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Changes of primary and secondary metabolites in barley plants exposed to CdO nanoparticles☆

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ABSTRACT

The environmental fate of airborne nanoparticles and their toxicity to plants is not yet fully understood. Pot-grown barley plants with second leaves developed were therefore exposed to CdO nanoparticles (CdONPs) of ecologically relevant size (7–60 nm) and concentration ($2.03 \pm 0.45 \times 10^5$ particles cm^{-3}) in air for 3 weeks. An experiment was designed to test the effects of different treatments when only leaves (T1); leaves and soil substrate (T2); and leaves, soil, and water supply were exposed to nanoparticles (T3). A fourth, control group of plants was left without treatment (T0).

Although CdONPs were directly absorbed by leaves from the air, a part of leaf-allocated Cd was also transported from roots by transpiration flow. Chromatographic assays revealed that CdONPs had a significant effect on total content of primary metabolites (amino acids and saccharides) but no significant effect on total content of secondary metabolites (phenolic compounds, Krebs cycle acids, and fatty acids). In addition, the compositions of individual metabolite classes were affected by CdONP treatment. For example, tryptophan and phenylalanine were the most affected amino acids in both analysed organs, while ferulic acid and isovitexin constituted the polyphenols most affected in leaves. Even though CdONP treatment had no effect on total fatty acids content, there were significant changes in the composition of saturated and unsaturated fatty acids in both the roots and leaves of treated plants. Although the results indicate the most pronounced effect in T3 plants as compared to T1 and T2 plants, even just leaf exposure to CdONPs has the potential to induce changes in plant metabolism.

1. Introduction

Nanoparticles (NPs), which are particles having dimensions smaller than 100 nm in at least one dimension and large relative surface area, occur naturally in the biosphere as a result of volcanic eruptions and hydrothermal activity (Luther and Rickard, 2005). Intensive burning of fossil fuels as well as the development and application of nanotechnologies in recent decades are, however, directly related to the additional release of NPs into the environment (Wiesner et al., 2006). Manufactured NPs can enter the environment unintentionally through atmospheric emissions, domestic wastewater, agriculture, and accidental release during manufacture and transport (Klaine et al., 2008). Monitoring studies have shown a substantial increase in concentrations of fine and ultrafine NPs in the atmosphere of large cities, industrial agglomerations and motorways with high traffic volume (Nowack and Bucheli, 2007; Shi et al., 2001; Weijers et al., 2004). In addition, the lifetime of fine NPs (>20 nm) is relatively long. That means these can be transported over long distances, thereby contributing to regional air quality degradation (Biswas and Wu, 2005; Dietz and Herth, 2011). Information concerning the impacts of these nanomaterials on the environment and, in particular, their toxicity to plants, animals, and humans is therefore needed.

Effects of NPs have been described in a variety of microbial and

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aquatic organisms (reviewed in Krysanov et al., 2010; Navarro et al., 2008), although studies exploring the interactions of NPs with higher plants are still rare. Transport of NPs through a growth environment and their penetration into plant tissues are the most critical parameters for evaluating NPs' impact on plants (Auffan et al., 2010). Although higher plants are exposed particularly to atmospheric NP depositions, the majority of experimental studies conducted on plants thus far have employed an aqueous solution, agar, or soil rather than air media (Kurepa et al., 2010; Lee et al., 2012; Yang et al., 2007). NPs occurring in the atmosphere can agglomerate on plant surfaces and penetrate into leaves through cuticle-free areas such as bases of trichomes and/or stomata (Dietz and Herth, 2011; Eichert et al., 2008; Navarro et al., 2008). NPs associated with soil water and soil colloidal particles can interact with roots (Zhu et al., 2008), penetrate into the xylem, and then be transported into plants' above-ground parts via a transpiration flow (Lin et al., 2009).

The primary site of interaction between a plant and NPs is the plant's cell wall, which is composed particularly of cellulose and proteins and offers a number of active sites for binding NPs. The cell wall is semipermeable, and its pore sizes ranging from 5 to 20 nm thus enable the penetration of small molecules (Navarro et al., 2008). Interaction of NPs with the lipid bilayer of plasma membrane, reported by Moore (2006), among other authors, are followed by changes in the production of reactive oxygen species and in metabolic processes (e.g. Jia et al., 2005).

The general consequences of plant exposure to NPs nevertheless remain unclear (Zhang et al., 2012). This is due to the fact that only a limited number of NPs, and in particular atmospheric NPs, and higher plant species have been studied (e.g. Battke et al., 2008; Lee et al., 2012; Lin and Xing, 2008). The majority of studies have focused on the phytotoxicity of metal-based nanomaterials (Ruffini Castiglione and Cremonini, 2009), whereas negligible attention has been given to NPs emitted into the environment from technological processes, including NPs of Cd and its oxides (Fernández Álvarez et al., 2004). Nevertheless, these comprise the most important NPs emitted by sintering plants (Oravisjärvi et al., 2003) and electric steel plants (Sammut et al., 2010). In addition, even as most studies have reported the effect of NPs on plants' early growth stages, and especially on seed germination (Shah and Belozerova, 2009), the effects on fully developed higher plants have received only limited attention.

To reduce the aforementioned uncertainties, we tested the hypotheses that CdO NPs (CdONPs) penetrate from the air into the leaf interior and that an ecologically relevant atmospheric concentration of CdONPs has the potential to change the metabolism of the roots and leaves of barley plants. Specifically, changes in total amount and composition of primary (saccharides and amino acids) and secondary (phenolic compounds, Krebs cycle acids, and fatty acids) metabolites were studied. Moreover, a unique design of dose-concentration chambers enabled us to separate the effects of contaminated air, soil, and water supply. To the best of our knowledge, this is the first comprehensive report on the effect of atmospheric CdONPs on plants.

2. Materials and methods

2.1. Preparation of CdO nanoparticles

CdONPs were generated continuously *in situ* in a hot wall tube flow reactor, using an evaporation–oxidation–condensation technique in which a ceramic crucible containing a small amount of bulk cadmium was placed inside the ceramic work tube of a vertically orientated Carbolite TZF 15/50/610 furnace (Clarkson Laboratory & Supply, Chula Vista, CA, USA). The cadmium was evaporated at the centre of the furnace at 340 °C. The metal vapour was formed in the furnace in a nitrogen gas stream (99.9995%) and then diluted with a stream of air that oxidized the cadmium into cadmium oxide. Both flow rates were set at 3 l min^{-1} with mass flow controllers. The CdONPs thusly formed were diluted in a second step with a stream of air (20 l min^{-1}) and used for experiments in a chamber made of glass and stainless steel. The CdONP concentration used in the experiment was stable at $2.03 \pm 0.45 \times 10^5$ particles per cm^3 air. Particle size was found to be within the range of 7–60 nm (Fig. 1). The distributions of nanoparticles with respect to size and number of particles per unit volume were measured directly using a Model 3936L72 Scanning Mobility Particle Sizer including as peripherals model 3772 and 3775 condensation particle counters, a 3080L differential mobility analyser, and a model 3085 nano differential mobility analyser (TSI, Shoreview, MN, USA). To analyse chemical composition of the generated CdONPs, the CdONPs were sampled on cellulose nitrate membrane filters (porosity 1.2 μm, EMD Millipore, Billerica, MA, USA). The exposed filters were digested in high purity concentrated nitric acid within a microwave device and the Cd content in digests was determined using an AAnalyst 600 atomic absorption spectrometer (Perkin-Elmer, Waltham, MA, USA). Magellan 400L XHR scanning transmission electron microscopy (FEI, Hillsboro, OR, USA) was used to study particle morphology. The micrograph (Fig. 1) revealed that the NPs observed in the gas phase are formed by agglomerates of particles primarily 2–7 nm in diameter.

2.2. Plants and experimental design

Barley (*Hordeum vulgare* L.) seeds were germinated at room temperature on wet filter paper for 48 h. Seeds of the variety Bonus had been provided by the barley gene bank of the Agricultural Research Institute Kroměříž, Czech Republic. Only germinating seeds were then transplanted into small pots (5 cm in diameter) filled with a mixture (1:1) of horticultural substrate and a substrate for potted plants (Agro CS, Česká Skalice, Czech Republic). Three seeds were transplanted into each pot in a triangular spatial distribution. Uniform watering was ensured through capillary action from plastic trays. Barley plants were pre-cultivated for 2 weeks in June 2014 under the controlled conditions of FS-SI-3400 growth chambers (PSI, Brno, Czech Republic): air temperature of 22 °C days/18 °C nights (12/12 h regime), relative humidity of 65/75%, and light intensity of 0/200 μmol m^{-2} s^{-1}. After the full development of second leaves, the pots were transferred for 3 weeks (30 June–22 July 2014) into dose-concentration chambers and grown under microclimatic conditions similar to those of pre-cultivation (light intensity 150 μmol m^{-2} s^{-1} with photoperiod 12/12 h, air temperature 22–24 °C, relative humidity 65–70%). During the whole experiment, all pots were placed in open plastic trays filled with water to ensure uniform watering through capillarity.

Ten pots (30 barley plants) were placed into a control dose-concentration chamber with atmospheric CdONP concentration of 0 (this treatment hereinafter referred to as T0). Thirty pots (90 barley plants) were placed into a dose-concentration chamber with a concentration of $2.03 \pm 0.45 \times 10^5$ CdONPs particles per cm^3 of air. Cellulose nitrate membrane filters (porosity 1.2 μm, EMD Millipore) were used together with polyvinyl chloride membrane foil to prevent contamination of the soil surface and the surface of the supplying water stored in open plastic trays. Three CdONP treatments were thus prepared: only above-ground biomass (leaves) was exposed to CdONPs (hereinafter T1); leaves and soil substrate surfaces were exposed to CdONPs (hereinafter T2); and leaves, soil, and supplying water were completely exposed to CdONPs (hereinafter T3) (see Fig. 2).

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