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Uptake and phytotoxicity of anthracene and benzo[*k*]fluoranthene applied to the leaves of celery plants (*Apium graveolens* var. *secalinum* L.)

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ABSTRACT

The above-ground parts of celery plants were exposed to two polycyclic aromatic hydrocarbons (PAHs): 3-ring anthracene (ANT) and 5-ring benzo[*k*]fluoranthene (BkF), and the combination of ANT and BkF. After 43 days of exposure (overall dose of 1325 μg/plant), celery plants retained only 1.4% of the total dose of ANT and 17.5% of the total dose of BkF. After exposure to a combination of ANT and BkF (1325 μg of each compound per plant), the average ANT concentrations were more than twofold higher in/on leaf blades, whereas BkF levels were insignificantly higher. Under natural photoperiod conditions equivalent to a normal day, the combined application of ANT and BkF to the above-ground parts of celery plants slowed down physicochemical transformations of ANT. A similar effect was observed when PAHs were applied to glass surfaces. The combination of both PAHs probably led to stacking interactions, which decreased volatilization, in particular of ANT. Phytotoxicity of ANT and BkF could not be unambiguously established based on the results of this study. In all analyzed treatments, the chlorophyll content of leaf blades remained unchanged. Foliar application of ANT reduced ascorbic acid levels in all analyzed plant parts and increased the total acidity of celery leaves. In all experimental treatments, the total phenolic content of leaves increased up to 15%. Interestingly, ANT and BkF did not produce cumulative effects when applied in combination (when total PAH concentrations per plant were twofold higher).

1. Introduction

Anthracene (ANT) and benzo[*k*]fluoranthene (BkF) are polycyclic aromatic hydrocarbons (PAHs) – a group of compounds composed of aromatic rings in linear or angular arrangements. PAHs are present in the environment as a result of incomplete combustion of organic matter and pyrolysis. PAHs, in particular BkF, are strong absorbers of UV-A (320–400 nm) and UV-B (290–320 nm) radiation spectra from sunlight. ANT comprises three linearly condensed benzene rings, where the central ring has a relatively unstable orbital π, and it is particularly reactive in positions 9 and 10 (Huang et al., 1996).

Sunlight initiates abiotic transformations in PAHs via two mechanisms: photosensitization, which involves the production of reactive oxygen species, and photomodification, which includes photooxidation and photolysis (Ren et al., 1996; Huang et al., 1996; Mallakin et al., 2000). In the gas phase in the atmosphere, PAHs participate in electrophilic substitution reactions in the presence of hydroxyl radicals, ozone and nitrate (V) radicals. Their half-life is inversely proportional to the gas-phase concentrations of radicals and ozone, and it lasts from several days to several minutes in polluted air. PAHs are continuously emitted during oil combustion in Diesel engines and during coal and wood combustion for residential and industrial purposes (Laroo et al., 2012), but due to rapid transformation of PAHs in atmospheric air, the least stable compounds, including anthracene, are maintained in relatively low concentrations. The reaction products of PAHs, hydroxyl radicals and nitrate (V) radicals, are potentially more powerful mutagens and carcinogens than the original compounds, and they also demonstrate phytotoxic activity (Arey and Atkinson, 2003; Huang et al., 1996; Mallakin et al., 2000; Atkinson, 2000; Babu et al., 2001; Boström et al., 2002; Arfsten et al., 1996; Aina et al., 2006).

Research results indicate that exposure to atmospheric PAHs influences plant biomass and physiological processes in plants,

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such as photosynthesis and transpiration (Huang et al., 1996; Wieczorek and Wieczorek, 2007; Oguntimehin and Sakugawa, 2008; Desalme et al., 2011b; G.J. Ahammed et al., 2012a). Model-based experiments revealed a decrease in the chlorophyll content of leaves when the above-ground parts of plants were exposed to ANT, benzo[*a*]pyrene (BaP), benz[*a*]anthracene (BaA) and phenanthrene (PHE) in the form of aerosol or as air pollution (Huang et al., 1996; Desalme et al., 2011b). The reduction in chlorophyll concentrations was accompanied by reduced biomass yield (Desalme et al., 2011b; G.J. Ahammed et al., 2012a).

The results of studies into the phytotoxicity of PAHs are difficult to interpret and compare due to considerable differences in dose, time of exposure, plant species and external factors in those experiments. Oguntimehin and Sakugawa (2008) reported changes in photosynthesis rate, stomatal conductance and chlorophyll content subject to the applied dose of PHE, time of plant fumigation and the use of effective scavengers of reactive oxygen intermediaries (ROI). J.G. Ahammed et al. (2012b) observed that PHE and pyrene (PYR) influenced the activity of selected antioxidant enzymes and the concentrations of malondialdehyde (MDA) and glutathione (GSH, GSSG). They modified the external factors by introducing the protective effects of brassinosteroids.

Unlike in experimental models, the primary source of PAHs uptake by plants is difficult to establish in environmental studies. According to Wild and Jones (1992), the values of bioconcentration factors calculated for the above-ground parts of plants relative to the concentrations of specific PAHs in soil do not account for the actual uptake of those compounds from atmospheric air. PAHs are significantly hydrophobic, and they are absorbed by plant roots in small amounts. According to research, the translocation of PAHs from the roots to the above-ground parts of plants is relatively low (Gao and Zhu, 2004; Kipopoulou et al., 1999; Wild et al., 2006; Wieczorek and Wieczorek, 2007). The adsorption of PAHs, polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) from gas-phase air and solid particles suspended in the air represents the main route by which organic compounds reach the above-ground parts of plants (Kipopoulou et al., 1999; Tao et al., 2006; Desalme et al., 2011a). In a study analyzing the uptake of persistent organic pollutants by plants growing in polluted soil, Kacálkova and Tlustoš (2011) reported higher levels of PHE accumulation in sunflower shoots than roots, but adsorption from gas-phase air could be the main source of the 3-ring volatile compound. In summer, the volatility of lighter PAHs from soils and vegetation could increase their concentrations in the above-ground parts of plants (Kipopoulou et al., 1999; Prevedouros et al., 2004; Tao et al., 2006). High PAH concentrations in internal leaf tissues can be attributed to the uptake of PAHs from atmospheric air by leaves and impaired translocation of those hydrophobic compounds, some of which are relatively persistent (Desalme et al., 2011a,b).

A knowledge of the routes by which PAHs are transported to plant tissues and the magnitude of PAHs bioaccumulation/bioconcentrations in the edible parts of plants is crucial for estimating the amounts of PAHs that are ingested by humans. When those compounds are continuously released from anthropogenic sources, plants are the key link responsible for the presence of PAHs in the food chain (Shen et al., 2013).

Based on the findings of numerous toxicology studies in animals, the EU Environmental Protection Agency classified selected PAHs as probable human carcinogens (B2). This list includes BaP, an indicator compound for a combination of carcinogenic PAHs present in the environment and produced during technological processes, as well as BaA, chrysene, BkF, benzo[*b*]fluoranthene, indeno[1,2,3–*cd*]pyrene and dibenz[*a,h*]anthracene. The Panel on Contaminants in the Food Chain of the European Food Safety Authority analyzed the results of 10,000 analyses of PAHs in food products and decided that PAHs and benzo[*g,h,i*]perylene, a total of eight compounds, are the most reliable indicators of the health risks associated with the ingestion of PAHs. Those findings were based on the results of a study examining the carcinogenic effects of coal tar administered to mice (Culp et al., 1998).

The objective of this study was to determine the effect of long-term foliar application of ANT and BkF to celery plants. PAHs that differ in the number of rings and molecular structure were selected for the study. ANT and BkF were applied individually and in combination to determine whether they exert an additive influence on celery plants despite their structural differences. The retained dose was quantified, and its distribution in various parts of celery plants was determined. Three-ring ANT was used in view of its experimentally verified phytotoxicity (Huang et al., 1996; Krylov et al., 1997; Wieczorek and Wieczorek, 2007). According to the Integrated Risk Information System (United States Environmental Protection Agency, 2014), 3-ring ANT is not classifiable as a human carcinogen, whereas 5-ring BkF is a probable human carcinogen. Phytotoxicity of BkF was not identified. The plant selected for the study was celery, a popular vegetable characterized by large leaf area, edible above-ground parts and naturally high levels of compounds participating in the non-enzymatic response of plant cells to chemical stress, including oxidative stress.

2. Materials and methods

2.1. Materials

A pot experiment was conducted in the greenhouse of the University of Warmia and Mazury in Olsztyn. During daytime, the side walls of the greenhouse were removed. Plants were grown under natural photoperiod conditions equivalent to a normal day: 15–16 h/9–8 h (day/night) in May and 16/8 h (day/night) in June. Three 7-week-old celery plants (*Apium graveolens* L. var. *Secalinum*) were planted per pot containing 10 kg of sandy substrate. The plants were regularly supplied with Hoagland's nutrient solution throughout the experiment. The moisture content of the substrate was maintained at approximately 70% of gravimetric water capacity.

Standard solutions of ANT and BkF were prepared by dissolving 4 mg of each compound (Sigma Chemical Co.) separately in 2 cm^3 dimethyl sulfoxide (DMSO) (POCH S.A. Gliwice, Poland). Working solutions of a single compound were obtained by diluting the standard solution with distilled water to 1000 cm^3 (Wieczorek and Wieczorek, 2007). To prepare the standard solution of the combined doses of ANT and BkF, 4 mg of each compound was dissolved in 1 cm^3 DMSO. Both solutions were quantitatively transferred to a single volumetric flask that was filled up with distilled water to 1000 cm^3.

2.2. Methods

Seven days after transplanting, celery plants were divided into four groups of 21 plants each, based on their habit characteristics. The following solutions were uniformly applied to the above-ground parts of celery plants: 0.2% aqueous DMSO solution (control group), 0.2% aqueous DMSO solution containing 4 mg/L of ANT (ANT group), 0.2% aqueous DMSO solution containing 4 mg/L of BkF (BkF group), and 0.2% aqueous DMSO solution containing 4 mg/L of ANT and BkF each (ANT + BkF group). The volume of the applied working solutions was increased at successive growth stages to ensure even coverage of the entire surface of the above-ground parts of celery plants. Working solutions were applied three times a day (at 8 a.m., 1 p.m. and 6 p.m.) for 43 consecutive days. The total dose administered per plant over 43 days was