Selenium protects plants from phloem-feeding aphids due to both deterrence and toxicity

Brady Hanson, Stormy Dawn Lindblom, Miriam L. Loeffler and Elizabeth A. H. Pilon-Smits
Department of Biology, Colorado State University, Anatomy/Zoology Building, Fort Collins, CO 80523, USA

Summary

• Certain plant species hyperaccumulate selenium (Se) to 1000 mg kg\(^{-1}\) d. wt, even from low-Se soils. It is not known whether Se hyperaccumulation offers these plants any advantage. In this study the hypothesis was tested that Se can protect plants from phloem-feeding herbivores.

• Indian mustard (Brassica juncea) grown with or without Se was subjected to colonization by green peach aphids (Myzus persicae).

• In choice feeding experiments the aphids clearly avoided Se-containing plant material, and were able to detect and avoid Se-containing leaves with levels as low as 10 mg Se kg\(^{-1}\) d. wt. In nonchoice feeding experiments aphid population growth was inversely correlated with leaf Se concentration. The leaf Se concentration leading to a 50% reduction in aphid population growth was 1.5 mg kg\(^{-1}\) d. wt, and \(\geq 10\) mg Se kg\(^{-1}\) d. wt was lethal.

• In summary, Se can protect plants from feeding by aphids at leaf levels two orders of magnitude lower than those found in hyperaccumulators in the field. These results shed light on the possible functional significance of Se hyperaccumulation.

Key words: selenium (Se), hyperaccumulation, herbivory, Brassica juncea, Myzus persicae.


Introduction

Hyperaccumulation in plants is the phenomenon that individuals of certain species accumulate metals or metalloids to concentrations several orders of magnitude higher than those found in other species on the same site (Baker & Brooks, 1989). Elements that have been reported to be hyperaccumulated include As, Cd, Co, Cu, Mn, Ni, Pb, Se, and Zn (Reeves & Baker, 2000; Guerinot & Salt, 2001). Selenium hyperaccumulator species have been reported in the genera Astragalus (Fabaceae), Stanleya (Brassicaceae), Oonopsis (Asteraceae) and Xylorhiza (Asteraceae) (Beath et al., 1939a,b; Brown & Shrift, 1982). These Se hyperaccumulators occur mainly on naturally selenium-rich soils such as in the western USA and typically contain 0.1% of d. wt (1000 mg kg\(^{-1}\)) Se in their tissue (Feist & Parker, 2001). Ingestion of these ‘locoweeds’ causes disease and death in animals (Rosenfeld & Beath, 1964). The toxicity of Se is thought to be due to its chemical similarity to sulfur, leading to nonspecific replacement of S by Se in proteins and other sulfur compounds (Stadtman, 1990; Anderson, 1993).

On the other hand, Se is also an essential element for many organisms, as a component of seleno-enzymes and selenotRNAs (Stadtman, 1990). Whether Se is essential for higher plants is still unknown (Fu et al., 2002; Novoselov et al., 2002).

It is not clear why plants hyperaccumulate these metals or metalloids. Several hypotheses have been proposed: metal tolerance, inadvertent uptake, allelopathy, drought resistance, and protection from herbivory and/or infection (Reeves et al., 1981; Boyd & Martens, 1992). No evidence was found for a role of Ni hyperaccumulation in drought resistance (Whiting et al., 2003). There is also no consistent correlation between metal tolerance and accumulation; the two traits often segregate independently (Macnair et al., 1999).

There is substantial evidence for a protective role of Ni and Zn against invertebrate herbivory. Zinc was shown to protect the Zn hyperaccumulator Thlaspi caerulescens from herbivory by caterpillars, slugs and locusts (Pollard & Baker, 1997; Jhee et al., 1999). Nickel protected the Ni hyperaccumulator Streptanthus polygaloides from caterpillar herbivory (Boyd et al., 1994; Martens & Boyd, 1994, 2002; Boyd & Moar, 1999).
Nickel also protected Senecio coronatus from herbivory by snails (Boyd et al., 2002). No Ni protection was found against aphids, perhaps because the phloem Ni concentration was subtoxic (Boyd & Martens, 1999). There is also evidence that Ni can protect plants from microbial infection by fungi (Pythium, Erisyphe polygoni, Alternaria brassicicola) and bacteria (Xanthomonas) (Boyd et al., 1994; Ghaderian et al., 2000). By contrast, Ni enhanced the susceptibility to turnip mosaic virus (Davis et al., 2001).

There is also evidence that Se can protect plants from biotic stresses. The moth caterpillar Spodoptera exigua showed a preference to feed on artificial diet without Se, and Se was toxic at levels of 50 mg kg\(^{-1}\) d. wt feed (Trumble et al., 1998; Vickerman & Trumble, 1999). Selenium was shown to protect plants from herbivory by Pieris rapae caterpillars both due to deterrence and toxicity, but to promote feeding by the snail Mesodon ferrissi (Hanson et al., 2003). In addition to protecting plants from invertebrate herbivory, Se may also protect them from fungal infection (Hanson et al., 2003) and from feeding by mammals (Franke & Potter, 1936).

In the present study, the hypothesis was tested that Se can protect plants from feeding by invertebrate phloem-feeders. The green peach aphid (Myzus persicae) was used as a generalist phloem feeder. M. persicae is a European native that is now worldwide in distribution. Its host range comprises over 40 plant families including Brassicaceae (Heathcote, 1962). Brassica juncea (Indian mustard) was used as a model plant species since it is quite susceptible to aphid herbivory, tolerant to Se, and a good Se accumulator. B. juncea is thought to originate in central Asia but now grows as a crop or weed worldwide including Asia, the Americas, Africa and Australia.

Materials and Methods

Biological material

B. juncea seeds (Indian mustard Czern., accession no. 173874) were obtained from the North Central Regional Plant Introduction Station (Ames, IA, USA). The seeds were germinated on coarse sand or soil. Seedlings grown on sand were transferred after 2 wk to a hydroponic set-up consisting of aerated 4 l-containers with half-strength Hoagland’s solution (Hoagland & Arnon, 1938). The nutrient solution was replaced every week. The plants were grown under glasshouse conditions at 25°C and a 16 h/8 h L/D photoperiod until use. After at least 1 week in hydroponics, the plants were supplied with Se as sodium selenate in half-strength Hoagland’s solution. The duration of the Se treatments and the Se concentrations used varied per experiment and are indicated in the text.

Green peach aphids (M. persicae Sulzer) were collected from the Colorado State University glasshouses and propagated on B. juncea plants until experimental use.

Choice feeding experiments

In an initial experiment, aphids from a neighbouring group of B. juncea plants were allowed to colonize B. juncea plants growing in half-strength Hoagland’s solution spiked with 0, 1, 5, 10 or 20 µM Se as sodium selenate (\(n = 12\) plants per concentration). When the aphid population on the most densely populated plants had reached c. 150 per plant, the aphids on each individual plant were counted. Leaf samples were collected for determination of Se concentration as described below under 'elemental analysis'.

In a subsequent experiment, designed to exclude a possible position effect of the plants on aphid choice, three cages were used each containing three plants pre-treated for 7 d with 0, 20 or 40 µM Se, respectively. At 0 d, 20 aphids were placed on a platform connecting all three pots from the middle. After 7 d the number of aphids on each plant was counted. Leaves were sampled and the leaf Se concentrations were determined as described below.

To determine whether Se in leaves has a deterrent effect on aphids, individual aphids were given the choice between leaves with and without Se. There were no visible differences between the ±Se plants or leaf sections used. Aerated 250 ml plastic containers were used, each containing two B. juncea leaf pieces of 2.5 x 2.5 cm, one from a Se pre-treated plant and one from a control plant. In each container one aphid at a time was placed in between the two leaf pieces and its movement was recorded over a period of 1 min. If the animal had moved onto one of the leaf pieces the choice was scored as ‘–Se’ or ‘+Se’, and if the aphid was not on a leaf it was scored as ‘no choice’. For each experiment 100 aphids were scored in this way. The Se levels in the leaves used were determined as described below under ‘elemental analysis’.

Nonchoice feeding experiments

To determine whether Se-containing plants are toxic to these aphids and at which concentration, nonchoice feeding experiments were performed using intact plants or detached leaves from plants containing different Se levels. For the intact plant experiment, four cages were used, each containing three plants of a given Se treatment (0, 10, 20, or 40 µM Se). The plants were pre-treated for 7 d before transfer to the cages. At 0 d 20 aphids per cage were placed on a platform connecting the three plants. After 7 d the number of aphids on each plant was counted. Leaves were sampled and the leaf Se concentrations were analysed.

The detached leaf nonchoice feeding experiments were performed over a period of 4 d in Petri dishes Each Petri dish
contained two leaves of a given Se treatment (0, 0.1, 1, 5, 10, or 20 µM Se for 7 d) with their petioles placed in wet cotton to keep the leaves fresh. At 0 d five aphids per Petri dish were placed on the leaves. After 4 d the number of aphids per dish was recorded. Eight Petri dishes were used for each Se concentration. After the experiment the leaves were analysed for Se concentration as described below under ‘elemental analysis’. When possible, aphids were also collected and analysed for Se concentration using the same method.

Selenium as an aphid control agent

Two experimental approaches were used to test the efficiency of Se as an aphid control agent for *B. juncea*. In one approach, Se was supplied systemically to the plants in a hydroponic system while in the other Se was applied topically via spraying. In each case aphid populations were established on the plants before the Se application.

For the systemic experiment, four cages were used, one for each treatment. Each cage contained 11 plants in hydroponics, with c. 200 aphids per plant. At 0 d the aphid population on each plant was quantified, and Se was added as sodium selenate to a final concentration of 0, 0.1, 5 or 10 µM. After 7 d the aphid population on each plant was measured and the change in population size from 0 d was calculated. For the topical application experiment, two cages were used, each containing 6 plants in pots. At 0 d the aphid population on each plant was measured, and the plants of each cage were sprayed every other day with either 20 µM sodium selenate, or with water as a control. After 7 d the aphid population on each plant was measured and the change in population size from 0 d was calculated.

Elemental and statistical analyses

Samples for Se analysis were dried for 2 d at 70°C. The material was acid-digested as described by Zarcinas *et al.* (1987) in order to extract the Se. The Se concentration in the digests was quantified by inductively coupled plasma atomic emission spectrometry (ICP-AES Fassel, 1978). The detection limit of this method is c. 0.05 mg kg⁻¹ d. wt.

For statistical analyses the software package JMP-IN version 3.2.6 was used (SAS Institute, Cary, NC, USA). Various statistical tests (ANOVA, t-test, χ²) were used for different experiments. Analysis of variance (ANOVA) was used for the data shown in Figs 1, 2, 4, 5, and 6. A Chi-square (χ²) test was used in the detached leaf choice experiment (Fig. 3), and a t-test was used for the data shown in Fig. 7. When one-way ANOVA was used, pairwise posthoc analyses were performed to determine which means differed significantly. Statistically significant differences (*P* < 0.05) are reported in the text and shown in the figures, as are mean, standard error of the mean, and number of replicates. Note: in some cases the standard error was too small to be recognized by the graphing program; in those cases no error bar is shown.

Results

Choice feeding experiments

After green peach aphids were allowed to invade a batch of hydroponically grown *B. juncea* plants containing different levels of Se, a difference in aphid population density was observed that was inversely correlated with the Se concentration supplied. There were seven times more aphids on the plants without Se compared with the plants treated with the lowest Se concentration of 1 µM; at treatments at and above 5 µM Se, few if any aphids were present (*P* < 0.0001, Fig. 1a).

It therefore appeared that plant Se accumulation can protect *B. juncea* against aphid colonization, and that a leaf Se concentration of 10 mg kg⁻¹ d. wt (as provided by the 1 µM treatment) is enough to have a pronounced negative effect on aphid colonization (Fig. 1b).

To test if these observed differences were indeed an effect of plant Se concentration and not due to another variable...
(e.g. position of the plants) the experiment was repeated using plants randomly positioned in cages, with the aphids placed in the centre among the plants. After 7 d almost all aphids were found on the control plants without Se \( (P < 0.01, \text{Fig. 2a}) \). The leaf Se concentration in the plants used (Fig. 2b) was comparable to the earlier experiment.

The reason why the aphids were found predominantly on the control plants may have been deterrence by the Se, toxicity...
of the Se leaves to the aphids, or both. To test whether the presence of Se in leaves deters aphid colonization, two choice experiments were performed in which individual aphids were given a choice between two leaf sections, one containing Se, and one without Se. In the initial choice experiment leaves were used from control plants and from plants pre-treated with 20 µmol selenate. The +Se leaves contained Se levels of 500 mg kg⁻¹ d. wt (Fig. 3a). The aphids showed a strong preference to move toward the leaf section without Se (P < 0.0001, Fig. 3a), avoiding the Se leaves. When the experiment was repeated using leaves from plants treated with 1 µmol Se, containing only 13 mg kg⁻¹ d. wt Se, a similar preference was found (Fig. 3b, P < 0.001). Thus, the aphids were able to detect Se-containing leaves, and avoided them.

Nonchoice feeding experiments
Another reason why the aphids were found predominantly on the control plants may have been toxicity of the Se leaves to the aphids. To test this, two nonchoice experiments were performed, one using intact plants treated with different Se concentrations (0–40 µmol) and the other using detached leaves from Se-treated plants. On the intact control plants without Se, the aphids were successful in colonizing and reproducing (Fig. 4a). However, on the intact plants containing Se at levels of 125–750 mg kg⁻¹ d. wt few if any aphids were found after 7 d (Fig. 4a,b, P < 0.01). Therefore, levels of leaf Se above 125 mg kg⁻¹ d. wt appear to be lethal to aphids.

In the follow-up nonchoice feeding experiment using detached leaves, toxicity could be observed at much lower leaf Se concentrations (Fig. 5). Aphid population size was inversely correlated with both supplied Se concentration and leaf Se concentration (Fig. 5a–c, P < 0.0001). From extrapolation (Fig. 5c, and 5c inset), the leaf Se concentration leading to a 50% reduction in aphid population growth compared to control plants appears to be 1.5 mg kg⁻¹ d. wt. At Se treatments of 1 µmol and higher dead aphids were observed, indicating that a leaf Se concentration ≥10 mg kg⁻¹ d. wt is lethal to these aphids. Aphids collected from the 1 µmol treatment contained 17.4 mg Se kg⁻¹ d. wt, i.e. more than 2-fold higher levels than in the leaves they were on. Thus, it appears the aphids did ingest Se from the phloem of the leaves and that Se may have been the cause of toxicity.

Selenium as an aphid control agent
The combined effect of Se as a deterrent and a toxin to aphids may make Se an efficient aphid control agent. To test this,
two approaches were used. In one approach, Se was supplied systemically to aphid-harbouring plants in a hydroponic system while in the other Se was applied topically via spraying. Selenium supplied systemically as selenate was quite efficient at reducing aphid population growth (Fig. 6a, \(P < 0.01\)). While the aphid populations on the control plants increased by 35% in 7 d, the aphid populations on the Se-treated plants decreased markedly as leaf Se concentration increased, up to 75% reduction for the 10 \(\mu M\) treatment (Fig. 6a,b). It can be concluded from this experiment that Se supplied systemically at a concentration as low as 0.1 \(\mu M\) (corresponding to a leaf Se concentration of c. 2 mg kg\(^{-1}\) d. wt) is effective in preventing aphid population growth.

Selenium applied topically via spraying also reduced aphid population growth although less effectively than systemic application. While the control aphid populations on \(B.\) \(juncta\) plants almost doubled in size over the test period, the number of aphids on plants sprayed with 20 \(\mu M\) selenate decreased by 20% (Fig. 7a,b). Thus, topical application of a 20 \(\mu M\) selenate solution resulted in a decrease in aphid population growth similar to a 0.1 \(\mu M\) systemic selenate treatment.

**Discussion**

The objective of this study was to investigate whether Se offers plants any protection from phloem feeding herbivores. \(B.\) \(juncta\) and the generalist aphid \(M.\) \(persicae\) were chosen as a model system for this study. Both species have a worldwide distribution, and Brassica species are known hosts to this aphid. \(B.\) \(juncta\) and \(M.\) \(persicae\) thus may interact in natural and agricultural settings. The results presented here show that Se accumulation in \(B.\) \(juncta\) is effective against colonization by \(M.\) \(persicae\). The protective effect of Se was both due to deterrence and toxicity: the aphids avoided Se leaves when given the choice, and when forced to feed on Se leaves the aphids died, likely due to Se ingestion. As little as 2 mg kg\(^{-1}\) d. wt of...
Se in leaves was enough to be toxic, and 10 mg kg\(^{-1}\) d. wt was enough to deter aphids (no lower levels were tested).

These results fit into a pattern of earlier reports that Se in plants can protect them from invertebrate herbivory (Trumble et al., 1998; Vickerman & Trumble, 1999; Bañuelos et al., 2002; Vickerman et al., 2002; Hanson et al., 2003). Selenium was also shown to protect plants against fungal infection (Hanson et al., 2003). These results shed light on the possible evolution of Se hyperaccumulation. The leaf Se concentration effective against aphid colonization in these experiments (10 mg kg\(^{-1}\) d. wt) is two orders of magnitude below those typically observed in the field in Se hyperaccumulators (Beath et al., 1939a,b; Feist & Parker, 2001). It is therefore feasible that the Se levels occurring in plants in the field are protecting the plants from herbivory by aphids. Indeed, similar results were obtained for the hyperaccumulator Stanleya pinnata. In a small-scale study, glasshouse-grown S. pinnata plants that were colonized by aphids were treated systemically with Se (20 µm selenate), or without Se as a control. In the absence of Se there was a 211% increase in aphid population size over a 7 d-period, while the application of Se resulted in a 28% decrease. Thus, aphids may be a natural herbivore of this hyperaccumulator and Se may protect the plant from aphid colonization in the field as well. Together, these results support the hypothesis that Se hyperaccumulation functions as a defence mechanism against invertebrate herbivory, and that protection from invertebrate herbivory may have driven the evolution of this trait.

The observation that the aphids contained Se after feeding on phloem fluid of selenate-treated plants suggests that Se was transported through the phloem. This is in agreement with the idea that selenate is assimilated into organic forms in the leaf and transported in the phloem to roots and other organs as organic Se (Pilon-Smits et al., 1999). The finding that plant Se accumulation is toxic to this phloem feeder is different from the report by Boyd and Martens (1999) that Ni accumulation in Streptanthus polygaloides offered no protection against the pea aphid (Acyrthosiphon pisum). This difference may be because Ni is less toxic to aphids, but also because the phloem Ni concentration may be lower than that of Se.

As already mentioned by Beath (1959), Se may have application as an insecticide. Our studies showed that systemically supplied Se as 0.1 µm selenate effectively inhibits aphid population growth. Topical application did not have as pronounced an effect: spraying with 20 µm selenate gave similar reduction in aphid population growth to 0.1 µm systemically applied selenate. The reason why spraying the plants with Se was less toxic to the aphids than systemic application of Se may be that when sprayed the Se is not necessarily ingested by the animals, in contrast to systemically applied Se, and Se needs to be ingested to be toxic. Also, in the systemic experiment Se was bioconcentrated by the plants to values c. 20-fold higher than in the external medium. Finally, the form of Se that the aphids consumed may have been different in the systemic experiment compared to the spraying fluid. As mentioned, Se is thought to be assimilated into organic forms in plant leaves and then transported in the phloem; organic Se is more toxic to animals than selenate (Wilber, 1980). Based on these findings, Se may indeed be useful as an insecticide, and systemic application appears to be more efficient than topical application. However, systemically applied Se leads to Se accumulation in plant tissue (e.g. 2 mg kg\(^{-1}\) d. wt when supplied with 0.1 µm selenate), which may be undesirable depending on the use of the plant material. On the other hand, Se is an essential nutrient for humans and animals, and Se deficiency occurs in many areas of the world, especially in livestock. Thus, Se application to crops could function in the dual roles of insecticide and food fortifier, provided the plant Se levels are monitored carefully and if needed the Se-containing material is mixed with low-Se material to prevent toxicity. The interaction of B. juncea with herbivores is also of applied significance in view of the fact that monocultures of this species are currently being grown for phytoremediation of Se (Bañuelos et al., 2002).

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