Food Chemistry 213 (2016) 163–168

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Interspecific transfer of pyrrolizidine alkaloids: An unconsidered source of contaminations of phytopharmaceuticals and plant derived commodities

Melanie Nowak [a], Carina Wittke [a], Ines Lederer [b], Bernhard Klier [b], Maik Kleinwächter [c], Dirk Selmar [a,*]

[a] Institut für Pflanzenbiologie, Technische Universität Braunschweig, Mendelssohnstraße 4, D-38106 Braunschweig, Germany
[b] Phytolab, Dutendorferstr. 5-7, D-91487 Vestenbergsgreuth, Germany
[c] Repha, Alt-Godshorn 87, D-30855 Langenhagen, Germany

ARTICLE INFO

Article history:
Received 9 February 2016
Received in revised form 20 May 2016
Accepted 21 June 2016
Available online 22 June 2016

Keywords:
Horizontal transfer of natural products
Pyrrolizidine alkaloids
Plant derived commodities
Contamination
Senecio
Ragwort

ABSTRACT

Many plant derived commodities contain traces of toxic pyrrolizidine alkaloids (PAs). The main source of these contaminations seems to be the accidental co-harvest of PA-containing weeds. Yet, based on the insights of the newly described phenomenon of the *horizontal transfer of natural products*, it is very likely that the PA-contaminations may also be due to an uptake of the alkaloids from the soil, previously being leached out from rotting PA-plants. The transfer of PAs was investigated using various herbs, which had been mulched with dried plant material from *Senecio jacobaea*. All of the acceptor plants exhibited marked concentrations of PAs. The extent and the composition of the imported PAs was dependent on the acceptor plant species.

These results demonstrate that PAs indeed are leached out from dried *Senecio* material into the soil and confirm their uptake by the roots of the acceptor plants and the translocation into the leaves.

1. Introduction

Within the last decade, it became quite evident that a tremendous number of plant derived commodities, including herbal teas, spices, or phytopharmaceuticals contain toxic pyrrolizidine alkaloids (PAs; EFSA Panel on Contaminants in the Food Chain, 2011). An actual evaluation of Mulder, Sánchez, These, Preiss-Weigert, and Castellari (2015), supported by the European Food Safety Authority (EFSA), revealed that PAs are present in more than 90% of all herbal tea samples. However, the origin of these PAs is still unknown. In Central Europe, up to now, only a limited number of species, including some widespread crop weeds, such as various *Senecio* species, are known to be able to synthesize pyrrolizidine alkaloids (Pichersky & Lewinsohn, 2001). Pyrrolizidine alkaloids, representing typical secondary plant products, are thought to protect the plants against herbivores and pathogens. In PA containing plants the alkaloid content varies between different plant organs (e.g., Frölich, Ober, & Hartmann, 2007) as well as plants species (e.g., Johnson, Molyneux, & Merrill, 1985). Moreover, the PA content is reported to be modulated by several abiotic factors such as soil quality, water availability, and temperature (Hol, Vrieling, & van Veen, 2003; Kirk, Vrieling, Van Der Meijden, & Klinkhamer, 2010; Vrieling, de Vos, & van Wijk, 1993). In typical PA-plants like *Senecio jacobaea*, the concentration of PAs could be extremely high, and values between 1000 and 10,000 mg alkaloids/kg d.w. are frequently reported (e.g., Johnson et al., 1985; Vrieling et al., 1993). Accordingly, in any case where just one *Senecio* plant is co-harvested with several thousands of PA-free crop or medicinal plants, the entire batch is contaminated, exhibiting PA concentrations of 1 mg/kg d.w. or more. Consequently, the accidental co-harvest of PA-containing weeds is considered to be the main source of the PA-contaminations of herbal teas, spices, or phytopharmaceuticals (e.g., Mulder et al., 2015). However, in various cases, where an accidental co-harvest of PA containing weeds could be excluded, e.g., when the plant material is obtained by thorough hand-picking like green or black teas (*two leaves and a bud*), the observed PA-contaminations must have another origin.

In this context, the recently discovered *horizontal transfer of natural products* comes into focus. This phenomenon, which describes the uptake of natural products leached out from rotting plants (Selmar, Radwan, & Nowak, 2015), is reported to be responsible, at least in part, for the nicotine contaminations of several plant derived commodities (Selmar, Engelhardt et al., 2015).

* Corresponding author.
E-mail addresses: m.nowak@tu-bs.de (M. Nowak), c.wittke@tu-bs.de (C. Wittke), ines.lederer@phytolab.de (I. Lederer), bernhard.klier@phytolab.de (B. Klier), m.kleinwaechter@tu-bs.de (M. Kleinwächter), d.selmar@tu-bs.de (D. Selmar).

http://dx.doi.org/10.1016/j.foodchem.2016.06.069
0308-8146/

Consequently, horizontal transfer of natural products could conceivably also be the cause for contaminations of plant derived commodities with PAs. In this paper, various plant species were employed that are commonly used as herbal teas or spices, i.e., melissa (*Melissa officinalis*), peppermint (*Mentha × piperita*), chamomile (*Matricaria chamomilla*), and parsley (*Petroselinum crispum*), to investigate the putative uptake of PAs from soil, mulched with dried plant material from *Senecio jacobaea*.

2. Materials and methods

2.1. Plant material

All experimental plants (*Melissa officinalis*, *Mentha × piperita*, *Matricaria chamomilla, Petroselinum crispum*) were grown during spring to early summer, outdoors, in pots. In the case of the peppermint, chamomile, and parsley plants, cultivation was performed in square pots (9.0 × 9.0 cm × 9.5 cm) containing about 0.75 L of a soil-sand mixture (3:1). For the melissa plants, due to their vigorous growth, round pots (Ø = 15 cm) containing about 1.5 L of the soil-sand mixture were used.

For the mulching, inanimate plant material of ragwort (*Senecio jacobaea*) was used. This was obtained as follows: aerial parts of mature flowering *S. jacobaea* plants were dried in a lab oven at 60 °C for three days. Subsequently, the dried material was milled in a blade grinder. In order to prevent any contamination by dust, the material was moistened before being applied carefully to the soil surface of the potted plants to avoid any direct contact of the mulch with the plants. In the case of melissa, 2 g of the powdered *Senecio* material was applied per pot, whereas the mulching of peppermint, chamomile, and parsley plants was performed using 1 g of the material. Subsequently, the mulched soil was covered with two layers of filter paper. The plants were watered every 2 days by pouring water thoroughly on the filter paper. The plants were harvested prior to the mulching (control) and 7 or 14 days after mulching. To boost the putative alkaloid uptake, in one third of the treated plants, mulching was repeated after 7 days. Seven days later, the plants were harvested (i.e., 14 days after the first mulching procedure).

2.2. Sampling

For each sample, the leaves of a distinct number of individual plants were pooled (5 for chamomile, 6 for melissa, and 7 for peppermint and parsley). However, the lower leaves (up to 5 cm) had been excluded in order to avoid any contamination by a direct contact of the leaves to the filter paper. For each time, 3 independent samples (each consisting of the pooled leaves of 5–7 plants) had been taken. In addition, control plants were cultivated without any mulching. Overall, about 60–80 plants had been harvested for the corresponding analyses. Directly after harvest, the leaves were shock-frozen in liquid nitrogen and then ground using a mortar and pestle and subsequently freeze-dried.

2.3. Chemicals and standards

Formic acid (suprapur) and acetonitrile (LC-MS grade) were obtained from VWR (Darmstadt, Germany). The following PA standards were purchased from PhytoLab (Vestenbergsgreuth, Germany): erucifoline, erucifoline *N*-oxide, jacobine, jacobine *N*-oxide, retrorsine, retrorsine *N*-oxide, senecionine, senecionine *N*-oxide, seneciphylline, seneciphylline *N*-oxide, senecivernine and senecivernine *N*-oxide.

2.4. Quantification of PAs

The lyophilized plant material was ground to a fine powder. The final particle size was about 200 μm. Two grams of these samples were extracted with 25 mL formic acid (2%), using ultrasonication. The obtained solution was directly used for analysis. Analysis were performed on a Agilent 1290 system (Agilent, Waldbronn, Germany), in combination with an electrospray ionisation tandem mass spectrometer (Sciex, Darmstadt, Germany). Chromatography was carried out on a 150 × 2.0 mm C18 reversed phase column, particle size 3 μm with an alkaline water-acetonitrile gradient at a flow rate of 300 μL/min and 10 μL injection volume. PAs were analysed in the positive ion mode. Two specific ion transitions (multi reaction monitoring mode) were recorded for each PA (Table 1). The identified PAs were quantified with external calibration.

3. Results and discussion

3.1. PA-uptake and translocation into the shoots

The plants had been mulched with dried plant material of *Senecio jacobaea*, which revealed about 3200 mg/kg of total PAs. When the plants were mulched with 1 g of this material, 3.2 mg PAs were added to each pot. One week after application, every mulched plant exhibited marked concentrations of PAs (Fig. 1a–d), whereas the untreated controls were totally free of these alkaloids. These results demonstrated that PAs indeed are leached out from the dried *Senecio* material into the soil and confirm that these alkaloids are taken up by the roots of experimental plants and are translocated into the leaves. Obviously, like nicotine, pyrrolizidine alkaloids could also be transferred by the means of a *horizontal transfer of natural products* from one plant species into another; however the extent of alkaloid import varies markedly. Whereas the maximal levels of PA concentrations in leaves of peppermint, melissa and chamomile are about 0.1–0.15 mg/kg dry weight, it is 3–5 times higher in parsley (>0.5 mg/kg). Assuming a passive import of PAs via a simple diffusion across biomembranes (see below), the observed variations should not be due to fundamental

Table 1
Measurement parameters for LC-MS/MS analysis.

Pyrrolizidine alkaloids	tR (min)	Ion transitions	
		Q1	Q3
Erucifoline	12.8	349.9	120.1
		349.9	67.1
Erucifoline N-oxide	9.1	365.9	119.1
		365.9	94.1
Jacobine	13.1	351.9	120.1
		351.9	77.0
Jacobine N-oxide	9.8	367.9	296.2
		367.9	120.2
Retrorsine	13.0	352.2	138.2
		352.2	67.0
Retrorsine N-oxide	10.2	368.3	94.2
		368.3	120.0
Senecionine	14.9	336.3	120.0
		336.3	94.1
Senecionine N-oxide	11.3	352.2	94.1
		352.2	136.2
Seneciphylline	14.0	334.3	120.2
		334.3	94.1
Seneciphylline N-oxide	10.6	350.2	94.2
		350.2	119.1
Senecivernine	15.7	335.9	120.2
		335.9	308.3
Senecivernine N-oxide	11.7	351.9	94.1
		351.9	119.1