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Horizontal Natural Product Transfer: The Origin of the Widespread Alkaloidal Contaminations of Herbal Products

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Abstract

Alarmed by numerous worrying reports by the European Food Safety Authority related to widespread contaminations of plant-derived commodities by poisonous alkaloids (nicotine, pyrrolizidine alkaloids), the sources of these alkaloidal contaminations were investigated. Our studies revealed that alkaloids, which have been leached out from decomposing alkaloidal donor plants, are taken up by the roots of acceptor plants growing in their vicinity. Based on these results the so-called *"Horizontal Natural Product Transfer"* was appointed. By now, it becomes evident that plants per se take up many other natural products, such as coumarins or stilbenes, from the soil. After the import by the roots, the compounds are allocated into the leaves. Actual research unveiled that alkaloids are also transferred from vital donor plants into plants growing in their vicinity. Furthermore, it was demonstrated that in various acceptor plants, the imported natural products are just accumulated, whereas, in others, they are modified. In the same manner, as it is known for xenobiotics these modifications comprise hydroxylation, methylation, and glucosylation processes. Indeed, in the past, these reactions had been considered as part of a deliberate detoxification mechanism of xenobiotics, the so-called "*green liver concept*". However, when taking into account that the mode and extent of these modifications massively vary among different plant species, a universal or general detoxification system as proposed by the "*green liver concept*" has to be excluded. This, however, would mean that the manifold observed modifications of typical xenobiotics could also be due to accidental reactions catalyzed by enzymes ordinarily responsible for the specialized metabolism of particular plant species. In addition to its relevance in preventing contaminations of plant-derived commodities, *"Horizontal Natural Product Transfer"* will also change our basic understanding of plant-plant interactions.

Keywords: Horizontal Natural Product Transfer; Specialized Metabolites, Natural Products; Pyrrolizidine Alkaloids; Alkaloids; Coumarins; Allelopathy; Xenobiotics

Introduction

In the recent past, it became obvious that a huge number of plant-derived commodities contain toxic alkaloids. Various alarming reports from the European Food Safety Authority (EFSA) expounded that many herbal products, herbal medicines, and phytopharmaceuticals are contaminated by nicotine [1], pyrrolizidine alkaloids [2-4], and tropane alkaloids [5]. In this context, the EFSA [1] unveiled that more than 70% of the tested herbal tea and spice samples contained nicotine significantly above the allowable limit. In the same vein, Mulder et al. [6] reported that pyrrolizidine alkaloids (PAs) are present in more than 90% of all herbal tea samples tested. First and foremost, because of their high toxicity for livestock, wildlife, and humans [7, 8], PAs - even in low concentrations - are of special concern when present in plant-derived commodities. Although the genuine PAs are not toxic, their related 1,2,-unsaturated derivatives, which are generated in the liver of vertebrates, are highly poisonous [8]. The corresponding toxicological data revealed a wide range of toxic effects: hepatotoxicity, developmental toxicity, genotoxicity and carcinogenicity. Moreover, some also exhibit pulmonary toxicity [2]. In the same manner, low amounts of tropane alkaloids occurring in foods had been shown to be toxic. Accordingly, an acute reference dose of 16 ng/ kg bw was established by the EFSA CONTAM panel [5].

Indeed, most notably, concerning the widespread contaminations by PAs, it was rapidly argued that they might be due to accidental co-harvest of alkaloid-containing weeds [9, 10]. Yet, when considering that nicotine-containing weeds are very rare and restricted in their occurrence, a corresponding origin of the nicotine contaminations could be excluded. In consequence, there must be another path of contamination for this alkaloid. Based on literature published more than half a century ago [11, 12] it seemed to be evident that the alkaloidal contaminations of the related staple plants are – at least in part – due to an uptake from the soil [13 - 15]. Up to recently, various reports had been published outlining the transfer of alkaloids between various donor and acceptor plants (Table 1).

Table 1: Transfer of Alkaloids Via Soil Between Various Donor and Acceptor Plants

Donor plant	Type of alkaloid	Acceptor plant	Authors
Chromolaena odorata	Pyrrolizidine alkaloids	*Zea mays*	[16]
Nicotiana tabacum	Nicotine	*Mentha× piperita*	[17]
Nicotiana tabacum	Nicotine	*Coriandrum sativum*	[18]
Nicotiana tabacum	Nicotine	*Mentha* x *piperita*	[18]
Nicotiana tabacum	Nicotine	*Ocimum basilicum*	[18]
Nicotiana tabacum	Nicotine	*Petroselinum crispum*	[18]
Secale cereale	Benzoxazinoids	*Vicia villosa*	[19]
Senecio jacobaea	Pyrrolizidine alkaloids	*Matricaria chamomilla*	[20]
Senecio jacobaea	Pyrrolizidine alkaloids	*Melissa officinalis*	[20]
Senecio jacobaea	Pyrrolizidine alkaloids	*Mentha* x *piperita*	[20]
Senecio jacobaea	Pyrrolizidine alkaloids	*Petroselinum crispum*	[20]
Senecio jacobaea	Pyrrolizidine alkaloids	*Melissa officinalis*	[21]
Senecio jacobaea	Pyrrolizidine alkaloids	*Petroselinum crispum*	[21]
Senecio spec.	Pyrrolizidine alkaloids	*Aspalathus linearis*	[22]
Solanaceae	Atropine	*Triticum aestivum*	[23]

In this context, the presence of alkaloids in the soil could have two possibilities: either they are leached out from rotting plant material or exuded from living alkaloid-containing weeds growing in the vicinity (see below). Meanwhile, the legacy data on the uptake of various alkaloids from the soil by plant roots are verified [24, 25]. Based on these insights, the so-called *"Horizontal Natural Product Transfer"* was

discovered and formulated, respectively [13, 14, 26, 27]. In this paper, the scientific background of this widespread phenomenon is outlined and its relevance for the contamination of plant-derived commodities is discussed. In addition, the analogies of the *"Horizontal Natural Product Transfer"* with uptake and modifications of xenobiotics in the proper sense are outlined and discussed.

The Uptake of Solutes from the Soil

The roots of plants take up a wide variety of substances from the soil. Because of their ionic character, the uptake of inorganic nutrients, such as nitrate, sulfate, or metal ions, requires specific transporters (e.g., Kobayashi and Nishizawa [28]). In contrast, most of the organic compounds diffuse passively into the root cells. However, inevitable preconditions for such simple diffusion through the plasmalemma of the root cells are appropriate physicochemical properties, i.e., the substances must be soluble in aqueous as well as in organic solvents. Research on the uptake of xenobiotics unveiled that the ability for such passive membrane transfer can roughly be deduced from the so--called K_{OW} value [29]. This parameter represents the distribution coefficient of a certain substance between octanol and water. Generally, in the related literature its decadal logarithm, i.e., pK_{OW}, is displayed [30], which is frequently also denominated as log*P* [31]. It is well accepted that all substances exhibiting log*P* values between -1 and 3 (at times up to 4) are generally able to diffuse easily through biomembranes [32, 33]. Although these coherences had been elaborated for the uptake of xenobiotics, they self-evidently also apply to all organic substances. In this respect, Hurtado et al. [34] documented that organic compounds exhibiting appropriate log*P* values, so-called *"emerging organic contaminants",* indeed are generally taken up by plants. Thus, there is no doubt that also all plant-derived natural products, revealing an appropriate log*P* value, will be taken up by plants when present in the soil.

Up to recently, in plant biology, the uptake of plant-derived natural products had only been considered for so-called allelochemicals, i.e., those plant-derived substances, which affect germination or growth of putative competitors. These active compounds are frequently exuded from donor plants and exhibit their effects on plants growing in the vicinity [35, 36]. To exhibit their inhibitory effect, the allelochemicals have to be taken up into the acceptor plants. Such import is well documented for systemic herbicides or fungicides [29, 37]. After their import into the roots, the compounds are generally translocated into the shoots [32, 38, 39]. Although the import of xenobiotics and allelochemicals was well established, an analogous uptake of typical plant-derived natural products was not taken into consideration. This is really surprising since there are various reports outlining that secondary metabolites - nowadays denoted as specialized metabolites - are leached out from rotting plant materials [40-42]. Indeed, the situation drastically changed when the origin of the widespread contamination of plant-derived commodities was investigated.

The phenomenon "Horizontal Natural Product Transfer"

Based on the coherences mentioned above, it was obvious that the widespread alkaloid contaminations of plant-derived commodities are due to the uptake of the alkaloids from the soil. In order to verify this assumption, tobacco leaf material was applied to test plants. These pot experiments expounded that high amounts of nicotine were leached out from the rotting tobacco material and subsequently accumulated in the acceptor plants [17]. This transfer of nicotine was vividly confirmed in corresponding field experiments by discarded cigarette butts on the acreage of various crop plants [18]. It turned out that only one cigarette butt per square meter suffices to generate nicotine concentrations in the crop plants which exceed the limit value set by the EU by the factor ten [18]. In the same manner, pyrrolizidine alkaloids (PAs), which had been leached out in the soil from rotting PA-containing weeds, i.e., *Senecio* jacobaea, are also taken up and accumulated in the acceptor plants [16, 20, 21]. These findings clearly show that the uptake of alkaloids from the soil – at least in part – is responsible for the widespread alkaloidal contaminations of plant-derived commodities. Hereafter, the uptake of many other alkaloids by plant roots has been demonstrated [24, 25]. Yet, not only alkaloids but also other specialized metabolites, belonging to various other groups of natural products, i.e., simple phenols [43, 44], coumarins [45], stilbenes [46], aristolochic acids [47, 48] or betalains [27] are imported by plant roots. Based on these insights and coherences, the concept of the *"Horizontal Natural Product Transfer"* was established and refined [13, 15, 17, 27, 49]: plants decompose after their death, and in consequence, their natural compounds, previously accumulated in these donor plants, are leached out into the soil, from which they are taken up passively by the roots of other plants growing in the vicinity. However, a precondition for such uptake is the ability of the substances to pass the plasmalemma of root cells. As already mentioned, all substances exhibiting a log*P* value in the range between minus one and roundabout three can diffuse across biomembranes [32, 33]. In contrast to the import of most ionic nutrients like nitrate, sulfate, or metal ions, which requires specific carriers [28], no transporters are involved in the uptake of respective natural products. Accordingly, the decision of whether or not a natural product is taken

up from the soil does not depend on the plant species but only on the physicochemical properties of the compound. This set of facts was confirmed by various investigations [24, 25] which show that all alkaloids revealing appropriate log*P* values are taken up by plants (Table 2).

Table 2a: Log*P* Values of Alkaloids Taken Up By Plant Roots

	log*P* / pK_{ow}	type	literature
anabasine	1.1	pyridine alkaloids	[12]
atropine	1.6	tropane alkaloid	[11, 12, 24]
berberine	-1.0	isoquinoline alkaloid	[11, 12]
caffeine	-0.1	purine alkaloid	[11, 12, 24]
cinchonine	2.2	quinoline alkaloids	[12]
codeine	1,3	isoquinoline alkaloid	[11, 12]
colchicine	1.5	colchicine alkaloids	[12]
cytisine	1.0	quinolizidine alkaloid	[12]
harmaline	2.7	indole alkaloid	[25, 50]
harmine	2.8	indole alkaloid	[25, 50]
hyoscyamine	1.8	tropane alkaloid	[11, 12]
jacobine	0.2	pyrrolizidine alkaloid	[20]
monocrotaline	-0.8	pyrrolizidine alkaloid	[50]
morphine	0.9	isoquinoline alkaloid	[11]
narcotine	2.3	isoquinoline alkaloid	[12]
nicotine	1.1	pyridine alkaloids	[11, 12, 26, 51]
noscapine	2.5	isoquinoline alkaloid	[24]
papaverine	3.5	isoquinoline alkaloid	[11, 12, 24]
pilocarpine	1.1	imidazole alkaloids	[12]
quinidine	2.7	quinoline alkaloids	[11, 12]
scopolamine	0.9	tropane alkaloid	[11, 12]
seneciphylline	1.0	pyrrolizidine alkaloid	[20]
sparteine	2.5	quinolizidine alkaloid	[12, 50]
strychnine	1.5	indole alkaloid	[12, 24]
thebaine	1,9	isoquinoline alkaloid	[12]
theobromine	-0.7	purine alkaloid	[11, 12, 24]
theophylline	-0.04	purine alkaloid	[11, 12, 24]
vincamine	3.6	indole alkaloid	[50]

Table 2b: Log*P* Values of Phenolic Compounds Taken Up By Plant Roots

	log*P* / pK_{OW}	type	literature
aristolochic acid	2.0	phenanthrene	[47]
catechol	1.0	catechols	[43]
hydroquinone	0.8	catechols	[43, 52]
phloroglucinol	0.5	catechols	[43, 52]
phenol	1.5	phenol	[44]
resorcinol	0.9	catechols	[43]
resveratrol	2.5	stilbene	[14, 45]
umbelliferone	1.5	coumarin	[45]

The data of log*P* were compiled and averaged from the databases "BioLoom," "ChemSpider," "chemicalize.org," and "ToxNet" as mentioned by [50]. In addition, the first studies on the uptake of phenolic compounds expounded the general validity of the entire issue. Since the process responsible for the import into the roots represents a quite general mechanism, a corresponding import is not restricted to some specialized plants but concerns all. In other words: all plants act as acceptors. In the same manner as known for xenobiotics [32, 33] also the imported natural products are translocated via xylem into the leaves [27, 53].

When considering the prerequisites for the uptake of alkaloids, in addition to the log*P*, a further factor, i.e., the pH has to be considered [50]. It is well known that the alkaloids in neutral or alkaline media - depending on their pKa values – exist as free bases, whereas they are extensively protonated in acidic solutions. In consequence, due to the positive charge, the protonated alkaloids exhibit a massively enhanced hydrophilicity, and their solubility in organic solvents is strongly decreased (Figure 1).

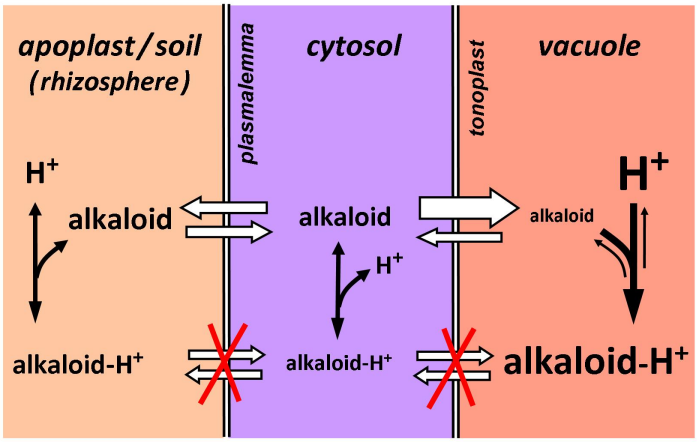

Figure 1: Ion trap mechanism according to Matile [54] modified by Hijazin et al. [50]: Whereas the free alkaloid bases easily diffuse across biomembranes, the protonated ones cannot. Thus, determined by differences in the degree of protonation in various media, the alkaloids are trapped in the most acidic compartment.

Accordingly, protonated alkaloids cannot cross biomembranes passively [53]. This effect and the corresponding consequences, for the distribution of alkaloids among cell compartments exhibiting different pH values, were originally outlined by Matile [54] and recently verified by [55]. This phenomenon, denoted as *"ion trap mechanism"*, plainly explains the passive accumulation of alkaloids in the acidic vacuole: within the neutral cytosol, a high share of alkaloids are present as free bases and thus are membrane permeable. In contrast, in the acidic vacuoles, most alkaloid molecules are protonated and consequently not able to cross membranes. As a result, within the vacuole, the actual concentration of the unprotonated alkaloids is drastically diminished, and the high concentration gradient between cytosol and vacuole is generated. This gradient causes a steady import of alkaloids into the acidic vacuole, where – due to their protonation - they are

accumulated and trapped [54]. In the same manner, differences in pH values between the rhizosphere and root cells determine the rate of influx into the root cells (Figure 2). In consequence, apart from the log*P* also the pKa values of the alkaloids have to be taken into account when evaluating the probability of their uptake [50].

When assessing the putative intensity of the uptake, in addition to the physicochemical properties of the compound, various other factors have to be considered. In this context, all phenomena are relevant, which influence the actual concentration gradient between the rhizosphere and root cells and thereby impact the uptake velocity and its extent. Above all, the properties of the soil have to be considered, for review see Miller et al. [56] as well as the capacity of soil microorganisms to degrade the relevant natural compounds (Figure 2).

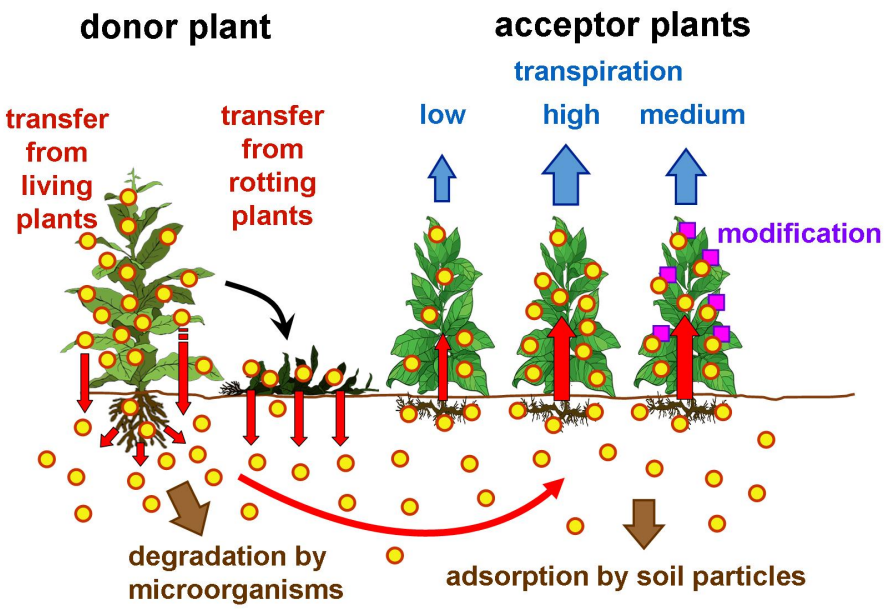

Figure 2: Horizontal natural product transfer. The scheme displayed by Selmar et al. [13] is supplemented with the impacts of transpiration and rhizosphere.

It is a matter of fact that various soils exhibit quite different abilities to adsorb alkaloids and other natural compounds on the soil particles [57]. Accordingly, in the soils that feature a high capability for absorbing and binding the natural products, their actual concentration is far lower than in those which are lacking this property. Consequently, the uptake, which directly depends on the concentration gradient between the rhizosphere and root cells, is massively determined by the related soil properties. Unfortunately, up to now, only limited information on this issue is available. Further investigations are required, which will facilitate the understanding of the complex interactions of the various natural products and distinct soils to predict their impact on the horizontal transfer of natural products.

Furthermore, the relevant concentration gradient could strongly be influenced by microbial degradation of the natural compounds. There is no doubt that many microorganisms efficiently degrade natural products including alkaloids and other specialized metabolites and use them as carbon sources. Already half a century ago, Winter et al. [58] showed that esculin, which was applied to soils, is cleaved. In this sense, *Pseudomonas* degrades atropine [59] and *Serratia marcescens* can metabolize caffeine and related methylxanthines [60]. Furthermore, Wang et al. [61] reported that nicotine is degraded by various *Pseudomonas* species. Moreover, pyrrolizidine alkaloids are metabolized by a wide variety of different microorganisms [62]. In other words, when addressing the uptake of specialized metabolites from the soil, their potential degradation by microorganisms has to be considered appropriately.

Notwithstanding, the concentration gradient between the rhizosphere and the root cells also depends on the actual concentration of the substance within the cells. In other words, its import is increased by diminishing its actual concentration within the root cells. In this context, the translocation of the imported substances from the roots into the shoots is of particular interest. Based on pressure chamber techniques, it was shown that xenobiotics are transferred from the roots into the shoots via the xylem [32, 63]. In the same manner, also for alkaloids, which had been taken up from the soil, a xylem-based translocation is verified [25, 53]. However, in this context it has to be considered that the acidic vacuoles impair the extent of alkaloids translocated. Allocation via xylem is mainly driven by transpiration [64]. In

consequence, the concentration of imported substances within the root cells - and thus the velocity of its overall uptake – is determined by the extent of transpiration. Yet, this property varies greatly among different plant species. Thus, although the passive import of the alkaloids across the plasmalemma is identical in all plants, its extent indeed depends also on the plant species. In this sense, a higher transpiration rate entails a faster allocation via xylem, and thereby enhances the alkaloid uptake from the soil (Figure 2).

In addition to allocation, the actual concentration of imported substances is significantly influenced by their biotransformation. Mid of the last century, Winter et al. [52] already reported that hydroquinone and phloroglucinol, which had been taken up by wheat plants, are subsequently glucosylated to yield arbutin and phlorin, respectively. Up to now, in particular, concerning the modification of imported xenobiotics, numerous information is available [65 - 67], and - in analogy - also the imported plant specialized metabolites are modified in the acceptor plants [25, 45]. Details of this fascinating issue are outlined below in the respective chapter. Yet, one of the most surprising results of this issue concerns strong heterogeneity of the modifications, e.g.: in barley seedlings, the imported umbelliferone is methoxylated to yield scopoletin and in garden cress, it is converted to esculin. In contrast, in flax, pea, or radish, no modification was observed at all [45]. These differences vividly outline that – in addition to variation in the allocation via xylem - also differences in the extent of modification of the imported substances, may strongly depend on the plant species. Accordingly, the actual concentration of a substance in the root cells and thereby the extent and velocity of its uptake from the soil is massively differing among plant species. In consequence: although the basic mechanism, i.e., the diffusion across the plasmalemma of the root cells is identical for all the different plant species, in various acceptor plants, massive differences in the extent and the velocity of uptake and accumulation could result (Figure 2). This nexus was nicely confirmed by corresponding variations in the extent of the accumulation of pyrrolizidine alkaloids in various acceptor plants, i.e., parsley, melissa, chamomile, peppermint, and nasturtium [21].

Transfer from Living Donor Plants

Based on our insights into allelopathy, it is well known that active compounds, which inhibit germination or growth of potential competitors, are released from various plants [68, 69]. In particular, there are several options for how these so-called allelochemicals are introduced into the environment. The active compounds can be exuded from living plants, either by their roots [35, 36] or by their leaves [70, 71]. Alternatively, the substances can be leached out from decomposing plant residues [72]. Accordingly, also concerning the phenomenon of horizontal natural product transfer, it seems to be evident that - in analogy to allelochemicals - also specialized metabolites might be released in the same manner from living donor plants. This assumption is supported by the fact that numerous of these natural products can diffuse - at least to a certain extent - through biomembranes. For verification that specialized metabolites are transferred between vital neighbouring plants, various co-culture experiments had been conducted. When *Senecio jacobaea* plants that contain high concentrations of pyrrolizidine alkaloids (PAs) were co-cultivated in the same pot together with various acceptor plants for several weeks, the *Senecio*-PAs indeed were also present in the non-alkaloidal acceptor plants [21, 27]; the corresponding PA-concentration was more than 200 µg PAs/kg d.w. Yet, due to intensive contact between the roots of donor and acceptor plants in pot experiments, a direct transfer via so-called root grafts [73] could not be excluded. Accordingly, co-culture experiments had been re-conducted under field conditions by cultivating plants of numerous species as potential acceptor plants in different spatial distances to the PA-containing *Senecio* donor plants (for experimental details see [20]). It became obvious that after several weeks of co-cultivation, the *Senecio*-PAs could indeed be detected in every plant [20], which genuinely did not contain these alkaloids. In the same manner, as outlined and explained for the experiments, in which various pure substances had been applied to different acceptor plants (see above), also in the related field experiments the PA concentrations varied markedly among the different acceptor plants [20]. All in all, these results clearly verify that the PAs, which originally had been synthesized and accumulated in the *Senecio* donor plants, were indeed transferred into the various acceptor plants.

As already mentioned for allelochemicals, basically there are several options for the introduction of natural products into the soil. First of all, it could be argued that the observed PA-transfer between living plants could be due to the shedding of *Senecio* leaves, from which the PAs then have been leached out. Indeed, in principle, this option particularly cannot be fully ruled out. However, in the course of the related pot experiments hardly any abscission of leaves was observed [21]. Accordingly, at least in the *Senecio*-experiments, this possibility could substantially be excluded. However, when considering an entire vegetation period, especially when the senescence-induced shedding of leaves takes place, this option certainly is relevant. Alternatively, the alkaloids could have been bled out from minor leaf injuries, especially from those generated in the course of herbivore or pathogen attack. However, the plants employed in the described experiments were healthy and no herbivory attack had been observed. Accordingly, plain bleeding of PAs from injured leaves seems to be unlikely,

too. This assessment is underlined by comparing the PA-spectra of donor and acceptor plants. Since these spectra are quite different, wound-induced bleeding can be ruled out. In such a case, all the various PAs present in the donor plant would have been leached out in the same manner and subsequently taken up by the acceptor plants to the same extent. The same coherences apply to wound-induced bleeding from putatively injured roots. In conclusion, it has to be assumed that the PAs are indeed released from vital and healthy *Senecio* donor plants. Again, there are two possibilities, i.e., an active exudation by the roots (or shoots) or a passive diffusion out of the cells. Unfortunately, up to now, it cannot reliably be answered, which one of these options is responsible for the PA transfer from the *Senecio* donor plants into the various acceptors. Yet, in this context, it is relevant to outline that ptaquiloside, a carcinogenic phytotoxin produced by *Pteridium aquilinum*, indeed passively diffuses out of the leaves of this fern [74]. According to their analogous ability to diffuse through biomembranes, all alkaloids and other specialized metabolites, which exhibit appropriate lop*P* values, could also be passively leached out either from the leaves or released by the roots into the soil. However, we have to consider that plant cells exhibit an efficient trapping system. The most famous is certainly the so-called *"ion trap mechanism"* [54, 55], which already is mentioned above: due to the strong acidity of the vacuoles, the alkaloids are protonated and in consequence, are not able to diffuse through the tonoplast anymore (Figure 1). Accordingly, they are trapped and accumulated in the vacuoles. However, these coherences are only valid for alkaloids exhibiting high pKa, which ensure ample protonation in the physiological pH range. The situation is different for all alkaloids revealing a very low pKa, e.g. caffeine (pKa = 0.7). Hence, caffeine - even in the acidic milieu of the vacuole - is not protonated. Consequently, the alkaloid would not be retained within this compartment, because the positive charge, which typically prevents membrane permeability, is lacking. Nonetheless, caffeine is trapped in the vacuole due to an alternative mechanism, i.e., an effective complex formation with chlorogenic acids [75]. Analogous to the protonated alkaloids, also the chlorogenic acid-caffeine-complex is not able to pass biomembranes passively, and thus, it is retained in the vacuole.

Most of the literature describing the release of alkaloids is based on organ or cell culture experiments. Bais et al. [76] reported that harmine and harmaline are exuded from root culture cells of Oxalis tuberosa. In the same manner, Ruiz-May et al. [77] showed that the indole alkaloid ajmalicine is secreted from cells of Catharanthus roseus hairy root cultures. Moreover, nicotine was found in the medium of root cultures from Nicotiana tabacum [78]. Indeed, based on the ability to diffuse across biomembranes, an occurrence of alkaloids in the medium is not a proof that they are actively exuded into the medium. However, Toppel et al. [79] showed that the alkaloid spectrum within the *Senecio* root culture cells is quite different in comparison to that of the culture medium, i.e., whereas a wide variety of PAs are present within the cultured cells, in the medium, nearly exclusively senkirkine was present. This difference could only be explained by an active and specific exsudation of senkirkine into the culture medium [79]. It is self-evident that such a process requires specific transporters, which enable the transfer of the protonated alkaloids out of the vacuole. In this context, it has to be mentioned that numerous transporters for alkaloids are described; e.g. [80 - 82]; for review see Yazaki et al. [83]. It is sad to say, in most of the reports dealing with membrane transfer of alkaloids, their ability to easily cross biomembranes is ignored, as well as the fact that membrane transfer of protonated alkaloids indeed requires transporters.

In contrast to the release of alkaloids into the medium of organ and cell cultures, only a few data are published which expound that alkaloids are liberated from genuine plants into the soil. Schulz et al. [84] reported that roots of Agropyron repens exude dihydroxybenzoxazinone into the soil. In the same manner, roots from *Oxalis tuberosa* release the carboline alkaloids harmine and harmaline into the soil [85], and roots of coffee seedlings exude caffeine [86]. The assumption that the release of alkaloids from roots is a quite general feature is supported by findings that alkaloids of various plants were detected in the soils, in which alkaloidal plants are grown: quinolizidine alkaloids (lupine alkaloids) are present in the soils overgrown with narrow-leaf and yellow lupines [87], and PAs were detected in the soils, in which the PA-containing *Senecio* plants were growing [88]. Indeed, when discussing the origin of these alkaloids, the same questions as already mentioned above arise, i.e., do these alkaloids result from the leaching of the shed or injured leaves or are they exuded from vital tissues? Furthermore, it has to be asked whether the related release is due to an active exudation or a passive diffusion. Although the actual path of the PA transfer from living donor plants into acceptor plants growing in the vicinity is still not identified, the co-culture experiments mentioned above [21] unambiguously display that specialized metabolites, i.e., pyrrolizidine alkaloids, indeed are transferred from vital donor plants to acceptor plants growing in the vicinity. Consequently, the original concept of horizontal natural product transfer had to be expanded by including the transfer from vital plants (Selmar et al. [21], Figure 2). However, much more research is required to soundly elucidate the exact path of alkaloids release into the soil.

The Imported Substances Could Be Modified in the Acceptor Plants

When half a century ago, Winter et al. [52] studied the uptake of hydroquinone and phloroglucinol by wheat and bean seedlings, the authors found that the imported phenolic compounds are glucosylated in the acceptor plants yielding arbutin and phlorin. Unfortunately, this knowledge got off the scientists' minds and was not considered later on, especially when the fate of imported xenobiotics had been analyzed. Meanwhile, it is well established that xenobiotics, which are taken up from the soil, could be modified within the acceptor plants. These corresponding modifications comprise oxidations, hydroxylations, and glucosylations [66, 89]. It is postulated that these reactions represent an efficient detoxification system for xenobiotics, denoted as the "*green liver concept*" [90]. Indeed, the manifestation and inherent relevances of such a fundamental detoxification system have to be challenged (see below), nonetheless, imported xenobiotics are modified in various plants. Thus, it was obvious that also the natural compounds, imported by the roots, could be modified within the acceptor plants in the same manner. Apart from the legacy data on the glucosylation of imported phenolic compounds [52], the first clue for corresponding modifications of alkaloids was expounded by the finding that the concentration of nicotine and pyrrolizidine alkaloids (PAs) in the acceptor plants strongly decreased by the time [17, 20]. In the case of PAs, the initial studies had been based on the standard HPLC quantifications by summing up the contents of all genuine alkaloids. Thus, potential derivatives could not be detected and quantified. However, when the quantification of PAs in the acceptor plants was performed by the so-called *"sum-parameter"* method [91], instead of a time-dependent decrease a continuous increase of alkaloids in the acceptor plants was evident. Since the sum-parameter method is based on a HPLC-ESI-MS/MS determination of the necine base, all PA-related structures, and thereby, also modification products of the genuine PAs, will be estimated. The comparison of the data from both approaches, i.e., a decrease of genuine PAs (determined by classical HPLC) and an increase of all substances revealing a necine base (quantified by the sum parameter method) unequivocally verified that the imported PAs indeed had been modified within the various acceptor plants [13]. It became obvious that more than two-thirds of the imported PAs had been modified to so far unknown derivatives [14]. Unfortunately, up to now, any clues to the related modification products are lacking as well as solid information on the potential toxicity of these unknown derivatives. Much more research is required to evaluate reliably the risk of the related PA contaminations. Although the reactions involved in the modification of PAs are not yet elucidated, it became obvious that - as known for xenobiotics - also alkaloids, which are imported, are modified to a large extent in the acceptor plants. Nonetheless, it has to be assumed that most of the modified PAs reveal an identical toxicity, since PAs commonly are converted in the liver of vertebrates to their related highly toxic 1,2,-unsaturated derivatives [8].

The main reason for the problems in isolation of the PA-metabolites is in particular related to their weak UV absorbance, which hampers their simple identification in the course of any HPLC separation. Consequently, it would be far more promising to study the putative modifications of imported natural compounds when such substances are applied, and their putative derivatives are more feasible to detect. In this respect, coumarins are quite promising, since the genuine substances as well as most of their derivatives could easily be detected due to their fluorescence [92]. By applying umbelliferone (log*P* = 1.5) Hijazin et al. [45] exemplarily studied the uptake of a typically specialized metabolite and its subsequent modification in various acceptor plants. As expected, all acceptor plants took up umbelliferone by their roots and translocated it into the leaves. Surprisingly, the further fate of the imported coumarin was quite different in the various plant species. Whereas umbelliferone was just accumulated in flax, pea, or radish seedlings, it was efficiently modified in barley and garden cress. However, the related modifications differed strongly. Whereas the imported umbelliferone was hydroxylated and glucosylated to yield esculin in garden cress (Figure 3), it was converted in barley seedlings by methoxylation to scopoletin [45]. Such modifications are known to be responsible for the modification of imported xenobiotics [66, 89].

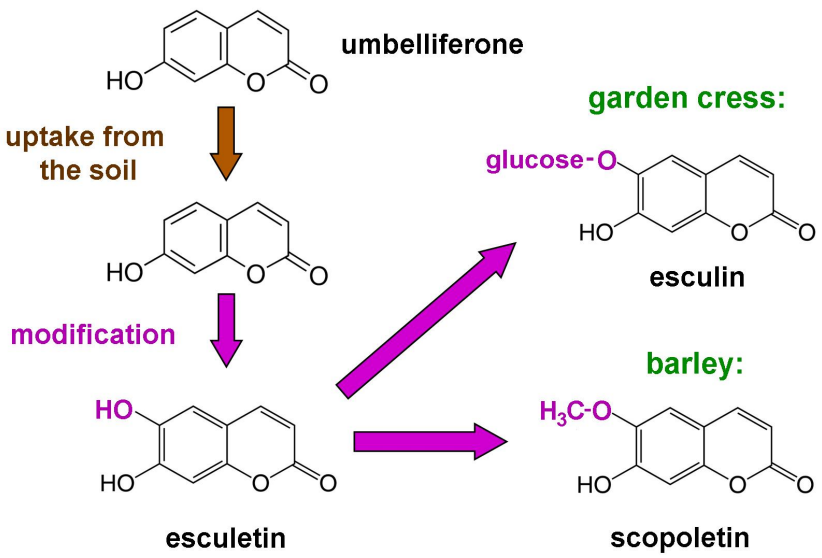

Figure 3: Modification of imported umbelliferone in barley and garden cress seedlings according to Hijazin et al. [45].

It is well established that these related oxidative reactions are frequently catalyzed by cytochrome P450 enzymes [93, 94]. To ascertain that the conversion of umbelliferone in barley and garden cress is also catalyzed by P450 enzymes, in an additional approach naproxen, a well-known inhibitor of P450 enzymes [95] was applied to the barley seedlings together with umbelliferone. The simultaneous application of umbelliferone and the P450 inhibitor strongly suppressed the conversion of the coumarin to both derivatives [45]. These results expound that – in analogy to the modification of xenobiotics – the conversion of umbelliferone to scopoletin in barley as well as to esculin in garden cress is also catalyzed by cytochrome P450 enzymes. Further evidence that specialized metabolites are modified within the acceptor plants was elaborated by Lewerenz et al. [25] who demonstrated that harmaline is oxidized to harmine in barley acceptor plants. All in all, it had to be stated that the concept of horizontal natural product transfer had to be broadened by including putative modifications of imported substances as outlined in Figure 2 according to Selmar et al. [13]. However, it has to be acknowledged that the extent and manner of modifying the imported coumarins massively differ between the various plant species.

As already outlined for the consequences of potential differences in the rate of transpiration, also variations in the ability to modify the imported substances will impact the actual concentration of the imported compounds within the roots. In consequence, the velocity and the extent of uptake might depend on the plant species although the basic principle, i.e., the passive diffusion across the plasmalemma is identical in all plants.

Species-related differences in related modifications are also described for typical allelochemicals. The isoflavone Biochanin A, produced in various clover species acts as a potent growth inhibitor for potential competitors. In different weed species, this allelochemical is converted diversely to yield various derivatives [96]. In the same manner, the ability to hydroxylate and glucosylate benzoxazolinone (a growth inhibitor released into the soil by numerous types of grass) varies markedly among different plant species [97]. The fact that the metabolic fate of imported natural products differs markedly among various plant species, demonstrates that the observed modifications cannot be part of a general and deliberate detoxification system as proposed for the modification of xenobiotics [66, 90]. On the contrary, these modifications seem to be due to accidental activities of enzymes, in particular those involved in the biosynthesis of specialized metabolites, which genuinely are present in the acceptor plants. Accordingly, enzyme promiscuity [98], gaining more and more attraction, seems to be responsible for the differential modification of natural products as well as of xenobiotics in the acceptor plants. Much more research is required for more clarification on whether the modifications of imported substances represent a specific "detoxification system" or are just catalyzed by *"side activities"* of promiscuous enzymes involved in the acceptor plant´s specialized metabolism.

Repercussions for Biochemical Ecology

The coherences presented here vividly demonstrate that the *"Horizontal Natural Product Transfer"* represents an endemic phenomenon,

which is far more spread than originally assumed. Accordingly, in prospective studies dealing with plant-plant interactions, this phenomenon has always to be considered. In other words, we have to realize that specialized metabolites synthesized in one plant species could be present in the soil, from which they are randomly taken up by other plants growing in the vicinity. However, inevitable preconditions for such exchanges are appropriate physicochemical properties of the substances, i.e., their log*P* value between -1 and about 3. When considering the vast number of specialized metabolites and the infinite number of permutations of donor and acceptor plants, it becomes obvious that the variation in the exchange of specialized metabolites is nearly unlimited. In consequence, for most of these exchanges, a certain or even specific ecological effect of this phenomenon can be ruled out - in categorical contrast to typical allelochemicals. There is no doubt that - based on their inhibiting impact on acceptor plants - allelochemicals exhibit a high relevance for the donor plant by suppressing potential competitors. In consequence, it is highly probable that the evolution of allelochemicals indeed was initiated by the random release of specialized metabolites. These coherences also apply to the various interactions with pathogens. Hence, the awareness of the phenomenon of *"Horizontal Natural Product Transfer"* will strongly influence our comprehension of chemical ecology, in particular concerning the evolution of specialized metabolites. All in all, far more research is required to comprehend the significance of this phenomenon for ecological biochemistry.

When reflecting on the entire issue of *"Horizontal Natural Product Transfer"* a particular scientific dissent gains center stage, i.e., the definition and meaning of "xenobiotics". In common usage, xenobiotics are considered as non-natural substances, being *"foreign to life"* [99]. Yet, the authors included in their definition already *"naturally occurring poisons"*. On the other side, in the last century, the natural substances taken up by the acceptor plants had been denoted as "allochthonous substances" [100]. As outlined in detail, the random uptake of specialized metabolites and their subsequent modification in the acceptor plants fully corresponds to the analogous processes described for xenobiotics. In consequence, either a re-evaluation of the classical definition of xenobiotics [13, 14, 15], or a strict differentiation between "non-naturally" and *"naturally derived compounds"* is required. In the first case, also specialized metabolites taken up by acceptor plants had to be included in the denomination of xenobiotics, i.e., xenobiotics would comprise all metabolites, which are foreign to the acceptor plants. In contrast, in the second case, the term xenobiotics would just comprise "non-natural" and would be contraposed to all substances generated by organisms, i.e., allochthonous substances, which adequately should be denoted as "allochtonics".

Moreover, in addition to the insights and reflections concerning basic science, the novel cognitions related to natural product transfer also reveal a high relevance for applied plant biology and agriculture. Indeed, up to now, this phenomenon explains away the alkaloidal contamination of food-derived commodities, however, it also might be the bases for increasing our understanding of various hitherto unexplained processes. In this regard, the release of specialized metabolites by donor plants into the soil might contribute explaining the beneficial effects of crop rotations or co-cultivation of certain vegetables.

Strategies to Prevent Contaminations Caused by Horizontal Product Transfer

As stated in the introduction, numerous alarming reports from the European Food Safety Authority unveiled that many plant-derived commodities are contaminated by toxic alkaloids [1-5]. In order to identify the origin of these pollutants, various research projects had been conducted. Meanwhile, it is verified that these contaminations-at least in part-are due to the horizontal natural product transfer. As a direct consequence of these insights, in the time to come a corresponding transfer of poisonous substances into crop plants should be markedly prevented. Indeed, in the case of PA contaminations, a major source of related contaminations is the accidental co-harvest of PA-containing weeds [9, 10]. Consequently, the farmers are urged to remove the PA-containing weeds efficiently from the fields. However, simple chopping of the weeds is not a suitable approach, since the PAs will be leached out from the rotting chopped shoots. Moreover, also the remaining roots of the chopped PA-containing weeds will continue to exude the toxic PAs into the soil. Thus, it is essential to remove the entire PA-containing weed plants from the field. In other words, the weeds should not be chopped off, but the entire plants must be extracted entirely and removed from the field.

Basically, these coherences pertain to all other alkaloidal contaminations. There is no doubt that the contaminations by poisonous specialized metabolites derived from collateral weeds could be prevented by removing the entire weed plants from the field.

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