

Response of Photosynthetic Characteristics in Broad Bean Seedlings to Volatile Substances Stress from *Chenopodium ambrosioides*: Post-print

Authors: Gu Congjing, Zhou Jian, Ma Danwei, flavin, Meng Qiaoqiao

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Abstract

Using broad bean (*Vicia faba*) as the recipient plant, pot experiments were conducted to evaluate the effects of essential oil from the invasive plant *Chenopodium ambrosioides* L. and its two main constituents, α -terpinene and p-cymene, on the photosynthetic characteristics of the recipient. The results showed that the essential oil of *Chenopodium ambrosioides* and its two main constituents affected the characteristics of broad bean leaves to varying degrees. Essential oil treatment significantly reduced net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), maximum photochemical efficiency (F_v/F_m), actual photochemical efficiency (Φ_P_s), and chlorophyll content, but increased intercellular CO_2 concentration (C_i). This effect exhibited both dose and time dependence, and was irreversible under high-dose essential oil treatment; Compared with the control, the α -terpinene treatment group showed decreased P_n , F_v/F_m , and Φ_P_s , and increased C_i , G_s , and T_r . After cessation of treatment, all parameters approached control levels; Overall, p-cymene had little effect on the photosynthetic characteristics of broad bean seedlings. These results indicate that the

Full Text

Preamble

Response of Photosynthetic Characteristics of *Vicia faba* Seedlings to Volatile Allelochemical Stress from *Chenopodium ambrosioides*

GU Congjing¹, ZHOU Jian^{1,2}, MA Danwei^{1*}, HUANG Su¹, MENG Qiaoqiao^{1}

¹College of Life Science, Sichuan Normal University, Chengdu 610101, China

²Chengxian Organization Department of the Communist Party of China, Chengxian, Gansu 742500, China

Abstract

Pot experiments were conducted to evaluate the effects of volatile oil from the invasive plant *Chenopodium ambrosioides* L. and its two main components, α -terpinene and cymene, on the photosynthetic characteristics of *Vicia faba* seedlings. The results showed that the volatile oil and its two main components differentially affected the characteristics of broad bean leaves. Volatile oil treatment significantly reduced net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), maximum photochemical efficiency (Fv/Fm), actual photochemical efficiency (Φ PSII), and chlorophyll content, but increased intercellular CO₂ concentration (Ci). These effects demonstrated both dose-dependent and time-dependent patterns, with high-dose volatile oil treatment causing irreversible damage. Compared with the control, α -terpinene treatment reduced Pn, Fv/Fm, and Φ PSII while increasing Ci, Gs, and Tr; after treatment cessation, all parameters gradually approached control levels. Overall, cymene had minimal impact on the photosynthetic characteristics of broad bean seedlings. These findings indicate that the effects of allelopathic stress from *C. ambrosioides* on receptor photosynthetic characteristics result from the synergistic action of multiple allelochemicals rather than being determined by a single component.

Keywords: *Chenopodium ambrosioides*, allelochemical stress, photosynthetic characteristics, α -terpinene, cymene

Introduction

Chenopodium ambrosioides is an herbaceous plant in the Amaranthaceae family that originates from tropical America. With strong adaptability and rapid dispersal, it has invaded most regions of China, posing a serious threat to the country's ecological security (Xu & Qiang, 2004). *C. ambrosioides* exhibits strong allelopathic effects (Jimenez-Osornio et al., 1996), and its volatile compounds possess significant cytotoxicity, causing excessive accumulation of reactive oxygen species and increased malondialdehyde content in receptor plant cells (Hu et al., 2012; Chen et al., 2016). These compounds also downregulate antioxidant enzyme gene expression, reduce antioxidant enzyme activity, and induce oxidative damage (Chen et al., 2015), leading to disorganized or destroyed cell structures, nuclear aberrations, reduced cell viability, and even apoptosis (Hu et al., 2011, 2012; Hu et al., 2015; Chen et al., 2016; Zhou et al., 2017; Li et al., 2018).

Photosynthesis is highly sensitive to environmental changes, and under stress conditions, reduced photosynthetic products inhibit plant growth and development (Xin & Huo, 2008). Previous studies have shown that allelochemicals released by plants significantly interfere with the photosynthetic processes of receptor plants (Hussain & Reigosa, 2016), yet few reports have addressed the effects of volatile compounds from *C. ambrosioides* on receptor plant photosynthetic characteristics. This study selected broad bean (*Vicia faba*), a widely cultivated crop in *C. ambrosioides*-invaded regions, as the receptor plant. Using pot experiments to simulate the action of volatile allelochemicals from *C. ambrosioides*, we investigated the effects of its volatile oil and two main components (α -terpinene and cymene) on gas exchange parameters, chlorophyll fluorescence parameters, and chlorophyll content in broad bean seedlings to comprehensively elucidate the mechanisms by which *C. ambrosioides* allelopathy inhibits the growth and development of neighboring plants.

Materials and Methods

1.1 Materials

Aerial parts of donor *C. ambrosioides* plants were collected near Baojiangqiao Community in Chengdu, Sichuan Province. Volatile oil was extracted by steam distillation (Singh et al., 2009) and dehydrated with anhydrous Na₂SO₄. The density of the obtained volatile oil was 843 mg · L⁻¹. Single-point determination revealed that its main components, α -terpinene and cymene, were present at concentrations of 151 mg · L⁻¹ and 156 mg · L⁻¹, respectively. Standard samples of α -terpinene and cymene were purchased from Chengdu Rui Kesi Biochemical Reagent Company. Broad bean seeds (cultivar Chenghu 14#) were obtained from the Wukuai Seed Market in Chengdu.

1.2.1 Experimental Design

Uniform, plump, pest-free broad bean seeds were selected and sterilized in 0.5% KMnO₄ for 15 minutes. After rinsing with distilled water, seeds were soaked for 24 hours and then germinated in darkness at 25°C until radicles emerged. Germinated seeds were sown in pots (10 cm diameter, 6 cm height) at one seed per pot, using quartz sand as the culture medium, and grown under a 14 h/10 h light/dark cycle at 25°C (light)/18°C (dark). During cultivation, 0.2% Hoagland nutrient solution was maintained. After five weeks, potted seedlings were randomly placed in specially designed sealed glass chambers (25 cm × 20 cm × 40 cm) at five pots per chamber.

Three treatment groups were established: volatile oil, α -terpinene, and cymene. Based on the concentrations of α -terpinene and cymene in the volatile oil and preliminary experiments, six treatment gradients were designed (Table 1). The volatile oil treatment concentrations were 0, 0.001, 0.002, 0.003, 0.004, and 0.005

$\text{L} \cdot \text{cm}^3$. Each treatment gradient of volatile oil, α -terpinene, or cymene was applied to a small petri dish placed at the bottom center of the glass chamber, which was then sealed with Vaseline. All treatment groups were placed in an illumination incubator and treated at 25°C for 3 hours (10:00–13:00). After three consecutive days of treatment, the chamber lids were removed to allow complete volatilization, followed by a 3-day recovery period in the illumination incubator. The entire experiment lasted 7 days.

1.2.2 Parameter Measurements

Gas exchange parameters were measured using a TPS-2 portable photosynthesis system. During the experiment, the fully expanded leaves at the apex of broad bean plants were selected daily at 13:00 to determine net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (Tr), and intercellular CO_2 concentration (C_i). Five plants were measured per treatment.

Chlorophyll fluorescence parameters were measured using an FMS-2 portable chlorophyll fluorescence meter. During the experiment, plants were dark-adapted for 30 minutes daily at 13:00. Fully expanded apical leaves were then selected to determine actual photochemical efficiency (Φ_{PSII}) and maximal photochemical efficiency (F_v/F_m). Five plants were measured per treatment.

Chlorophyll content determination: Leaves were harvested on day 4 (end of treatment) and day 7 (end of recovery). After removing major veins and cutting into small pieces, chlorophyll solution was prepared following Zhang et al. (2009). Absorbance values at A_{663} and A_{645} were measured using a SpectraMax M2 multi-mode microplate reader, and chlorophyll content ($\text{mg} \cdot \text{g}^{-1}$) was calculated as:

$$C = 8.02 \times A_{663} + 20.21 \times A_{645}$$

$$\text{Chlorophyll content} = \frac{C \times V \times N}{W \times 1000}$$

where C is chlorophyll concentration ($\text{mg} \cdot \text{L}^{-1}$), V is total volume (mL), N is dilution factor, and W is fresh leaf weight.

1.3 Data Analysis

Data were analyzed using ANOVA in SPSS 17.0, and figures were prepared using Microsoft Excel 2007.

Results

2.1 Changes in Gas Exchange Parameters Under Volatile Oil, -Terpinene, and Cymene Treatments

Under *C. ambrosioides* volatile oil treatment, the net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), and intercellular CO concentration (Ci) of broad bean seedling leaves changed to varying degrees (Tables 2-5). Compared with the control, Pn, Gs, and Tr decreased significantly ($P < 0.05$), while Ci increased significantly ($P < 0.05$), demonstrating both dose-dependent and time-dependent effects. After treatment cessation, the differences in gas exchange parameters between treated and control leaves gradually narrowed with extended recovery time. However, leaves treated with higher doses recovered more slowly, with some parameters even lower than during the treatment period, indicating that high-dose volatile oil treatment severely damaged the photosynthetic process in broad bean seedlings.

When broad bean seedlings were exposed to -terpinene, leaf Gs, Ci, and Tr increased to varying degrees, while Pn generally decreased (Tables 2-5). Most treatments showed significant differences from the control in Gs and Tr ($P < 0.05$), whereas Ci reached significant levels only at higher doses (gradients 4 and 5) ($P < 0.05$). The reduction in Pn was modest, with significant differences from the control observed only at gradients 4 and 5 ($P < 0.05$). After treatment stopped, most treatments maintained their status during the treatment period, though differences from the control narrowed.

Compared with the control, cymene had minimal overall effect on gas exchange parameters of broad bean seedling leaves. Only transpiration rate (Tr) at higher doses during the treatment period (days 2-4) was significantly greater than the control group ($P < 0.05$), and parameters essentially returned to control levels after treatment cessation (Tables 2-5).

In summary, *C. ambrosioides* volatile oil had the greatest impact on gas exchange parameters of broad bean seedlings, followed by -terpinene, with cymene having the smallest effect. The effects of the two main components (-terpinene and cymene) differed from those of the complete volatile oil.

2.2 Changes in Chlorophyll Fluorescence Parameters Under Volatile Oil, -Terpinene, and Cymene Treatments

C. ambrosioides volatile oil treatment caused significant reductions ($P < 0.05$) in both maximal photochemical efficiency (Fv/Fm) and actual photochemical efficiency (Φ PSII) of broad bean seedling leaves, showing dose-dependent and time-dependent effects (Tables 6-7). After treatment ended, Fv/Fm and Φ PSII in most treatments failed to recover to normal levels and remained significantly lower than the control ($P < 0.05$). In gradient 5, Fv/Fm and Φ PSII were even lower than during the treatment period. In the -terpinene treatment group, Fv/Fm and Φ PSII showed similar patterns to the volatile oil treatment but with

smaller magnitude (Tables 6-7). Only gradient 5 showed significant differences from the control ($P < 0.05$). After treatment cessation, Fv/Fm and Φ PSII in all treatments gradually recovered to control levels. Cymene had no significant effect on Fv/Fm or Φ PSII of broad bean seedling leaves ($P > 0.05$) (Tables 6-7). Among the three treatments, volatile oil had the greatest impact on chlorophyll fluorescence parameters, followed by α -terpinene, with cymene having almost no effect.

2.3 Changes in Chlorophyll Content Under Volatile Oil, α -Terpinene, and Cymene Treatments

As shown in Figure 1A, after 3 days of *C. ambrosioides* volatile oil treatment, chlorophyll content in broad bean seedling leaves decreased significantly ($P < 0.05$) in a dose-dependent manner. Compared with the control, chlorophyll content in gradient 5 decreased by 54.58%. After 3 days of recovery (Figure 1B), chlorophyll content in all treatment groups increased to varying degrees but remained lower than the control. In gradient 5, chlorophyll content was even lower than during the treatment period, reaching only 37.0% of the control level. In α -terpinene and cymene treatment groups, total leaf chlorophyll content decreased slightly but not significantly compared with the control ($P > 0.05$), and returned to near-control levels after treatment cessation. These results indicate that volatile oil had the greatest effect on chlorophyll content, followed by α -terpinene, with cymene having the smallest effect.

Discussion

3.1 Effects of Volatile Allelochemicals from *C. ambrosioides* on Photosynthetic Characteristics of Broad Bean

Photosynthesis is the fundamental source for plant organic synthesis and energy acquisition, and is highly susceptible to temperature, light, water, and microbial factors (Li et al., 2016; Schmid et al., 2016). Allelochemicals released by plants directly affect numerous physiological and biochemical processes in neighboring plants, thereby influencing their growth and development (El-Kenany et al., 2017). For example, the allelochemical benzoxazolin-2-(3H)-one (BOA) affects carbon assimilation efficiency in receptor plants by reducing stomatal conductance (Parizotto et al., 2017). Generally, factors affecting photosynthetic rate include both stomatal and non-stomatal components (Zhang et al., 2016). Our previous research found that volatile allelochemicals from *C. ambrosioides* induced programmed cell death in guard cells of broad bean (Zhou et al., 2017). The present study showed that under volatile oil treatment, Pn, Gs, and Tr decreased significantly while Ci increased significantly. After α -terpinene treatment, Gs, Ci, and Tr increased to varying degrees while Pn generally decreased. Cymene had no significant effect on photosynthetic characteristics of broad bean. These results suggest that when broad bean seedlings are stressed by *C.*

ambrosioides volatile oil, stomatal closure or guard cell programmed cell death reduces Gs, thereby inhibiting Tr and Pn. Alternatively, reduced RuBP carboxylase activity and slowed RuBP regeneration may decrease CO₂ affinity (Sun et al., 2017). Whether the photosynthetic decline induced by *C. ambrosioides* volatile oil is related to RuBP carboxylase activity requires further investigation. The effects of α -terpinene on photosynthetic characteristics may be caused by non-stomatal factors.

Chlorophyll content and chlorophyll fluorescence parameters are highly sensitive to environmental stress, with any environmental change affecting PSII Fv/Fm and Φ PSII (Cosgrove & Borowitzka, 2010; Xu, 2002). Previous studies found that allelochemicals such as phenolic acids (Xie et al., 2018) and BOA (El-Kenany et al., 2017) reduce chlorophyll content and chlorophyll fluorescence parameters in receptor plants. Our results showed that volatile oil and its two main components (α -terpinene and cymene) reduced chlorophyll content and chlorophyll fluorescence parameters (Fv/Fm and Φ PSII) in broad bean seedling leaves to varying degrees, with high-dose volatile oil treatment causing nearly irreversible effects. This suggests that *C. ambrosioides* allelopathic stress may interfere with chlorophyll synthesis or cause chlorophyll degradation, damage the photosystem, inhibit light reactions, and thereby reduce photosynthetic rate and suppress plant growth and development.

Although volatile oil, α -terpinene, and cymene all reduced chlorophyll content, chlorophyll fluorescence parameters, and net photosynthetic rate in broad bean seedling leaves, the effects on stomatal conductance, transpiration rate, and intercellular CO₂ concentration differed substantially between treatments.

3.2 Comparison of Allelopathic Effects Among Volatile Oil and Its Main Components α -Terpinene and Cymene

Vasilakoglou et al. (2013) used a full-series evaluation method to assess the toxic effects of chemical components in ryegrass (*Lolium rigidum* Gaudin) volatile oil and found synergistic effects among components, resulting in greater toxicity of the complete volatile oil than of individual components. *C. ambrosioides* volatile oil has a complex composition (Chu et al., 2011; Jardim et al., 2008; Huang & Kong, 2002), generally including monoterpenes, sesquiterpenes, and oxygenated derivatives. However, the main components in volatile oil from different geographical origins are typically α -terpinene, cymene, and ascaridole (Kandpal et al., 2016; Pan et al., 2007). Compared with the allelopathic effects of the complete volatile oil, the effects of its main components are generally weaker.

C. ambrosioides volatile oil and its main components α -terpinene and cymene increased ROS levels in maize (*Zea mays*) root tip cells, causing membrane lipid peroxidation and antioxidant enzyme inactivation, thereby inhibiting maize radicle growth (Chen et al., 2016; Li et al., 2018). The oxidative damage induction effect was greatest for volatile oil, followed by α -terpinene, and smallest for

cymene (Chen et al., 2015). Additionally, volatile oil, cymene, and α -terpinene from *C. ambrosioides* significantly inhibited the viability of maize root border cells (Hu et al., 2015) and leaf epidermal guard cells (Zhou et al., 2016), with cymene and α -terpinene showing weaker inhibitory effects than the complete volatile oil. Our results demonstrate that the effects of *C. ambrosioides* volatile oil on receptor photosynthetic characteristics are far greater than those of its two main components α -terpinene and cymene, consistent with previous findings.

This outcome may occur because α -terpinene, cymene, and other components in the volatile oil simultaneously affect the photosynthetic process of receptors, with the additive effects of each component resulting in a much greater impact on photosynthetic characteristics than the individual components alone. Of course, the possibility cannot be excluded that the volatile oil contains components with stronger allelopathic effects than α -terpinene and cymene. Our laboratory will conduct further research to identify other major allelochemical components in *C. ambrosioides* volatile oil.

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