No evidence for cerium dioxide nanoparticle translocation in maize plants

Birbaum, K; Brogioli, R; Schellenberg, M; Martinoia, E; Stark, W J; Günther, D; Limbach, L K
No evidence for cerium dioxide nanoparticle translocation in maize plants

Karin Birbaum\textsuperscript{1}, Robert Brogioli\textsuperscript{1}, Maya Schellenberg\textsuperscript{2}, Enrico Martinoia\textsuperscript{2}, Wendelin J. Stark\textsuperscript{3}, Detlef Günther\textsuperscript{1}, Ludwig K. Limbach\textsuperscript{3,*}

\textsuperscript{1} Laboratory of Inorganic Chemistry, ETH Zurich, Wolfgang-Pauli Strasse 10, 8093 Zurich, Switzerland
\textsuperscript{2} Laboratory for Molecular Plant Physiology, University of Zurich, Zollikerstrasse 107, 8008 Zurich, Switzerland
\textsuperscript{3} Institute of Chemical and Bioengineering, ETH Zurich, Wolfgang-Pauli Strasse 10, 8093 Zurich, Switzerland

* Corresponding author: ETH Zurich, Department of Chemistry and Applied Bioscience, Institute for Chemical and Bioengineering, 8093 Zurich, Switzerland. E-mail: limbach@chem.ethz.ch; Fax: +41 44 633 15 71; Tel: +41 44 633 63 69
Abstract
The rapidly increasing production of engineered nanoparticles has raised questions regarding their environmental impact and their mobility to overcome biological important barriers. Nanoparticles were found to cross different mammalian barriers, which is summarized under the term translocation. The present work investigates the uptake and translocation of cerium dioxide nanoparticles into maize plants as one of the major agricultural crops. Nanoparticles were exposed either as aerosol or as suspension. Our study demonstrates that 50 microgram cerium per gram leaves was either adsorbed or incorporated into maize leaves. This amount could not be removed by a washing step and did not depend on closed or open stomata investigated under dark and light exposure conditions. However, no translocation into newly grown leaves was found when cultivating the maize plants after airborne particle exposure. The use of inductively coupled mass spectrometer allowed detection limits of less than 1 nanogram cerium per gram leaf. Exposure of plants to well characterized nanoparticle suspensions in the irrigation water resulted also in no detectable translocation. These findings may indicate that the biological barriers of plants are more resistant against nanoparticle translocation than mammalian barriers.

Keywords: Nanotoxicology, fate, nanoparticles, uptake, environmental risk assessment.
Introduction

The broad and interdisciplinary field of nanoparticle engineering has witnessed an unprecedented expansion of research and product development within the last decade. Recent developments in nanoparticle production will lead to an increased use of nanoparticles in commercial products and industrial applications. Nevertheless, their potential ecotoxicological impact remains to a large extent unknown (1-2). Therefore, a better understanding of the environmental behavior of engineered nanoparticles (3-5) is required. Because the distribution behavior of nanoparticles is different, when compared to molecules or larger particles, the realm of nanotoxicity was derived.

The unexpected behavior of nanoparticles has been underlined by a study of Oberdörster et al. showing the translocation of inhaled nanoparticles into the brain of rats which was an unexpected pathway for particles (6). In general, it can be concluded that great attention has been paid to the effects of nanoparticles on mammal cells (7-8), bacteria (9-11) and aquatic organisms (12-15). However, only a few studies report the exposure of nanoparticles to plants. Nanoparticles may enter plants via the roots or via air exchange into the leaves (3). Different studies investigated the uptake of metal oxide nanoparticles into plants via their roots using hydroponic culture models. These studies reported an increased uptake of nanoparticles into roots and translocation into the leaves (16-18). Similarly carbenous nanomaterials have been found to translocate into the leaves (19) after uptake via the roots. These studies in hydroponic culture models provide clear evidence for nanoparticle uptake through the roots exposed to liquid media. The uptake of nanoparticles through the leaves has only been investigated qualitatively. Corredor et al. found an uptake of carbon coated magnetic nanoparticles in pumpkin plants and reported that movements over short distances are favored (20). Eichner et al. found that polymeric nanoparticles of 43 nm diameter penetrate into leaves. However, particles of larger diameters (e.g. 1.1 µm) cannot enter leaves (21). Unfortunately, a quantitative description of the uptake processes has not been reported.

Based on these qualitative descriptions we considered that a quantitative investigation of the flow pattern and translocation of nanoparticles is of major importance for a full life cycle assessment.
This study focused on the quantitative investigation of the uptake and translocation of ceria nanoparticles into maize plants. Using inductively coupled plasma mass spectrometry (ICP-MS), the quantitative description is not hindered by the detection limits of this technique. Cerium oxide nanoparticles are considered to be a representative member of the industrial important class of metal oxide nanoparticles, and combine both a low background signal in plants and insolubility under environmental conditions.

To simulate various scenarios, ceria nanoparticles were exposed to leaves as airborne aerosols and as aqueous suspensions, similar to a most recent air exposure setup used for human lung cells uptake studies, established by Rothen-Rutishauser et al. (22). Maize plants were exposed to nanoparticles under artificial daylight which results in open stomata and under dark conditions, where stomata are closed. In a second experiment, the plants were exposed to aqueous suspended nanoparticles by irrigation, which simulated rain forced nanoparticle transport into plants.

**Experimental section**

**Particle production** The cerium dioxide nanoparticles were produced by a flame spray pyrolysis in a totally closed production setup (23) which was previously used as exposure chamber for human lung cells (22). The CeO$_2$ nanoparticles were produced using cerium 2-ethylhexanoic acid diluted in xylene (8 wt %) which was introduced as precursor into a methan/oxygen flame. The flow rate of the precursor was 5 mL·min$^{-1}$ and 5 L·min$^{-1}$ oxygen gas was used to disperse the liquid leaving a capillary. Particle size distribution and specific primary particles diameter were determined by X-ray disk centrifugation (XDC, Brookhaven Instruments, BI-XDC), N$_2$ adsorption (Brunauer-Emmett-Teller, Micromeritics Tristar 3000), transmission electron microscopy (CM30 ST, Philips, LaB$_6$ cathode, operated at 300kV, point resolution ~4 Å), and X-ray diffraction (Siemens Powder X-ray diffractometer using Ni-filtered CuK$_\alpha$ radiation in stepmode with a step size of 0.3 Å).

**Suspensions** The CeO$_2$ nanoparticle suspensions with a concentration of 10 µg ceria per ml water were diluted from a freshly prepared stock suspension. CeO$_2$ nanoparticles were dispersed in ultra pure water (>18 MΩ cm) for 15 minutes using an ultrasonic device (UP400S, Hielscher, Germany) to ensure that the suspension were in a homogeneous state. Ceria suspensions in water were characterized by X-
ray disk centrifugation and for their zeta-potential (Dispersion Technologie DT 1200).

**Plants** The exposure experiments were carried out on 3-5 weeks old maize plants (Birko) which were grown in a greenhouse at ambient temperature and mercury lamp illumination. Depending on the experiment, the plants were directly sampled, washed, dried and measured after exposure. In some experiments the plants were grown further after initial sampling of the leaves. All plants were grown under similar conditions and equally treated (fertilizer and water in a regular way). The plants were seeded and grown in groups of 3-4 plants per pot. Standardized soil (Einheitserde type ED 73) was used for all experiments.

**Air exposure** The 3-5 week old plants were placed in an in-house built glove box of 2.25 m³, wherein the nanoparticles were produced. The exposition unit consists of the nanoparticle production unit and an exposure chamber where the plants were hosted (see figure 1). The particle production was switched on for one minute. This leads to a total exposure of 0.4 g ceria nanoparticles in the glove box with the dimension of 2.5 m × 1 m × 0.9 m. After turning off the flame, a fan was started to distribute the particles homogeneously over the plants in the box. Agglomerate size distribution and mass deposition was characterized in an earlier study (22). Temperature remained constant during the exposure between 27°C and 31 °C and the relative humidity was between 60% and 70%. The plants were kept within the box for an exposure time of 20 minutes. In order to measure differences in nanoparticle uptake with open and closed stomata, daylight and a night experiments were simulated. Lamps (OSRAM 400 W Deluxe, HQL (MBF - U) Germany) with an intensity of 80 µmol m⁻² sec⁻¹ were used with a distance of 1.4 meters. 48 plants were divided into two groups, one was exposed at simulated daylight and the other at night conditions. In order to differentiate total deposition on and uptake into the leaves, half of the plants of both groups were additionally washed thoroughly with deionized water (>18 MΩ cm) before digestion to remove particles weakly deposited on the leaf surface. The leaves were separately rinsed with deionized water and abraded with a glove, simulating a possible naturally occurring washing procedure by rain and wind. The second group was treated in the same way without the washing step as a measure of the total deposition. Two additional plant pots (containing 3-4 maize plants each) from each experiments and a control sample were placed back in the greenhouse for further growth of up to 12 weeks in order to
determine translocation of cerium dioxide nanoparticles into future grown leaves. The newly grown leaves were collected, dried, digested and analyzed by ICP-MS.

Figure 1: Cerium dioxide nanoparticles were exposed either as in situ prepared aerosol (left) or as suspension (right). Aerosol exposure was preformed in a setup developed for the exposure of human cells at the air-liquid interface. a) shows the nanoparticles production unit. b) shows the ventilator for the well characterized and homogeneous particles deposition and c) are the maize plants within the totally enclosed setup.

Suspension exposure of leaves. Additionally to the aerosol exposure, leaves were exposed to freshly prepared ceria suspensions for further investigations on the particle-leaf interactions. Viable plant’s leaves were directly immersed in 10 ppm ceria suspensions in 50 ml Eppendorf tubes. The leaves were fixed to the tubes with tape and kept in the suspension for different time intervals. After the exposure, the exposed parts of the leaves were cut with a scalpel, weighted and transferred into digestion vials. For every time interval three replicates were measured.

Irrigation soil exposure. The soil exposure was carried out by adding particles to the soil around the plant roots in order to study the uptake via the roots. 50 ml of 10 µg g⁻¹ cerium dioxide suspension were added twice a day to the soil over a period of 14 days to three individual plants. This leads to a total exposure of 14 mg cerium dioxide per pot. After the exposure, the leaves and the stem were collected, digested and measured by ICP-MS. In addition, when plants were cut the xylem sap was pumped out from the stem and 100µl of sap were collected with a pipette.

Sample preparation. The leaves were cut into small pieces and dried in an oven to constant dry mass. The digestion of 0.1 g of the dried leaf samples within 4 ml of
HNO$_3$ (subboiled, Sigma-Aldrich, Buchs, Switzerland) and 0.4 ml H$_2$O$_2$ (trace Select Ultra, Sigma-Aldrich, Buchs, Switzerland) was performed in a microwave digestion unit (Ultraclavell II, Milestone GmbH, Shelton, CT, USA) at a temperature of 230°C and a pressure of 90 bar for a period of 1 h. Yttrium was added prior to digestion to determine the digestion recovery (0.1 g of a 1 µg·g$^{-1}$ or 10 µg·g$^{-1}$ Y solution). After the digestion, the samples were diluted to 10 g and further diluted by a factor of 10. Following this, 10 ng·g$^{-1}$ Indium were added as internal standard during a second dilution step. Blanks were determined analyzing unexposed plant leaves. The digestion of collected xylem sap was not necessary; it was diluted by factor 1000 with 1% HNO$_3$ and directly analyzed by ICP-MS

**Elemental Analysis.** All concentrations were measured using an Element2 (Thermo-Finnigan, Bremen, Germany) sector field inductively coupled plasma mass spectrometer (ICP-SF-MS). The instrument was tuned daily for maximum signal intensity using a multielement solution at a concentration of 1 ng·g$^{-1}$. The mass calibration was also performed daily. This type of ICP-MS can be run in three different resolution modes (LR, low resolution R=400; MR, medium resolution R=4000 and HR high resolution R=10 000; R=$\Delta$m/m). Measurements were carried out in LR mode. The ICP-MS instrument provided a sensitivity of 2·10$^6$ cps for 1 ng·g$^{-1}$ Indium in LR mode when introducing the sample as liquid.

Every measurement sequence contained the calibration solutions at the start. The range of the calibration standards was adjusted to the concentrations expected in the samples. The operating parameters for all the measurements, the calibration ranges used and the applied measurement conditions are summarized in the supporting information.

**Electron microscopy.** The leaves were cut from the plants and, in order to preserve the leaf structure, slowly frozen to approx. -170 °C with liquid nitrogen. After freezing, the samples were transferred under vacuum atmosphere to a sputter coater device (Baltec BAF60, BAL-TEC AG, Balzers, Liechtenstein) where they were coated with a thin layer (ca. 2 nm) of platinum. The coating enhances the contrast when measuring such type of samples by scanning electron microscopy. After being coated, the samples were transferred to the microscope, a Zeiss Gemini 1530FEG (Carl Zeiss AG, Germany). During the whole procedure, coating and measuring with the electron microscope, the temperature of the samples was maintained at -170°C.
**Statistical Analysis.** For all figures data were represented as mean values ± standard error; n indicates the number of individual plants used for the experiment. Statistically significant data sets were indicated by a star * for p < 0.05 using a student’s t-test.
Results

Ceria nanoparticles showed the typical lognormal size distribution with an average primary particle size of 37 nm and a BET-surface area of 110 m$^2$ g$^{-1}$. The aerosol deposition and characterization was performed at identical conditions as reported in earlier work where ceria nanoparticles have been exposed to human cells (22). The nanoparticles and agglomerates were deposited homogeneously within the glove box as characterized previously by Rothen-Rutishauser et al. (22). Additional particle characterization showing size distribution measurements, suspension characterization and the phase purity of the materials are presented in the supporting information.

Exposure as aerosol. In a nanoparticle production unit, maize plants were exposed to in situ produced ceria nanoparticles to determine the nanoparticle uptake into the leaves (see Figure 1). The experiment was carried out both under additional light from a mercury lamp as well as in the dark to differentiate between open and closed stomata. Figure 2 shows both the total deposition on the leaves immediately after exposure and the amount which remains on the leaves after applying a washing procedure. The washing step removed deposited nanoparticles which were neither adsorbed nor integrated into the leaves. The opening of the stomata had no significant influence on the amount of nanoparticles adsorbed on the leaves. (Light: 44 ± 8 µg·g$^{-1}$ n=6; dark: 51 ± 10 µg·g$^{-1}$ n=11). Although the deposition including artificial light was significantly increased (Deposition by light: 270 ± 70 µg·g$^{-1}$ n=12; dark: 145 ± 11 µg·g$^{-1}$ n=12); the additional deposited nanoparticles were removed by the washing step and are not a result of a higher uptake of ceria nanoparticles. The increased deposition may be explained by light induced electrostatic interactions of the particles. In spite of the increased deposition upon ceria exposure on light, the adsorption of ceria nanoparticles was not significantly increased between open or closed stomata. We therefore concluded that the ceria nanoparticles get in contact with maize leaves and that this process is independent on the open/close state of the stomata.
Figure 2: Deposition and adsorption of nanoparticles exposed as aerosol. Exposure during light increased the deposition of nanoparticles on the leaves. Each data point is the mean value of n<5 replicates, error bar 1SD. Asterisk (*) denote significant different from control cell cultures (p < 0.05).

Nanoparticles adsorption on leaves. Scanning electron micrographs were taken in order to gain insights into the interaction of nanoparticles with the leaf surface. The structure of maize leaves is heterogeneous as shown in figure 3a. The leaf surface consists of wax regions which offer a protection for mechanical damage or UV radiation and adaption to moisture (24-25). This rough structure of the leaves made it difficult to find single particles. Bigger agglomerates were found nearby closed stomata (figure 3b) or integrated into the surface wax (figure 3d) + 3e).

As a control experiment, leaves were directly submerged in well characterized ceria suspensions in order to investigate interactions of leaves with liquid suspensions. In supporting figure 4 and 5a steadily increasing uptake was found over 12 hours. With increasing time leaves incorporated more ceria nanoparticles. It is therefore concluded that the incorporation of nanoparticles within the leaf structure may be the predominant uptake process. However, aggregates were found on the leaf surface indicating that particles adsorption also contributes to the total process (figure 3f).
was more difficult to find the aggregates on the leaf surface than in the aerosol exposure experiments.

Figure 3 Scanning electron micrograph of maize leaves. a) Overview on the unexposed maize leaf, stomata and surface wax areas are visible. b), d) and e) show different ceria agglomerates after exposure to aerosols. c) Transmission electron micrograph of as prepared ceria nanoparticles. f) shows a ceria agglomerate after the suspension exposure on the surface of the leaf.

**No translocation of nanoparticles.** To investigate possible translocations of nanoparticles, plants were cultivated for additional three months in the greenhouse after aerosol exposure. Leaves were cut and numbered starting from the bottom with the oldest leaf (see figure 4). The highest concentrations of 30-40 µg cerium·per g leaf have a similar concentration incorporated to the washed samples in figure 2. This shows the quality of the washing procedure, which lead to similar ceria concentrations as a cultivation in the greenhouse. The third leaf has a lower amount of particles incorporated. This is due to the fact that the first two leaves were already fully developed and present at the time of exposure. The third leaf was also already present, but not completely developed and continued to grow after the exposure. The ceria nanoparticle concentration is below the limit of detection in the leaves which
grew after the exposure. In this experiment, the instrumental limit of detection for cerium in the leaves was 0.4 ng·g⁻¹.

Figure 4: Four plants were cultivated after nanoparticle exposure for additional three months. Leaves were assigned according to their formation. Single data points represent cerium concentration of a leaf in ppm. Plants were exposed to ceria nanoparticles having 3 leaves; leaf 4 to 10 grew post-exposure. Circles represent exposure without additional light and squares including light.

**Irrigation exposure of suspension.** A second scenario is that nanoparticles are exposed to maize plants by irrigation of nanoparticle suspensions. The soil was exposed to 10 µg ceria / ml water over 14 days. No cerium concentration above the limits of detection was found in the different compartments of maize plants. In this experiment, the instrumental limit of detection was 1.7 pg·g⁻¹. Due to different sample preparation of the individual matrices, the detection limit varies along the different plant compartments, (leaves: 3.6 ng·g⁻¹, stem: 10.1 ng·g⁻¹ and xylem sap: 4.0 ng·g⁻¹).

We can thus state that no significant uptake and no accumulation of ceria nanoparticles was found in the maize leaves sequentially to an exposure in the irrigation water. After the exposure, the soil showed a cerium concentration of 40.1 µg·g⁻¹ at the top surface. This indicates that most of the nanoparticles dispersed in water are filtered by the soil compartment. Overall, 14 mg Ceria were exposed over this period of time.

**Discussion**

Nanotoxicology achieved its current importance primarily due to the different behavior of nanoparticles compared to molecules or larger particles (26). Inhaled nanoparticles have been shown to translocate within the body and can penetrate
different biological barriers such as the blood-brain barrier or the placenta (27). This has highlighted the importance for a reevaluation of biological barriers regarding the exposure for nanoparticles especially in undisturbed systems. Within our experiments we found no significant difference in growth and appearance of the maize plants even several months after ceria nanoparticle exposure. This is in agreement with Ma et al. which have found that ceria nanoparticles did not affect root growth in seven higher plant species (radish, rape, tomato, lettuce, wheat, cabbage, and cucumber) (28). Ceria nanoparticles are known to have no acute toxic effects but are highly suited to investigate nanoparticles mobility when crossing biological barriers as the cell wall of plants.

**Integration of nanoparticles into the leaves.** Following an aerosol exposure, 50 µg ceria per g of leaf could not be removed by a washing step. Two different mechanisms are proposed, the adsorption of ceria nanoparticles on the leaf surface and the incorporation into the leaf structure. It was not possible to differentiate quantitatively between adsorbed and incorporated nanoparticles. Adsorption on the leaves is reasonable as nanoparticles prefer to locate in the interphases of systems (29) and the wax lipids may quickly adsorb on the large surface of the particles (30). Using electron microscopy ceria nanoparticles were found on the surface of the leaves in a strongly agglomerated form.

The incorporation of nanoparticles into leaves has been reported earlier by Eichert et al. who found that polymeric nanoparticles penetrated into the epidermis of *Vicia faba* (21). Corredor et al found nanoparticle penetration into the first cell layer of pumpkin plants (20). In order to increase the understanding between the nanoparticles and the leaf surface, we incorporated maize leaves into a well characterized ceria suspension. This artificial exposure scenario enables the sampling of more time points and therefore an investigation of the time dependent uptake of ceria nanoparticles (Supporting Information Fig 4+5). The ceria concentration in the leaves increased over 12 hours. If sole adsorption of particles on the surface was the predominant process, saturation would be expected within two to four hours which is a relevant timeframe for adsorption processes (31). Incorporation of nanoparticles into leaves may contribute to the measured uptake as the uptake still increases after four hours. However, with our analytical methods we were not able to distinguish between these two mechanisms. Most likely smaller particles may be incorporated into the leaf whereas large agglomerates are trapped on the surface wax.
Interactions of nanoparticles with leaves have to be further evaluated in continuative studies.

**Translocation of nanoparticles.** A possible translocation of nanoparticles into newly grown leaves or into the corn would have more impact as maize is one of the most important agricultural crops. Using analytical equipment for ultra sensitive Ce analysis we found no evidence for a translocation within maize plants. The cell wall of plants is comparably thicker than membranes of mammalian cells and the membranes of bacteria are in between these two biological barriers. A different uptake into plants or mammalian cells as proposed by Chen et al. is a plausible explanation (32). Nanoparticles may be both adsorbed and integrated into the leaves, but the translocation is hindered compared to mammalian cells due to the larger biological barrier and their different composition. The uptake into mammalian cells was found to be limited by diffusion and sedimentation (33). Increasing the thickness of the biological barrier will therefore reduce the mobility of the nanoparticles and consequentially their ability for translocation. Uptake of nanoparticles into plant leaves has been proposed to occur through small pores in the cuticle (< 5 nm) or through stomatal apertures (21). Cell wall pores are smaller than ten nanometers and the cell wall is expected to be a tight sieve which does not allow nanoparticles migration (34). Furthermore, it should be considered that translocation of nanoparticles from one leaf to another would require complex translocation mechanisms. They would have first to be excluded from a cell by exocytosis and then loaded into the phloem. The phloem is constituted by living cells which are tightly controlled to allow sugars and amino acids to migrate from the source to the sink tissue. As we measured no translocation, these transport mechanisms may not allow for the migration of nanoparticles.

Another exposure scenario for nanoparticles to plants is the pathway via the roots. Suspension experiments were conducted using the same plants and nanoparticles dispersed in water. Nanoparticles were exposed for 14 days in the irrigation water. Again, no translocation of ceria nanoparticles was found into the leaves, the xylem liquid or the stem using ICP-MS. Nanoparticles were exclusively found in the potting soil. Further studies have to investigate the role exerted by roots and rhizosphere soil. This finding is in good agreement with a recent study by Wild *et al.* where ceria and titania nanoparticles were found as aggregates on the surface of the roots (18). In literature different groups reported translocation of nanoparticles through the roots into the whole plants. Iron oxide was found to translocate into the leaves of
pumpkins, but not into lima bean plants (17). Soluble zinc oxide nanoparticles were taken up by the roots of ryegrass more effectively than corresponding zinc ions, although the translocation to the leaves was higher in the case of ion exposure compared to the particles exposure (16). These studies were performed in hydroponic cultures where the roots are surrounded by a liquid media. It is obvious that the nanoparticle migration is faster from a liquid surrounding when compared to soil. Irrigation experiments of soil grown maize plants with titania nanoparticles suspension had mostly insignificant inhibitory effects whereas same exposure in hydroponic cultures reduced the hydraulic conductivity (34). Hydroponic cultures help to understand mechanistic behavior of nanoparticles into plants but can not perfectly reflect nature.

It is well known that living plants can be used for the generation of nanoparticles (35-36). This is mainly attributed to precipitation of ions during an over-exposition. The resulting generation of nanoparticles in different plant compartments is connected to the dependence of the solubility on the conditions (pH, redox state, ionic strength). In case of soluble nanoparticles it difficult to distinguish between ion uptake and nanoparticle uptake (37). The use of insoluble nanoparticles as ceria overcomes this difficulty and enables to attribute detected Ce concentrations through ICP-MS to nanoparticles. The detection limit of 0.5 ng·g⁻¹ for Ce in plant samples correspond to 3·10⁶ particles per gram. No evidence was found for ceria nanoparticles translocation within maize plants. Nanoparticles were adsorbed on and incorporated in leaves but were not able to translocate into not exposed, newly grown leaves. In irrigation experiments of soil grown maize plants no translocation was detected. Further studies have to focus on the behavior of nanoparticles in soil and the validity of experiments with hydroponic cultures.

**Acknowledgment**

We thank F. Krumeich for TEM analysis, R. Wepf for the scanning electron micrographs and the Electron Microscopy Center of the ETH Zurich (EMEZ). Financial support from the ETH Zurich (TH 21- 08-3) is gratefully acknowledged.

**Supporting Information available**

Extended particle characterization and control experiments on particle-leaf interactions are presented in the supporting information. This information is available free of charge via the internet at http://pubs.acs.org.
Literature


nanoparticles can inhibit leaf growth and transpiration via physical effects on root surface wax composition.

Asli, S.; Neumann, P. M. Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root surface wax composition.


Asli, S.; Neumann, P. M. Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root surface wax composition.


Brief

Ceria nanoparticles were taken up by maize plant leaves but no translocation within the plants was measurable using ultra-low detection methods.