−0.19±0.13 d	−0.52±0.16 f
Lactuca sativa	Germination	0.02±0.09 ab	−0.08±0.06 abc	−0.12±0.04 bc	0.06±0.03 a	−0.09±0.10 abc	−0.17±0.11 c
	Root length	0.01±0.27 a	−0.15±0.31 b	−0.24±0.18 c	0.05±0.12 a	−0.17±0.14 bc	−0.34±0.18 d
	Shoot height	0.04±0.17 a	−0.10±0.17 b	−0.17±0.16 bc	0.01±0.22 a	−0.19±0.31 c	−0.28±0.18 d
Mikania micrantha	Germination	0.02±0.04 a	−0.11±0.04 b	−0.15±0.07 b	0.06±0.09 a	−0.13±0.07 b	−0.19±0.04 b
	Root length	0.03±0.33 a	−0.17±0.43 bc	−0.24±0.19 bc	0.05±0.19 a	−0.16±0.19 b	−0.28±0.11 c
	Shoot height	0.04±0.21 a	−0.11±0.20 b	−0.17±0.21 bc	0.02±0.17 a	−0.15±0.18 bc	−0.20±0.19 c

emission of β-caryophyllene and reached the maximum level at 9 d after the exposure (Fig. 2). β-Caryophyllene emission capacity may depend on many factors, such as the rate of its synthesis, the rate of its conversion to other sesquiterpenes, and the rate of emission as volatile compounds from intact tissue. Our data showed clearly that elevated CO_2 concentration induced the expression of the β-caryophyllene synthase gene and increased the emission of β-caryophyllene in *M. micrantha*. These findings support our hypothesis that emission of VOCs from plants is particularly sensitive to changes of environmental factors such as CO_2. Leaves of *Quercus ilex* also increase volatile emission of monoterpenes at elevated CO_2 levels (Staudt et al., 2001). Elevated CO_2 typically has both physiological and biochemical effects on VOC emissions (Rosenstiel et al., 2003; Niinemets et al., 2004).

Allelochemicals play a role in mediating interspecific interactions (Dicke et al., 1990; Legrand et al., 2003; Duke, 2007). Terpenoids, a diverse group of secondary compounds, have a variety of ecological functions, including allelopathy (Gershenzon and Croteau, 1993; Sharkey and Singsaas, 1995). Allelopathy is a phenomenon whereby various plants enhance competitiveness and fitness (Singh et al., 2003; Kegge and Pierik 2010). The allelopathic potential of a given plant may vary in different habitats. Environmental stresses such as light, or nutrient or water deficiency may enhance allelopathic potential of plants (Anaya, 1999). β-Caryophyllene, a well-known volatile sesquiterpene with allelopathic potential, has been reported previously to inhibit development of seedlings of various plant species (Kil et al., 2000; Wang et al., 2009). A previously characterized β-caryophyllene synthase gene of *A. annua* also was induced previously by a fungal elicitor, suggesting a role for β-caryophyllene in plant defense (Cai et al., 2002). Emission rates of β-caryophyllene from orange trees (*Citrus sinensis* (L.) OSBECK, var. *Navel* and *Navel Late*) vary with light intensity and temperature (Hansen and Seufert, 2003). Here, at elevated CO_2 levels (750 ppm), phytotoxic effects of β-caryophyllene were enhanced on all tested plants, especially on *R. sativus*. Hence, CO_2 concentrations not only affected the emission of β-caryophyllene from *M. micrantha* leaves (Fig. 2), but also increased its phytotoxicity (Table 1). In other words, an increase in CO_2 concentrations may have multiple effects on plant-plant interactions.

Many studies suggest that climate changes may affect structure and function of ecological systems (Walther, 2003; Jump and Penuelas, 2005). Temperature changes in the subtropical north Indian plains seem to influence biosynthesis of essential oil constituents in *A. annua* (Bagchi et al., 2003). It has been suggested that rising atmospheric CO_2 concentrations increase abundance, biomass, and fitness of vines that may interfere with regeneration and diversity of forest ecosystems (Sasek and Strain, 1991; Granados and Körner, 2002; Zotz et al., 2006; Song et al., 2009). Increasing CO_2 concentration has been suggested previously as a factor that could facilitate exotic plant invasion (Song et al., 2009). Our experiment was conducted over 12 days. In nature, however, an increase in

CO_2 concentration is likely to occur over a long period of time for which the plant may even acquire adaptation. Recently, Klironomos et al. (2005) reported on the over estimation of plant responses in experiments that use abrupt increase of CO_2 concentration. Although 100 years (predicted time for doubling of CO_2 concentration) is a short time (in evolutionary terms) for acquiring such an adaptation, there is some evidence, however, that shows micro-evolution occurring in plants over short periods of time (Collins and Bell, 2004; Ward and Kelly, 2004). Based on such reports and our findings, we postulate that a future increase in atmospheric CO_2 levels may alter allelopathic potential of the invasive vine *M. micrantha*. Future studies are, of course, required to determine how the release of volatile allelochemicals and CO_2 concentrations will affect invasion of *M. micrantha* in Southern China.

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