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Short communication

Horizontal natural product transfer: A potential source of alkaloidal contaminants in phytopharmaceuticals

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ABSTRACT

Background: It was recently shown that nicotine and pyrrolizidine alkaloids that leach out from decomposing plant material (donor plants) are subsequently taken up by the roots of acceptor plants and translocated into their leaves. Furthermore, it is well established that plant roots take up xenobiotics, generally by simple diffusion, and that this passive import depends on the physico-chemical properties of the substances.
Hypothesis: Based on the well-known uptake of xenobiotics, we assumed that in analogy, the uptake of alkaloids, which are leached out from plant material (donor plants) represents a quite general feature of plant biology.
Methods: Using barley as a model plant, we analyzed the uptake of alkaloids by applying them to *Hordeum vulgare* seedlings. Based on HPLC analyses, the presence of the particular alkaloids in the acceptor plants was determined.
Results: We demonstrated that numerous alkaloids of different structural types are able to diffuse through biomembranes and are taken up by acceptor plants. In contrast, an uptake of quaternary alkaloids, with a permanent positive charge, could not be detected.
Conclusion: As most alkaloidal plants generally die back afield, and the corresponding natural products are leached out into the soil. Our findings have substantial relevance for all plant-derived commodities, especially for the production of phytopharmaceuticals and the related safety issues. Moreover, the evidence that plants are inherently able to take up alkaloids from the soil, which are derived from other plants, will alter our appraisal of plant–plant interactions. In this context, the classical definition of xenobiotics, which are considered as "non-natural" substances, might be also extended by including natural products leached out into the soil.

Introduction

It is well established that numerous xenobiotics,[1] such as systemic herbicides, fungicides, and veterinary medicines, are taken up by plant roots from the soil and translocated into their shoots (Boxall et al., 2006; Trapp and Legind, 2011). However, until recently, there have been no studies on the uptake of natural compounds. When examining or exploring potential sources for contamination of plant-derived commodities, such as herbal teas or medicinal plants by nicotine and pyrrolizidine alkaloids (PAs), it was demonstrated that these alkaloids are taken up from the soil (Selmar et al., 2015a; Nowak et al., 2016). This phenomenon was termed "*Horizontal Natural Product Transfer*" (Selmar et al., 2015b). When the alkaloids are leached out from decomposing plants - denoted as donor plants - into the soil, they could be taken up by the roots of acceptor plants and are translocated into their leaves. Accordingly, the substances must pass the plasmalemma of root cells in the acceptor plants. The required import into the symplast may occur within the rhizodermis or, at the latest, in the passage cells of the endodermis. This uptake could be accomplished either by active transport, facilitated by the action of carriers, or by passive diffusion through the biomembranes. On a first glance, the involvement of transporters seems to be required (for review see Yazaki, 2006). However, many substances simply diffuse across biomembranes. The ability of substances to penetrate biomembranes is related to their physico-chemical properties and can be roughly estimated from the distribution of these substances between octanol and water. Membrane permeability can be extrapolated from the corresponding distribution coefficient, the K_{OW} value, or its decadal logarithm, denoted as pK_{OW} (e.g., Trapp, 2000, 2004), also termed log*P* (e.g., Cronin and Livingstone, 2004). The ability of a substance to permeate membranes is linked to solubility in

Abbreviations: ACN, acetonitrile; HPLC, high performance liquid chromatography; MeOH, methanol; PAs, pyrrolizidine alkaloids; TFA, trifluoroacetic acid; UV, ultra violet

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[1] According to IUPAC, a xenobiotic is a compound that is foreign to a living organism and which has been introduced into the environment by artificial means (DeBolster, 1997).

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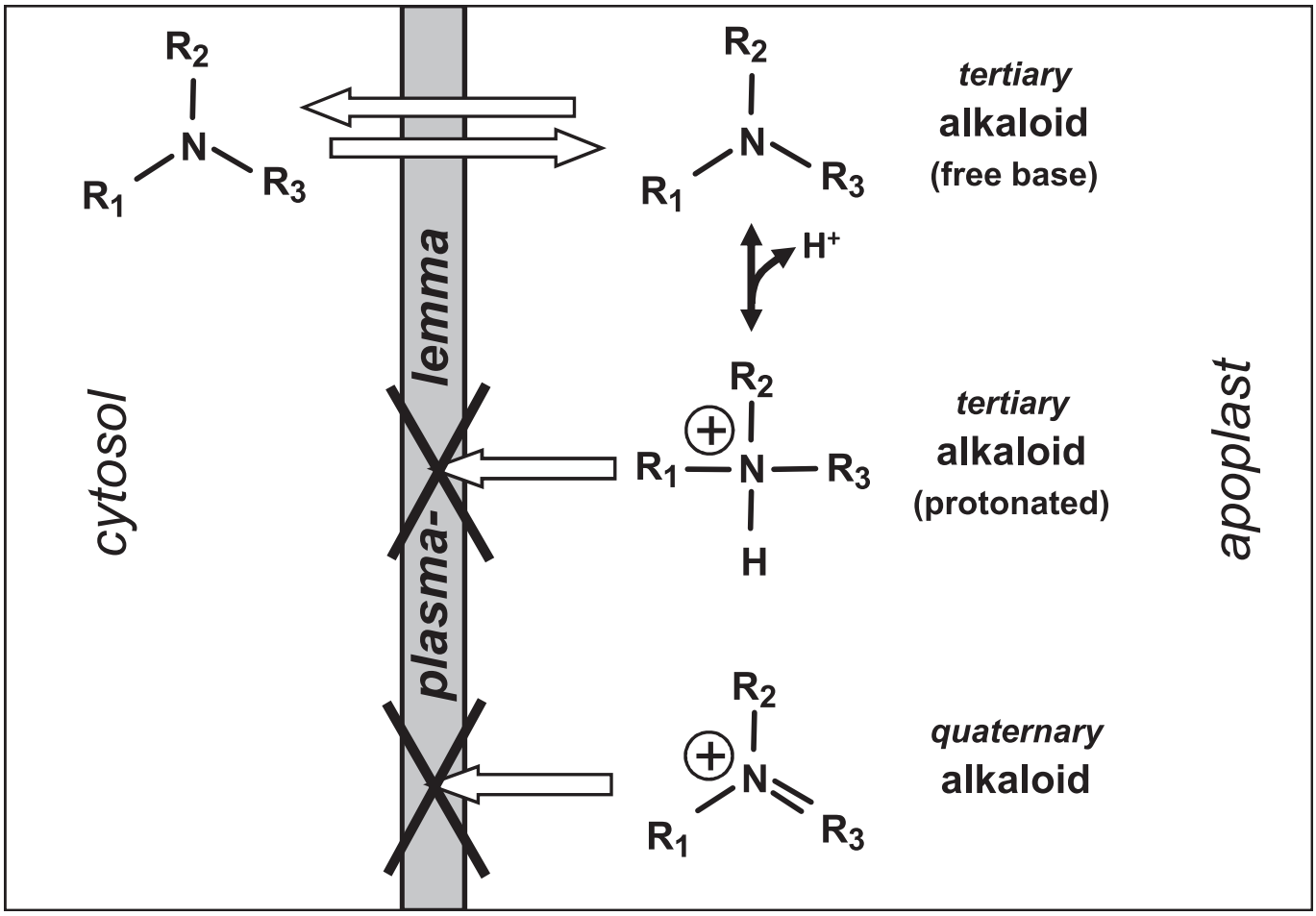

Fig. 1. Inherent differences in membrane permeability of alkaloids. Whereas the free bases of tertiary alkaloids easily diffuse across biomembranes, protonated tertiary alkaloids as well as quaternary alkaloids, due to their positive charge, cannot pass the membranes.

aqueous as well as in organic media. To a first approximation, substances that have log*P* values between −1 and 3 normally diffuse easily through biomembranes (Trapp, 2000; Trapp and Legind, 2011). Although these generalizations are based on studies of xenobiotics, they are also relevant for natural products such as alkaloids. However, in the case of alkaloids, an additional factor must be considered. The free bases of alkaloids (e.g., nicotine or PAs) are able to pass through membranes (Fig. 1), but their protonated forms reveal a positive charge and therefore cannot penetrate through the biomembranes. This is confirmed by the strongly negative log*P* values of charged alkaloid salts. Thus, in addition to the log*P* values of the alkaloids, the membrane permeability is also determined by the pH of the medium.

The ambivalent properties of alkaloids have been previously considered by Matile (1976) who outlined the ion trap model that serves as a basis for our understanding of the accumulation of alkaloids in vacuoles. Because of their membrane permeability, unprotonated alkaloids present in the neutral cytosol can pass freely through the tonoplast into the vacuole by passive diffusion. However, once inside the acidic vacuole, the alkaloids are protonated and the resulting hydrophilic cations are unable to pass through the membranes and are trapped. Although these observations are well established, until recently, they have not been considered to explain the uptake of alkaloids from the soil, and related experimental studies thoroughly investigating these phenomena have not been conducted. In this study, we present evidence for the wide-ranging validity of the concept of horizontal natural product transfer by demonstrating that numerous alkaloids, representing several important structural types, are taken up by acceptor plants, e.g., barley seedlings.

Material and methods

Plant material and application of alkaloids

Barley seedlings were sown in vermiculite. Two weeks after germination, the alkaloids were added during watering. The alkaloid concentration was 200 µg/ml; overall, 10 mg of each alkaloid (50 ml of each alkaloid solution) were applied to 500 plants in the course of watering. Special diligence was applied to avoid any direct contact of the watering solution with the plantlets. In order to prevent any flooding of the vermiculite, the volume of solution added never exceeded the capacity of vermiculite. Accordingly, any anoxia or leaching of highly water-soluble substances could be excluded.

Analysis and quantification of alkaloids

To exclude any contamination with alkaloid-containing substrate (vermiculite), harvesting was performed by cutting the seedlings at 1–2 cm above the ground. The aerial parts were directly transferred into liquid nitrogen and freeze dried. For the extraction of alkaloids, 600 mg of the lyophilized plant material were blended with 10 ml of water adjusted with TFA to pH 2.0 and ultrasonicated for 30 min at 55 °C. After centrifugation, water was evaporated using a steam of air. To dissolve the alkaloids, 1 ml MeOH was added to the dried residue. After ultrasonication (10 min, room temperature) and centrifugation (10 min; 10,000 × *g*), the sample was used for HPLC.

HPLC separations were performed on a Nucleosil RP-18 column using a tertiary gradient, starting with 10% MeOH, 10% ACN, 80%