REVIEW ARTICLE

Ecological aspects of plant selenium hyperaccumulation

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ABSTRACT

Hyperaccumulators are plants that accumulate toxic elements to extraordinary levels. Selenium (Se) hyperaccumulators can contain 0.1–1.5% of their dry weight as Se, levels toxic to most other organisms. In this review we summarise what is known about the ecological functions and implications of Se (hyper)accumulation by plants. Selenium promotes hyperaccumulator growth and also offers a plant several ecological advantages through negative effects on Se-sensitive partners. High tissue Se levels reduce herbivory and pathogen infection, and high-Se litter deposition can inhibit neighbouring plants. There is no evidence for a cost of hyperaccumulation in terms of reproductive functions or pollinator visitation. Hyperaccumulators offer a niche for Se-tolerant herbivores, pollinators, microbes and neighbouring plants. They may even facilitate these partners through Se enrichment: neighbouring plants with elevated Se levels enjoy enhanced growth and reduced herbivory. Through combined negative and positive effects on ecological partners, Se hyperaccumulators likely affect local plant, microbial and animal species composition and richness, favouring Se-tolerant species at different trophic levels. By locally concentrating Se and altering its chemical form, Se hyperaccumulators likely play an important role in Se entry into, and Se cycling through, seleniferous ecosystems. These findings are of significance since they provide insight into the ecological reverberations of Se hyperaccumulation, and shed light on the possible selection pressures that have led to the evolution of this fascinating phenomenon. Better ecological insight will also help in the management of seleniferous areas and the agricultural production of Se-rich crops for phytoremediation or biofortification.

INTRODUCTION

Hyperaccumulation is the intriguing phenomenon through which some plant species accumulate one or more toxic elements to extraordinarily high concentrations, typically 100-fold higher than other vegetation on the same site (Baker et al. 2000). The criterion used to distinguish a hyperaccumulator ranges from 0.01% to 1.00% of leaf dry matter, depending on the element. Elements that can be hyperaccumulated include arsenic (As, >0.1%), cadmium (Cd, >0.01%), cobalt (Co, >0.1%), copper (Cu, >0.1%), lead (Pb, >0.1%), manganese (Mn, >1%), nickel (Ni, >0.1%), selenium (Se, >0.1%) and zinc (Zn, >1%). The element levels accumulated in these plants would be lethal to other organisms, yet cause no toxicity in hyperaccumulators. About 450 plant species from over 40 families have been reported to hyperaccumulate, but hyperaccumulation is most prevalent in the Brassicaceae (Baker et al. 2000). Hyperaccumulators are found predominantly on soils that contain elevated levels of the toxic element mentioned above, suggesting they have a physiological or ecological requirement for the element. Boyd & Martens (1992) proposed several possible physiological or ecological functions of hyperaccumulation, including roles in herbivore or pathogen defence, elemental allelopathy, drought resistance or metal tolerance. Several of these hypotheses have been tested using different hyperaccumulator models. In this review, we provide an overview of our current knowledge regarding the ecological effects of plant element hyperaccumulation, using Se hyperaccumulation as a model system.

Selenium naturally occurs as a trace element in most soils. Soil Se levels are typically below 1 mg kg⁻¹, but may be up to 100 mg kg⁻¹ in seleniferous soils (Beath et al. 1939). The main forms of bioavailable Se in oxidising and reducing environments are selenate and selenite, respectively (White et al. 2007). Selenium has not been shown to be an essential element for higher plants, although it can have a growth-promoting effect for many species, particularly Se hyperaccumulators (Pilon-Smits et al. 2009). Since Se is chemically similar to sulphur (S), it is taken up and metabolised via the same mechanisms by organisms. Plants can inadvertently take up selenate and selenite via transporters for sulphate or other anions (Arvy 1993). Selenium uptake is dependent on Se concentration and speciation, the concentration of competing anions, rhizosphere pH and redox conditions (Mikkelsen et al. 1989). Roots take up selenate faster than selenite (de Souza et al. 1998; Zhao et al. 2005). Selenate and selenite can be further reduced to selenide and assimilated into the amino acids selenocysteine (SeCys) and selenomethionine (SeMet) via the S assimilation pathway (Terry et al. 2000). Both SeCys and SeMet can be incorporated into
proteins. Non-specific incorporation of SeCys into proteins, in the place of cysteine, is toxic (Stadtman 1990). However, organisms that require Se as a micronutrient, including mammals, can also specifically incorporate SeCys into a small set of selenoproteins, which contain SeCys in their active site and perform redox functions (Zhang & Gladyshev 2009). Some selenoproteins play a role in scavenging free radicals. In humans, Se deficiency has therefore been associated with enhanced risk of cancer (Rayman 2005). The window between Se deficiency and toxicity in animals is extremely narrow, and both are problems worldwide (Terry et al. 2000). Selenium-containing plants may be used both as a dietary source of Se (biofortification) and as a means to remove excess Se from the environment (phytoremediation) (Pilon-Smits 2005; Bañuelos et al. 2011).

SPECIAL PROPERTIES OF SE HYPERACCUMULATORS

Most plant species growing on seleniferous soils contain <10 mg Se kg\(^{-1}\) DW, and experience toxicity at levels above ~100 mg Se kg\(^{-1}\) DW; these are considered Se non-accumulator species (Rosenfeld & Beath 1964; Brown & Shrift 1982; White et al. 2004). Plants that can accumulate and tolerate moderately elevated Se levels (up to 1000 mg Se kg\(^{-1}\) DW) are called Se accumulators. True Se hyperaccumulator species, from the genera Stanleya, Astragalus, Xylorhiza and Oonopsis, can accumulate 1000–15,000 mg Se kg\(^{-1}\) DW (0.1–1.5\% Se) without any toxicity symptoms (Beath et al. 1939). Hyperaccumulators differ from non-accumulators in several ways. Selenate uptake by hyperaccumulators is not inhibited by high sulphate concentration (Feist & Parker 2001). Hyperaccumulators also tend to become enriched with Se relative to S: they have a higher Se/S ratio in their tissues compared to their growth medium (White et al. 2007). Hyperaccumulators of Se often also contain elevated S levels, compared to non-hyperaccumulators (El Mehdawi et al. 2011a). Inside a plant, Se hyperaccumulators show a relatively high degree of root-to-shoot Se translocation compared to other plants. The form of Se accumulated by hyperaccumulator plants (Se speciation) is mainly organic methyl-SeCys (Freeman et al. 2006a), while in non-accumulators and accumulators, the majority of Se remains as selenate (de Souza et al. 1998; Van Hoewyk et al. 2005). Perhaps because of this difference in speciation, the tissue Se sequestration pattern is different for hyperaccumulators: they store Se mainly in the leaf epidermis (sometimes in leaf hairs) and in reproductive tissues, particularly pollen, ovules and seeds (Freeman et al. 2006a; Quinn et al. 2011b). Non-hyperaccumulators mainly store Se in vascular tissues of leaves, and have higher Se levels in leaves than flowers (Quinn et al. 2011b). Methyl-SeCys can be converted to dimethylselenide, the main form of volatile Se produced by hyperaccumulators. Non-hyperaccumulators, on the other hand, produce volatile dimethylselenide, using selenomethionine (SeMet) as a starting point (Terry et al. 2000). Plant levels of Se also show different seasonal fluctuations in hyperaccumulators and non-accumulators: the leaf Se concentration is highest in the early spring for hyperaccumulators, but peaks in summer for non-hyperaccumulators (Galeas et al. 2007). The seasonal fluctuations in Se levels are correlated with S levels for non-hyperaccumulators, but not for hyperaccumulators. Table 1 summarises these physiological differences between hyperaccumulators and non-hyperaccumulators.

<table>
<thead>
<tr>
<th>property</th>
<th>Se hyperaccumulators</th>
<th>non-hyperaccumulators</th>
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<tbody>
<tr>
<td>Se uptake</td>
<td>Sulphur-independent</td>
<td>Inhibited by sulphur</td>
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<tr>
<td>root-to-shoot Se translocation</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Se accumulation and tolerance</td>
<td>1000–15,000 mg Se kg(^{-1}) DW</td>
<td>&lt;1000 mg Se kg(^{-1}) DW</td>
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<tr>
<td>Se volatilisation</td>
<td>Higher, as dimethylselenide</td>
<td>Lower, as dimethylselenide</td>
</tr>
<tr>
<td>Se sequestration (organ level)</td>
<td>Highest in reproductive organs</td>
<td>Highest in leaves</td>
</tr>
<tr>
<td>Se sequestration (tissue level)</td>
<td>Highest in epidermis, pollen, ovules</td>
<td>Highest in vascular tissues</td>
</tr>
<tr>
<td>main Se form in tissues</td>
<td>Methyl-SeCys</td>
<td>Selenate</td>
</tr>
<tr>
<td>seasonal fluctuations of Se and S</td>
<td>Highest in spring for Se, summer for S</td>
<td>Highest in summer for Se and S</td>
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up-regulation of the transporter that exports sulphate from root cells into the root xylem (leading to enhanced translocation) and from shoot cells into the shoot phloem (resulting in enhanced remobilisation to reproductive tissues). There may also be methyl-SeCys transporters in hyperaccumulators that mediate leaf phloem loading and sequestration in specialised epidermal cell types. If Se is (re)mobilised in different forms in hyperaccumulators and non-hyperaccumulators – organic versus inorganic Se – this may also explain the observed differences in sequestration patterns, both temporal and spatial. The molecular mechanism underlying the capacity of hyperaccumulators to accumulate Se as methyl-SeCys is a SeCys methyltransferase (SMT) (Neuhierl & Bock 1996). Methyl-SeCys can be safely accumulated because it is not incorporated into proteins, and therefore does not disrupt protein function. This explains the extreme Se tolerance of hyperaccumulators.

Assuming that Se hyperaccumulation is a derived trait, not all hyperaccumulators are likely to have evolved from a single ancestor, since they occur in several unrelated genera and are often considered derived taxa (Brown & Shrift 1982; White et al. 2004). It is therefore more likely that Se hyperaccumulation and hypertolerance arose independently in different genera via convergent evolution (Brown & Shrift 1982). This raises the question as to what has/have been the selection pressure(s) for the evolution of Se hyperaccumulation? More than one selection pressure may act simultaneously. Since hyperaccumulators of Se are rarely observed in non-seleniferous areas (Brown & Shrift 1982), it has been hypothesised that Se may play an essential physiological role in hyperaccumulators. Although Se is clearly a beneficial nutrient for hyperaccumulators and many other plant species, through improved protection against oxidative stress (Cartes et al. 2005; Djanaguiraman et al. 2005; Hartikainen 2005; Kong et al. 2005), to date there is no convincing evidence that supports an essential role for Se in any higher plant species. Hyperaccumulators may also have an ecological requirement for Se. As mentioned above, Boyd & Martens (1992) proposed several possible functions of hyperaccumulation, including roles in herbivore or pathogen defence, elemental allelopathy, drought resistance or metal tolerance. As for Se hyperaccumulation, there is supporting evidence for both the elemental defence hypothesis and the elemental allelopathy hypothesis, as described in the next sections.

EFFECTS OF SE ON ECOLOGICAL PROCESSES IN SELENIFEROUS AREAS

Since Se is a toxic element, Se hyperaccumulation in plants can be envisioned to have ecological implications at many levels (Fig. 1). Belowground, Se hyperaccumulator plants may affect the local soil Se distribution and chemical speciation via litter deposition, root turnover and root exudation. This Se may affect soil microbial composition and abundance, soil fauna, as well as neighbouring vegetation. Selenium accumulation in plant tissues (root and shoot) as well as Se volatilisation may affect plant–pathogen, plant–herbivore and plant–pollinator interactions. Below we summarise what is known about the ecological implications of Se (hyper)accumulation.

Plant–herbivore interactions

Since Se is toxic to animals at high levels, ingestion of Se-rich plant material may be expected to have toxic effects on herbivores. As a result, herbivores may learn to avoid high-Se plant material. Through such toxicity and/or deterrence, Se accumulation may serve to defend plants against herbivory. To investigate this so-called elemental defence hypothesis (Boyd & Martens 1992), different approaches have been used. In one approach, plants were pre-treated under controlled conditions with different Se concentrations, and offered to herbivores in choice or no choice experiments, to test for deterrence and toxicity, respectively. In another approach, herbivores or herbivory were surveyed in the field as a
function of natural plant Se concentration. Since the 1930s, Se has been known to be the toxic component of so-called ‘locoweed’, responsible for substantial livestock losses in the western USA (Beath et al. 1939). Six decades later, we now know that Se in plants is toxic to a variety of insect and vertebrate herbivores, and also deters many herbivores. Varieties of Atriplex that accumulated more Se support lower insect growth and survival of Spodoptera exigua (Vickerman et al. 2002). Caterpillars of the cabbage looper (Trichoplusia ni) prefer to feed on low-Se rather than high-Se Brassica juncea (Bañuelos et al. 2002). Selenium also protects Brassica juncea from caterpillars of the cabbage white butterfly (Pieris rapae), due to both deterrence and toxicity (Hanson et al. 2003). Furthermore, Se protects plants against other leaf chewing herbivores (crickets and grasshoppers) via deterrence and toxicity, both in Brassica juncea and in the hyperaccumulator Stanleya pinnata (Freeman et al. 2007). In laboratory studies B. juncea plants were protected by Se at levels as 230 and 447 mg kg\(^{-1}\) DW, and in a subsequent manipulative field study, Se levels as low as 50 mg Se kg\(^{-1}\) DW already protected S. pinnata plants. As described above, a difference between the two plant species is that the Se is sequestered specifically in the epidermis of S. pinnata, along the leaf edges, while B. juncea contains most Se in its vasculature. Sequestration in the periphery of the leaves, as found for S. pinnata, may offer additional protection from invertebrate herbivores. The main forms of Se in B. juncea and S. pinnata are selenate and methyl-SeCys, respectively. Since Se accumulation protects both plants, both forms of Se appear to be toxic to herbivores. The mechanism of methyl-SeCys toxicity was revealed in a comparative study using two populations of the diamondback moth (Plutella xylostella). In a lab study, a Se-sensitive moth variety from a non-seleniferous area was shown to accumulate SeCys after feeding on Se-rich S. pinnata plants, while a Se-tolerant variety from a seleniferous area accumulated unaltered methyl-SeCys (Freeman et al. 2006b). Thus, the demethylation of methyl-SeCys likely causes toxicity via non-specific incorporation of the resulting SeCys into proteins. The Se-tolerant diamondback moth population may have lost the capacity to demethylate methyl-(Se)Cys. It appears to have also lost its aversion to feed and oviposit on high-Se plants: while the moth population from the non-seleniferous area preferred to oviposit and feed on low-Se plants, the population from the seleniferous area had no preference. Together, these adaptations enable this herbivore to overwhelm the element plant defence and occupy the niche provided by Se hyperaccumulator plants. It is even feasible that the moth, which contained around 250 mg Se kg\(^{-1}\) DW uses the Se for its own defence against predators and parasites. If so, this defence likely will be overcome by Se-tolerant predators and parasites. Indeed, a parasitic wasp (Diaegma insulare) was found to complete its life cycle in larvae of the Se-tolerant diamondback moth, and to contain similar Se levels as the moth, also in the form of methyl-SeCys (Freeman et al. 2006b).

In addition to leaf chewers, Se was also shown to protect plants from invertebrates with other modes of feeding. Phloem-feeding aphids were deterred by high-Se B. juncea plants, and suffered toxicity at plant Se levels as low as 10 mg Se kg\(^{-1}\) DW (Hanson et al. 2004). This indicates that Se is present in the phloem of this plant; in agreement with X-ray absorption spectroscopy (XAS) data, which showed predominant accumulation of Se in the vasculature. Selenium also protects the hyperaccumulators S. pinnata and Astragalus bisulcatus from two herbivores with a cell-disrupting feeding mode, the two-spotted spider mite and the Western flower thrip (Quinn et al. 2010). Furthermore, in a preliminary study addressing the effect of root-accumulated Se on nematode colonisation, high-Se S. pinnata plants harboured fewer nematodes than low-Se plants (Prins and Pilon-Smits, unpublished results). Besides protecting plants from a variety of invertebrate herbivores, Se was also shown to protect B. juncea and S. pinnata from a vertebrate herbivore: the black-tailed prairie dog (Quinn et al. 2008; Freeman et al. 2009). When high- and low-Se plants were offered to prairie dogs in the field, they preferentially fed on the low-Se plants, and when high- and low-Se S. pinnata plants were planted and followed over a 2-year period, the high-Se plants survived significantly better and suffered less herbivory.

In a different approach to address the element defence hypothesis for Se, a field survey was done comparing arthropod load on hyperaccumulator and non-hyperaccumulator species in a seleniferous habitat. The two Se hyperaccumulator species (A. bisulcatus and S. pinnata) harboured significantly less arthropods (both in terms of animals per plant and number of species) compared to similar-sized non-Se hyperaccumulators (Medicago sativa and Helianthus pumilus; Galeas et al. 2008). In another field survey, it was found that the hyperaccumulator A. bisulcatus containing 120–600 mg Se kg\(^{-1}\) DW was one of the few species capable of growing on prairie dog towns. It was equally abundant on prairie dog towns compared to outside these towns in seleniferous areas, and when growing on prairie dog towns it was rarely eaten (Quinn et al. 2008).

In summary, there is ample support for the Se element defence hypothesis, which states that Se hyperaccumulation has evolved as a defence mechanism. Even levels as low as 10 mg Se kg\(^{-1}\) DW can protect plants from herbivores, due to deterrence and toxicity. As with any plant defence modes, over time it is bound to be overcome by some herbivores, and this also appears to be the case for Se hyperaccumulation, as described in the case study on the diamondback moth.

Plant–pollinator interactions

A high content of Se in flowers may be hypothesised to have a negative effect on plant reproduction if it impairs plant reproductive functions or plant–pollinator interactions. High Se levels may, for instance, impair pollen germination, pollen tube growth, number and size of seeds or seed germination. Selenium in flowers may also deter pollinators or be toxic to them when ingested, since Se has already been shown to be toxic to many herbivorous insects and to deter them (Quinn et al. 2007). Before addressing these questions it is helpful to understand the distribution and chemical speciation of Se in reproductive tissues. In the Se hyperaccumulator S. pinnata, flowers and seeds were found to have the highest Se levels of the entire plant, reaching levels up to 4000 mg kg\(^{-1}\) DW (Quinn et al. 2011b). Within flowers, the pistil and anthers contain the highest Se levels, particularly the pollen and ovules. The nectar of S. pinnata also contained significant Se
levels (~200 mg kg\(^{-1}\) FW). The predominant form of Se in the S. pinnata flowers is an organic C-Se-C compound, presumably MeSeCys, since the same compound was found earlier in its leaves (Freeman et al. 2006a). In contrast, the related non-hyperaccumulator B. juncea did not show particularly high floral Se levels compared to that in other organs (Quinn et al. 2011b). The majority (67%) of the Se in B. juncea flowers was also MeSeCys, with relatively minor fractions of selenocystine (16%), selenate (11%), and selenite (6%). This Se speciation in B. juncea flowers is different from the predominant form in leaves, which is selenate (Pilon-Smits et al. 1999). Within B. juncea flowers, the Se was evenly distributed (Quinn et al. 2011b). Thus, tissue-specific Se sequestration appears to be a trait that is unique to the hyperaccumulator. It is intriguing why the hyperaccumulator preferentially stores its Se in its pollen, ovules and seeds, perhaps the Se functions to protect these valuable reproductive structures from herbivores and pathogens.

Overall, the high Se levels in flowers, particularly in hyperaccumulators, may well have physiological and ecological implications. Prins et al. (2011) tested the effect of floral Se accumulation on reproductive functions in the Se accumulator B. juncea and the Se hyperaccumulator S. pinnata. At levels above ~500–1000 mg Se kg\(^{-1}\) DW, pollen germination was significantly affected in B. juncea but not in S. pinnata. In addition, the number, size and germination rate of seeds started to be affected in B. juncea around the same concentration range. Thus, in non-hyperaccumulators high Se levels may negatively affect reproductive functions. Incidentally, in the field when grown for phytoremediation or biofortification, B. juncea typically does not accumulate more than 100 mg Se kg\(^{-1}\) DW, and this level does not impair reproduction. There was no evidence that the high Se levels impaired reproductive functions in S. pinnata, perhaps because it does not store toxic forms of Se.

In another study, the ecological impacts of floral Se accumulation were investigated. B. juncea and S. pinnata plants containing high or low Se levels were monitored for floral visitation by honeybees and other potential pollinators (Quinn et al. 2011b). No differences in floral visitation were observed for either plant species, even at extremely high (4000 mg kg\(^{-1}\) DW) floral Se concentrations. Thus, results so far do not show evidence of a reproductive cost for Se hyperaccumulators due to deterrence of pollinators. A question that remains is whether the high-Se nectar and pollen ingested by the pollinators has a health effect on these animals. If there is a health impact of the ingested Se on pollinators such as the European honeybee (Apis mellifera), it could be positive or negative. Selenium is an essential trace element for insects (Zhang & Gladyshev 2009), and therefore a Se-enriched diet may promote bee health. On the other hand, if ingested in excess, Se will have a toxic effect on the bee. When 2–5 animals per species of floral visitor were collected while foraging on S. pinnata, (non-native) honeybees were found to contain around 20 mg Se kg\(^{-1}\) DW while (native) bumblebees contained around 250 mg Se kg\(^{-1}\) DW (Quinn et al. 2011b). While the bumblebee contained predominantly C-Se-C (presumably the non-toxic MeSeCys), the honeybee also contained more toxic forms of Se. Based on these limited data, it is hard to predict whether these Se levels are likely to be beneficial or toxic. Tissue Se levels of 10–90 mg Se kg\(^{-1}\) DW were found to be lethal to Se-sensitive Lepidoptera larvae of Pieris rapae and Plutella xylostella (Hanson et al. 2003; Freeman et al. 2006b). The Se-tolerant P. xylostella population, on the other hand, accumulated 250 mg Se kg\(^{-1}\) DW, without ill effects, as non-toxic MeSeCys (Freeman et al. 2006b). It is possible that the European honeybee, which is native to a seleniferous area, has evolved Se tolerance; it may even have specialised to forage on Se hyperaccumulators. Indeed, this species contained the same high Se level and the same non-toxic form of Se as found in larvae of the Se-tolerant moth herbivore (P. xylostella). The lower Se levels and presence of different forms of Se in the European honeybees may reflect different foraging behaviour (visiting both hyper- and non-hyperaccumulators), or may be an indication of reduced Se tolerance. More studies are needed to address this question and to assess the potential health effects of high-Se plants on local bee populations.

Honey from seleniferous areas in Colorado, USA, contain around 1 mg Seg\(^{-1}\) DW (Quinn et al. 2011b), which is higher than Se levels reported for honey from Turkey (Tuzen et al. 2010). At 1 mg Seg\(^{-1}\) DW, honey may be considered nutritionally enhanced. The recommended daily intake of Se for humans is 50–75 µg, corresponding to about three teaspoons of the Se-enriched honey. Thus, Se in flowers and the resulting Se-enriched honey may benefit human health via the reported capacity of Se to prevent cancer (Rayman 2005). Since not all forms of Se are equally potent in this regard, future studies investigating the chemical form of Se in honey could help to better determine the health benefits of Se in honey.

Plant–plant interactions

Phytoenrichment is the process by which plants concentrate certain elements in their surrounding soil, and may be the result of deposition of litter, root exudation and root turnover (Morris et al. 2009). Since Se hyperaccumulators typically concentrate soil trace elements around 1000-fold, and are perennials that shed their leaves annually, they may be hypothesised to phytoenrich their surrounding soil. In support of this hypothesis, the Se concentration in soil surrounding hyperaccumulators A. bisulcatus and S. pinnata was seven- to 13-fold higher (up to 266 mg Se kg\(^{-1}\)) than Se in soil surrounding non-hyperaccumulators, Medicago sativa and Helianthus annuus, growing on the same site (El Mehdaoui et al. 2011a,b). Moreover, the levels of Se were up to 20-fold higher in neighbouring species (Artemisia ludoviciana and Symphyotrichum ericoides) when growing next to hyperaccumulators than when growing >4 m away from Se hyperaccumulators (El Mehdaoui et al. 2011a,b). These enhanced Se levels in soil and plants around hyperaccumulators may be the result of phytoenrichment by the hyperaccumulator, but it cannot be excluded that soil Se distribution is simply heterogeneous and that Se hyperaccumulators are more abundant in Se ‘hot spots’.

The high Se levels in Se hyperaccumulator plants and their associated soil may be hypothesised to have a negative effect on germination and growth of Se-sensitive neighbouring species. Indeed, soil collected around hyperaccumulators significantly reduced the germination and growth of a Se-sensitive plant species, Arabidopsis thaliana, and enhanced its Se level.
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compared to soil collected from non-hyperaccumulators (El Mehdawi et al. 2011a). This may point to elemental allelopathy, processes by which plants concentrate toxic elements as a means to better compete with neighbouring plants. Elemental allelopathy is one of the hypothesised functions of hyperaccumulation (Boyd & Martens 1992). If hyperaccumulation serves an allelopathic function, we would expect to find a difference between the plant community growing around Se hyperaccumulators compared with that around non-hyperaccumulators on the same site. The vegetative ground cover was ~10% less and species diversity slightly lower around Se hyperaccumulator species A. bisulcatus and S. pinnata than around non-accumulators (El Mehdawi et al. 2011a). Thus, it is feasible that Se hyperaccumulators benefit from their accumulated Se through decreased competition from surrounding vegetation; they may also affect the species composition in this vegetation.

Interestingly, some neighbours, such as the abovementioned A. ludoviciana and S. ericoides, appear to thrive next to hyperaccumulators, despite – or perhaps even because of – elevated Se levels (El Mehdawi et al. 2011b). Both A. ludoviciana and S. ericoides were twofold larger, harboured fewer arthropods and showed less herbivory damage when growing in proximity (<1 m) to hyperaccumulators, as compared to plants of the same species growing further away (>4 m) from hyperaccumulators. These Se-enriched neighbours of hyperaccumulators, which contained over 1000 mg Se kg⁻¹ DW, were used in controlled herbivory studies in comparison with their low-Se counterparts collected next to non-accumulators. In choice experiments, grasshoppers collected from the same site preferred to feed on the low-Se A. ludoviciana and S. ericoides plants, and when given no choice, the grasshoppers showed high Se accumulation and mortality after feeding on the high-Se plants. Therefore, Se phytoenrichment associated with growing next to Se hyperaccumulators appears to facilitate Se-tolerant neighbours via reduced herbivory (El Mehdawi et al. 2011b). In addition to this ecological benefit, A. ludoviciana and S. ericoides plants may experience a physiological benefit from their hyperaccumulator-mediated Se enrichment. Selenium has been shown to enhance growth for a variety of higher plant species (Pilon-Smits enrichment. Selenium has been shown to enhance growth for a variety of higher plant species (Pilon-Smits 2009), and the growth of S. ericoides also responded favourably to selenium treatment in herbivore-free greenhouse experiments (El Mehdawi and Pilon-Smits, unpublished results). Therefore, it appears that Se hyperaccumulators facilitate their Se-tolerant neighbours A. ludoviciana and S. ericoides by promoting their growth and decreasing their herbivory, both via enhanced Se supply. It is interesting that the same process of Se phytoenrichment by hyperaccumulators can have either a negative or a positive effect on neighbouring plants, depending on whether a neighbour is sensitive or tolerant to Se. Through these combined competitive and facilitating forces, hyperaccumulators may significantly affect local plant species composition and, consequently, species composition at other trophic levels.

Plant–microbe interactions

Plants live in close association with bacterial and fungal microbes. Some live inside a plant (endophytes), some on the plant surface and some in the sphere of influence of a plant, such as the rhizosphere. From a plant perspective, the nature of the plant–microbe interaction may be positive (mutualistic), negative (parasitic) or neutral (commensalistic). In mutualistic relationships, a plant typically provides a microbe with organic carbon, and in return obtains better access to mineral nutrients or protection from abiotic or biotic stresses. In parasitic relationships, a microbe exploits a plant and may cause reduced growth, disease or death. Selenium accumulation in plant tissues may affect any or all plant–microbe interactions. Compared to other ecological interactions, the effects of Se on plant–microbe interactions are relatively little studied. In one study by Hanson et al. (2003) it was found that Se accumulation in B. juncea at 300–750 mg Se kg⁻¹ DW reduced fungal infection by two pathogens, one a leaf pathogen (Alternaria alternata) and the other a stem-root pathogen (Fusarium sp.). Both pathogens are fairly Se-sensitive, showing 50% growth inhibition at 40–60 mg l⁻¹ selenate when grown on Petri dishes. Thus, plant Se accumulation may protect plants from Se-sensitive fungal pathogens. It will be interesting in future studies to test whether Se also protects plants from bacterial and viral pathogens.

High-Se plant material may also have a profound effect on mutualistic relations with microbes, selecting for more Se-tolerant symbionts. The most extreme Se levels are likely experienced by hyperaccumulator endophytes and litter decomposers, which may experience levels >1000 mg Se kg⁻¹ DW. Rhizosphere Se levels can also be quite elevated in soil around hyperaccumulator species (up to 600 mg kg⁻¹; El Mehdawi et al. 2011a). This is likely to have a profound effect on microbial species composition and perhaps density. Indeed, in a survey of rhizosphere fungi collected from seleniumiferous and non-seleniumiferous habitats, the isolates from seleniumiferous habitats were significantly more Se tolerant than those from a non-seleniumiferous habitat (Wangelin et al. 2011). The rhizosphere of hyperaccumulator plants harboured many highly Se tolerant fungi, some of which appear to benefit from Se in terms of overall growth and stress tolerance (Wangelin and Pilon-Smits, unpublished results). Moreover, it was found that several Astragalus spp. hyperaccumulators (Fabaceae) have root nodules containing high Se concentrations (Lindblom, Alford and Pilon-Smits, unpublished results), suggesting the presence of a Se-tolerant Rhizobium species. The high Se levels associated with hyperaccumulator habitats may lead to the evolution of specialised microbes that have evolved Se tolerance and therefore can occupy niches provided by hyperaccumulator plants. In future studies it will be interesting to test the nature of these plant–microbe interactions, and the potential effects of associated microbes on plant Se accumulation and speciation. In the next section, we discuss another class of microbe that interacts with high-Se plant material: the litter decomposer.

Litter decomposition and Se cycling

Decomposition of leaf litter in soil around plants is a significant ecosystem process that plays an important role in chemical cycling of elements (Aerts 2006). Many factors affect leaf litter decomposition rate, e.g. physical, chemical and biological factors. Physical factors include geography and climate (temperature, precipitation, evapotranspiration) (Silver & Miya 2001; O’Neill et al. 2003; Aerts 2006), and chemical
factors include the elemental composition and pH of the soil. Biological factors include the quality of the litter and the local decomposer community (Swift et al. 1979; Smith & Bradford 2003). Among these factors, litter quality is considered one of the most important (Swift et al. 1979). The high Se levels observed in hyperaccumulator litter (>1000 mg kg⁻¹ DW) may be hypothesised to slow down decomposition, due to toxicity to the microbial and microarthropod decomposer communities. However, the opposite was found when plant material with varying Se concentrations was left to decompose in a seleniferous area. High-Se decomposition, due to toxicity to the microbial and microarthropod and culturable microbes (Quinn et al. 2011a). The A. bisulcatus material (around 600 mg Se·kg⁻¹ DW) decomposed faster than low-Se M. sativa material and contained more microarthropods and culturable microbes (Quinn et al. 2011a). The A. bisulcatus material had a higher N/C ratio than the M. sativa litter, potentially making it a higher-quality litter for decomposers. The fast decomposition of the high-Se litter in seleniferous habitat suggests that the local microbial and microarthropod decomposer community has substantial Se tolerance. Perhaps there are even specialist decomposers that specifically target hyperaccumulator litter. These Se-tolerant decomposers may contribute to the local Se cycling, by recycling hyperaccumulator-bound Se and moving it into the food chain. Some may also volatilise Se, or promote Se leaching into groundwater. The presence of Se-tolerant decomposers enables hyperaccumulators to have a profound effect on the overall Se cycling in the local ecosystem. Such plants concentrate Se many-fold in their tissues, change its speciation from inorganic to organic, redepot the Se to the soil, and through their tissues and litter, offer a significant portal for organic Se into the food chain. Organic Se is often taken up more readily by organisms, and affects organisms differently to inorganic Se; hyperaccumulators may therefore mediate not only a quantitative but also a qualitative effect on Se fluxes through the local ecosystem.

**A SYNTHESIS: ECOLOGICAL AND EVOLUTIONARY ASPECTS OF SE HYPERACCUMULATION**

**Ecological effects – common trends**

Selenium hyperaccumulators show a general trend, in that they have a negative effect on Se-sensitive ecological partners, while offering a niche for, and potentially even facilitating, Se-tolerant partners. This has been found for herbivores, plants, microbes and perhaps also pollinators. There is ample support for the Se element defence hypothesis, which states that Se hyperaccumulation may have evolved as a defence against herbivores and pathogens. Even at low levels (10–50 mg·kg⁻¹ DW), Se can protect plants against some herbivores, due to a combination of deterrence and toxicity. At higher levels, Se protects against additional herbivores as well as microbial pathogens. In addition, hyperaccumulator plants appear to be able to use their accumulated Se in their competition with neighbouring plants. While Se in or around hyperaccumulators deters or is toxic to Se-sensitive neighbouring organisms, it may benefit Se-tolerant organisms via enhanced growth or stress resistance. Through these negative and positive effects, Se hyperaccumulators may affect plant, microbial and animal species composition and species richness within the area under their influence. In this way, Se hyperaccumulators may favour the evolution of Se tolerance at different trophic levels. It appears that specialised Se-tolerant herbivores, detritivores, microbial symbionts and perhaps also pollinators have evolved to live in symbiosis with Se hyperaccumulators. Through their tendency to locally concentrate Se, change its speciation and then disperse it to the Se-tolerant ecological partners, Se hyperaccumulators likely play an important role in Se cycling through seleniferous ecosystems.

**Implications for the evolution of hyperaccumulators and their ecological partners**

Selenium hyperaccumulation has evolved independently in different plant lineages. Thus, the selection pressures that have driven the evolution of this trait may be single or multiple, and may be different or the same in different genera. If we incorporate the results from physiological and ecological studies into an ecological framework, we can envision a possible scenario for the evolution of Se hyperaccumulation in a plant lineage (Fig. 2). Assuming that hyperaccumulation is a derived trait, the evolution of a hyperaccumulator such as Stanleya pinnata probably started with a non-accumulator species similar to Arabidopsis thaliana. Within A. thaliana there is substantial genetic variation in Se accumulation and tolerance, probably due to differential expression of genes related to S uptake and assimilation (Zhang et al. 2006a,b,c). Even at the low Se levels present in non-accumulators, the highest Se (and S) accumulators may have had a selective advantage in seleniferous areas due to enhanced protection from Se-sensitive herbivores. This selection pressure may have led to a gradual evolution of Se accumulator species, with properties similar to Brassica juncea. Boyd (2007) described such proposed gradual evolution of element accumulation the ‘defensive enhancement hypothesis’. As Se levels in plants increased, such plants offered additional protection from other species of herbivore, as well as from microbial pathogens. However, at some point, a further increase in Se accumulation may have been prevented as the plant Se tolerance was reached. Above this Se tolerance ceiling (500–1000 mg·kg⁻¹ DW), plant growth and reproductive functions in Se accumulators could be impaired. This may be overcome by metabolic conversion to less toxic forms of Se. In B. juncea the main form of Se in leaves is selenate, while in flowers it is the less toxic MeSeCys. The next evolutionary step, from a Se accumulator to a true hyperaccumulator, may have involved more universal expression of SeCys methyltransferase activity, leading to accumulation of MeSeCys in all organs. The MeSeCys is then sequestered in specific tissues and organs in hyperaccumulators (leaf periphery, reproductive organs), which may further contribute to Se tolerance and defence. As a result, hyperaccumulator plants do not seem to have any Se tolerance ceiling, and can reach levels (>1000 mg Se·kg⁻¹ DW) that protect them from even more herbivores and pathogens, and further provide additional selective advantage through elemental allelopathy. Over time, many ecological partners of Se hyperaccumulators appear to have co-evolved Se tolerance. These include herbivores, pathogens, litter detritivores, plants, mutualistic symbionts such as Rhizobium, other rhizosphere and endophytic microbes and pollinators. Because of these co-evolved symbionts, hyperaccumulator plants may experience no net...
evolutionary cost of hyperaccumulation. The only apparent limitation of Se hyperaccumulation is that it limits the geographic distribution to seleniferous soils: hyperaccumulators appear to be physiologically or ecologically dependent on Se for their competitive strength, perhaps because they have lost other mechanisms to defend themselves against biotic or abiotic stresses.

Selenium hyperaccumulators appear to promote the evolution of Se-tolerant symbionts, as evidenced at the microbial, animal and plant level. The toxic Se levels associated with hyperaccumulators select against Se-sensitive individuals and favour Se-tolerant forms. Thus, ecological partners of Se hyperaccumulators may co-evolve with their host/neighbor. Evolution of Se tolerance in these partners not only enables them to live in the Se-rich environment created by a hyperaccumulator, but may even benefit them in the form of better growth or stress resistance, as observed for Se-tolerant neighbouring plants. Some neighbours of Se hyperaccumulators even reach hyperaccumulator Se levels themselves (>0.1% DW), but only when growing next to hyperaccumulators. They benefit from their higher Se levels through reduced herbivory and probably also a physiological growth response to the Se. It is an interesting thought that hyperaccumulators may in this way facilitate the evolution of hypertolerance and hyperaccumulation in neighbouring plant species, as hyperaccumulators promote the reproductive success of those individuals among their neighbours that best accumulate and tolerate Se.

IMPLICATIONS FOR MANAGING SELENIFEROUS HABITATS AND APPLICATIONS IN AGRICULTURE AND PHYTOREMEDIATION

Selenium is both an essential trace element and a toxin, dependent on the concentration. Selenium deficiency and toxicity are problems found throughout the world. Plants may be used to remove Se from natural or polluted Se-rich areas and as a food source to alleviate Se deficiency in humans or animals. The first process is called phytoremediation (Pilon-Smits 2005), and the second is known as biofortification (Bañuelos et al. 2011). The two may even be combined: plants that have accumulated Se from polluted soil may be used as fortified food. To optimise these technologies and avoid environmental problems, it is important to have better knowledge of the ecological implications of growing Se-rich plants. In 2002, Bañuelos et al. reported that Se from phytoremediation crops can be transferred biologically to insects and mammals (Bañuelos et al. 2002). If Se in flowers negatively affects honeybee health, this may have serious consequences for honeybee populations and agricultural productivity. It is also possible that Se positively affects bee health, e.g. by protecting bees from pathogens. In this context, it is interesting to note that Se has been reported to protect the Lepidopteran herbivore *Trichoplusia ni* from baculovirus infection (Popham et al. 2005). Since Se is toxic to generalist herbivores and pathogens, Se-rich crops may have a reduced need for pesticides and fungicides, and higher overall productivity. If Se hyperaccumulators enhance Se levels in neighbouring plants, this may be utilised in the form of co-cropping or intercropping. The Se-tolerant microbes that are found in association with Se hyperaccumulators may perhaps be used for bio- or phytoremediation, by themselves or in concert with plants. If Se-rich plants form a portal for Se into an ecosystem, and perhaps also change the form of Se in the environment, this may also have complex ecological implications. When using Se-accumulating plants in agriculture or environmental restoration, it is advisable to consider these various ecological implications and incorporate them into the management plan.

PROSPECTS

The same ecological and evolutionary implications observed for Se hyperaccumulation may be the case for other hyperaccumulated elements, such as As, Ni, Cd and Zn. These elements have already been shown to protect hyperaccumulators from herbivores (Boyd 2007, 2010), and there may be other parallels with Se. In future studies, it will be interesting to compare elemental effects on ecological processes in different...
hyperaccumulator systems. As for Se hyperaccumulator ecology, it will be interesting to look in more detail at ecosystem-wide effects, such as effects of the hyperaccumulators on species composition at different trophic levels, and Se cycling through the food chain and ecosystem. In future studies it will also be interesting to investigate rhizosphere processes in more detail, such as the mechanisms of phytoenrichment, root–root interactions and interactions with rhizosphere and endophytic microbes. From an applied perspective, future research could focus on the potential effects of plant Se on herbivory, pollination and pollinator health in the context of biofortification and phytoremediation. In addition, different co-cropping practices could be explored between hyperaccumulators and crops. If hyperaccumulators not only increase their neighbour’s Se concentration but also change the form of Se accumulated to a more organic form of Se, this could be beneficial for biofortification, since organic Se is thought to be a potent anti-carcinogen (Ellis et al. 2004). Finally, Se-tolerant microbes isolated from Se hyperaccumulators should be investigated for their capacity to affect growth, Se accumulation and speciation in crops.

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