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Effect of enhanced ultraviolet-B on allelopathic potential of *Zanthoxylum bungeanum*

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ABSTRACT

The effect of enhanced ultraviolet-B on allelopathic potential of *Zanthoxylum bungeanum* was investigated. A significant inhibitory effect on germination rate of crop seeds under bioassay was observed at 25 g l^{-1} and 50 g l^{-1} by extracts from Zanthoxylum leaf both treated with enhanced ultraviolet-B radiation and untreated control. *Medicago sativa* and lettuce were more sensitive than radish to the extract from Zanthoxylum leaf treated with enhanced UV-B radiation, as the germination rates of *M. sativa* and lettuce were significantly reduced compared to control at 25 g l^{-1} and 50 g l^{-1}, and so did alfalfa at 12.5 g l^{-1}. However, as for radish (*Raphanus sativus*) there was no significant reduction in germination rate at any concentration under bioassay compared to control. Content of UV-B absorbing compounds and total phenols in Zanthoxylum seedlings responded positively to enhanced UV-B radiation. The results suggest that the allelopathic potential of *Z. bungeanum* was generally improved under enhanced UV-B radiation.

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1. Introduction

In spite of the current efforts to restrict the production of ozone-depleting substances, thinning of the stratospheric ozone layer and increased penetration of ultraviolet-B radiation to the earth's surface will continue for decades (De La Rose et al., 2001). Atmospheric ozone remains depleted and the annual average ozone loss is approximately 3% globally (Executive summary, 2003). Researches have shown that enhanced UV-B radiation reaching the surface of the earth has many adverse impacts on plant growth parameters (Jordan, 2002; Jansen, 2002). These combine together to reduce plant fitness and function. When plants are exposed to UV-B radiation stress, some protective mechanism can also form in plants. For example, an increase in UV-B absorbing compounds and proline content has been reported (Fedina et al., 2006; Moorthy and Kathiresan, 1999). Enhanced UV-B radiation can induce overproduction of free radicals and result in oxidative stress eventually (Yu et al., 2004). The harmful effects of UV-B radiation on plants are often a consequence of reactive oxygen species (ROS) production (Strid et al., 1994). Recent reports have showed that ozone layer is attenuating in Qinghai–Tibetian Plateau zone (Qi et al., 2001; Bian et al., 2006), which must bring great influence on the subalpine vegetation, especially seedlings.

A plant may interfere with the growth of neighboring plant through competition and/or allelopathy which is defined by Molisch (1937). Allelopathy is an interference mechanism in which living or dead plants release allelochemicals exerting an effect (mostly negative) on the associated plants, and can play an important role in natural and managed ecosystems (Fitter, 2003; Inderjit and Duke, 2003). It plays a significant role in agro-ecosystems, and affects the growth, quality and quantity of the produce (Kohli et al., 1998; Singh et al., 2001). A number of plant species have been reported to have an allelopathic effect on other plant species (Mallik, 1987; Martin and Smith, 1994; Kato-Noguchi, 2003; Jefferson and Pennacchio, 2003; Oueslati, 2003; Djurdjevic et al., 2004). Most recent studies, however, have focussed on the inhibitory effect of allelopathic substances (Gross, 2003). Allelochemicals produced by one crop species can influence the growth, productivity, and yield of other crops or the same crop (Batish et al., 2002). The allelopathic potential of many plants is intensified by exposure to various environmental stresses (Einhellig, 1987, 1996) and induces phytochemical variation (Josep and Joan, 1997; Kong and Xu, 2000). Several studies have shown that the amount of allelochemicals produced and released by plants is correlated to environmental factors (Dicke, 1994; Loughrin et al., 1995; Rose et al., 1995; Pare and Tumlinson, 1997; Agrawal, 1998). As a result, an increasing number of studies have focussed on the

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relationship between environmental variation, plant chemical response, and the ecological function of plant allelochemicals. So far, few studies have been conducted to investigate the effect of enhanced ultraviolet-B on allelopathic potential of plant.

Zanthoxylum is widely distributed throughout the southeastern part of China, and could also be found in India, northern Queensland and Australia as well (Kong et al., 1996). *Zanthoxylum* is the largest and most widespread genus in the Rutaceae containing about 100 New World and 100 Old World species, and includes small to large trees, most species of which have spiny trunks and/or branches (Gentry, 1993). Members of the genus are used in traditional medicine around the world. For example, *Z. limonella* is used in India to promote digestion and as a treatment for wounds (Shiva et al., 2002). The essential oils from the fruits of *Z. limonella* have shown anthelmintic (Kalyani et al., 1989) as well as gastrointestinal stimulant effects (Itthipanichpong et al., 2002). *Z. capense* is used in Africa as parasiticide (Hutchings et al., 1996) and exhibits antiprotozoal activity (McGaw et al., 2000). It is a multipurpose horticultural plant and is becoming an integral part of agriculture in agroforestry programs. This practice increases productivity, improves soil quality, microclimate, nutrient cycling, soil conservation and increases overall productivity (Singh et al., 2001). However, *Zanthoxylum* does negatively affect performance of crops through allelopathy.

Previous studies have confirmed *Zanthoxylum* was potentially allelopathic (Wang et al., 2005; Lv et al., 2006). This study was conducted to investigate the effects of a 30% increase in UV-B radiation on allelopathic potential of 2-year-old *Zanthoxylum* seedlings. We hypothesise that seedlings treated with enhanced UV-B radiation were more allelopathic than control seedlings.

2. Materials and methods

2.1. Plant material and experiment design

The experiment was conducted in open semi-field for one growing season in Maoxian Ecological Station of Chinese Academy of Sciences, Sichuan province, China (31°41′N, 103°53′E, 1820 m a.s.l.). Uniform 2-year-old *Zanthoxylum* seedlings from a local nursery were selected based on plant height, basal diameter and fresh weight. Seedlings were transplanted into plastic pots (25-cm diameter and 35-cm depth) with a 12-h photoperiod and a daily average 1200 μmol m^{-2} s^{-1} photosynthetic photon flux density, one seedling per pot. The substrate used for growing the seedlings was sieved topsoil from a *Zanthoxylum* forest. In a preliminary experiment, the plastic pots did not affect growth of seedling root during a 2-year growth period. The experiment consisted of two treatments in the paper: (1) ambient UV-B (control) and (2) enhanced UV-B (+UV-B). Each treatment had three blocks, and each block had ten pots. The pots within blocks were rotated approximately every 20 days.

2.2. UV-B treatments

Supplementary UV-B was supplied by UV-B fluorescent lamps (Beijing Electric Light Sources Resource Institute, Beijing, China). In the control, UV-B from the lamps was excluded by wrapping the tubes with 0.125 mm polyester film, which transmits UV-A. In enhanced UV-B treatments, lamps were wrapped with 0.10 mm cellulose diacetate film, which transmits both UV-B and UV-A. Vertical polyester curtains were placed between the frames in order to prevent the UV-B radiation from reaching the control seedlings. The lamps were suspended 100 cm above the top of plant apex and this distance was kept constant throughout the experiment. The lamps were mounted in metal frames with minimum shading, and cellulose acetate and polyester films were replaced every 1 week. The lamp duration was modified monthly and replaced in times. The spectral irradiance from the lamps was determined with an Optronics Model 742 (Optronics Laboratory Inc., Orlando, FL, USA) spectroradiometer. The ambient UV-B dose weighted by Caldwell's generalized plant action spectrum normalized at 300 nm (Caldwell 1971) was 11.02 kJ m^{-2} day^{-1} in sunny day. The biologically effective level of a 30% increase in UV-B radiation was 14.33 kJ m^{-2} day^{-1}.The seedlings were exposed to supplemental UV-B radiation beginning from mid April (the starting of growing season) to the end of October 2007. All pots also received natural solar radiation. Seedlings were irradiated for 8 h daily centered on the solar noon.

2.3. Sampling and preparation of extracts

Topgrowth of 2-year old *Zanthoxylum* plants leaves were harvested at the experiment field. The plant leaves samples were oven dried at 60 °C for 3 days, ground with a Wiley mill to pass a 1 mm screen and then stored in a refrigerator at 2 °C until further use (Chon and Nelson, 2001). Fifty grams of dried leaves were extracted by soaking in 1 l deionized water at 25 °C for 24 h in a shaker to give a concentration of 50 g dry tissue l^{-1} (g l^{-1}). The extract was filtered through four layers of cheesecloth to remove the fiber debris, and centrifuged at 3000 rpm for 30 min. The supernatant was vacuum filtered again through Whatman no. 42 paper. Stock extracts were made fresh for each experiment.

2.4. Allelopathic effect of Zanthoxylum leaf extracts

Stock extract was diluted appropriately with sterile distilled water to give the final concentrations of 0, 12.5, 25 and 50 g l^{-1} (Inderjit, 2006; Tran et al., 2004). Five milliliters of the diluted extract were pipetted to the 2-layer filter papers (Whatman no. 2) placed in sterilized 9-cm diameter Petri dishes. Alfalfa (*Medicago sativa*), Lettuce (*Lactu sativa*) and Radish (*Raphanus sativus*) seeds were surface sterilized with 0.525 g l^{-1} sodium hypochlorite for 15 min. The seeds were rinsed four times with deionized water.

2.5. Bioassay

Fifty imbibed seeds were evenly placed on a filter paper containing extract in each Petri dish. The Petri dishes were covered and placed flat in a growth chamber held at 25 °C during the 14 h light period and 24 °C during the 10 h dark period. Plates were illuminated at 400 μmol photons m^{-2} s^{-1} PAR provided by a mixture of incandescent and fluorescent lamps. Cumulative germination was determined by counting the number of germinated seeds over 132 hs (alfalfa), 168 hs (radish) and 192 hs (lettuce) and transformed into percent germination for analysis as needed. The data were subjected to one-way analysis of variance, and treatment means were compared applying post hoc Tukey's test at $p < 0.05$. The statistical analysis was done with SPSS 11.0 for Windows statistical software package. The experiments were duplicated, each with three replications.

2.6. UV-B absorbing compounds and total phenols

UV-B absorbing compounds of the fully developed leaf were extracted with a MeOH:H_2O:HCl (79:20:1, v/v/v) solution. Samples were heated in a water bath (90 °C) for 1 h. The absorbance from 260 to 410 nm was recorded using a scanning spectrophotometer (Unicam UV-330, Thermo spectronic, England, UK). Results were expressed as A g^{-1} FW.

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