Selenium Biotransformations in an Insect Ecosystem: Effects of Insects on Phytoremediation

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Phytoremediation of selenium-contaminated soils may be influenced by higher trophic levels including insects. We examined how selenium affects the behavior, survival, and development of the wasp parasitoid *Cotesia marginiventris*, parasitizing its natural host, the beet armyworm *Spodoptera exigua*, feeding on alfalfa *Medicago sativa*, irrigated with water containing selenate. X-ray absorption spectroscopy was used to quantify the selenium chemical forms in each trophic level. Alfalfa partially transformed selenate to organoselenium, both directly absorbed from *M. sativa* and transformed from selenate. *C. marginiventris* cocoons collected shortly after larval emergence contained only organoselenium derived from the host. The surprising finding of trimethylselenonium-like species in adult parasitoids and the cocoons from which they emerged suggests that adults and pharates can detoxify excess selenium through methylation and volatilization. Adult parasitoids do not discriminate against selenium-containing alfalfa, even though alfalfa generates selenium volatiles. Parasitoids raised on selenium-fed larvae emerged later and pupae weighed less than their selenium-free counterparts. We conclude therefore that *C. marginiventris* can be used to control *S. exigua* damage to *M. sativa* being used to remove selenium from soils. Moreover, the presence of such insects may improve phytoremediation by increasing biotransformation of inorganic selenium and release of volatile selenium species.

Introduction

The element selenium (Se), which is a necessary micronutrient for humans and other vertebrates, is known to be toxic at levels only slightly higher than those required to support life (1, 2). This is also true for insects; control diets lacking Se have been shown to decrease weight, fertility, and survival compared to low-level Se-amended diets, but high Se levels also caused these parameters to decrease (3, 4). Toxicity of Se to terrestrial insects varies by species and developmental stage as well as with the form and concentration of Se (3–10).

Selenium contamination is a global problem originating from a multitude of sources including the following: mine tailings; combustion of fossil fuels; production of glass, pigments, inks, and lubricants; and leaching and concentration of Se in drainage water through agricultural irrigation or rainfall on naturally seleniferous soils (11–13). Although sometimes occurring from point sources, Se contamination is not always localized. For example, in the western United States, soil contamination from agricultural irrigation has become a serious problem affecting 1.5 million acres of farmland in eight states (14).

Remediation strategies for Se-contaminated soils include volatilization or reduction by soil bacteria (15) and phytoremediation—the use of plants to accumulate and volatilize Se with ultimate removal of the Se-containing plant tissues (16). However, to date, the effect of insect populations on phytoremediation has not been explored. Insects in the next trophic levels may biotransform, accumulate, or volatilize selenium. Phytoremediation strategies which accumulate Se in plant tissue at the same time increase the availability of Se to herbivorous insects (9) and therefore to their natural enemies. The resulting insect excreta, and eventually the insects, in turn become available to detritivores. A reduction in plant growth resulting from herbivore feeding could alter remediation efforts, change the distribution of selenium species, and thereby change the time that Se remains biologically available at contaminated sites.

Selenium irrigation may increase plant resistance to some herbivorous insects (9, 10, 23). However, this does not seem to be the case with the beet armyworm *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) because first-instar larvae will feed on plants containing selenium and fourth-instar larvae readily consume alfalfa containing selenium at potentially lethal levels (10). Oviposition is also not reduced on selenium-rich plants (10), suggesting that *S. exigua* might continue to immigrate into Se-contaminated sites creating a population "sink". Parasitic insects in the third trophic level could therefore prey upon a continuous supply of immature herbivores.

Here, we seek to study the effect of selenium on phytoremediation by investigating three trophic levels—the crop plant alfalfa, fed upon by the beet armyworm larva, which in turn is parasitized by a wasp. Alfalfa (*Medicago sativa* L., Condor CT) takes up the largest amount of selenium of any forage crop and is known to volatilize Se (17, 18). It is one of many plants proposed for phytoremediation of Se-contaminated soils, as a soil amendment for Se-deficient soils (19), and as a livestock feed supplement (11, 20–22). The range of the beet armyworm, *S. exigua*, broadly overlaps regions of North America where Se contamination occurs. The toxicity of Se to *S. exigua* has been established for both Se-supplemented alfalfa and artificial diets (7, 10). The wasp *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae) is a solitary endoparasitoid known to parasitize the first and second larval instars of *S. exigua* (24, 25). The parasitoid larva develops inside the host and emerges through the body wall of the third-instar caterpillar, thereby killing the host. It spins a cocoon and pupates away from the host (25). The major life stages can be rapidly and precisely documented due to the short larval cycle and easily observed larval–pupal transition.
The chemical form of selenium is rarely considered in studies of natural systems, in part due to the lack of an effective in vivo probe. X-ray absorption spectroscopy (XAS) is ideal for studies of biological tissues and intact organisms because the samples can be examined essentially without pretreatment; XAS is element-specific and can yield information on the local electronic and atomic environment of the element being studied. Near-edge XAS can be used to quantitatively determine the chemical types of selenium present, including analysis of mixtures (26). The limitations of the method are that compounds present in very small quantities might escape identification and that specific information about the exact chemical form is generally not available (e.g., all alkylselenides look alike). In this study, for the first time, we use near-edge XAS analysis to determine the chemical form of the selenium found in vivo as it is taken up through three trophic levels—from the crop plant alfalfa irrigated with water containing sodium selenate, through the herbivorous beet armyworm S. exigua to the wasp parasitoid C. marginiventris. We also discuss how selenium biotransformation from inorganic selenate to organic selenium to volatile methylated selenium species may influence the viability of insects and their role in phytoremediation efforts.

Materials and Methods

Study Organisms. Alfalfa plants were grown in greenhouses at the University of California, Riverside, using coarse-grained silica sand cultures as previously described (10). The selenium irrigation level chosen for treatments (3.3 mg/L) represents a known toxicity level to the herbivore (10) but is below levels reported from parts of central California and the west side of the San Joaquin Valley (SJ V), CA, where levels exceeding 4 mg/L have been reported (27-29).

All insects were maintained in laboratory colonies at 27 ± 1 °C and a 16:8 (L:D) photoperiod with fluorescent lighting. The S. exigua colony was field collected in Ventura Co., CA, and maintained on an artificial diet (BioServ, Frenchtown, NJ). The C. marginiventris colony was obtained from a USDA colony (Phoenix, AZ) and maintained on S. exigua fed artificial diet. Adult wasps were provided a 25% honey solution on cotton wicks.

Olfactometer Bioassays. C. marginiventris use herbivore-induced plant volatiles for host location and respond strongly to plants with insect damage and frass (30, 31). Therefore, olfactometer treatments included (1) two blanks, water-filled micropipet tubes covered with Parafilm, pierced to add humidity; (2) four control alfalfa cuttings (four trifoliolate leaves each) with feeding damage and frass with the cut end placed into a water-filled micropipet tube covered with Parafilm, pierced to allow for insertion of the stems; (3) Se-irrigated alfalfa with feeding damage and frass prepared as described for the control.

 Newly eclosed wasps were mated on day two. On day three, following at least 1 h of acclimation to ambient light and temperature (24 ± 1 °C), individual females in tubes were transferred to the arena and assayed once. Females had no prior adult contact with plant materials, host larvae, or frass. The assay arena was an 8 cm diameter glass central chamber with four connecting 10 cm long (2.5 cm diameter) glass tubes (32). At the terminus, each tube was attached to a glass dish containing one of the three treatments. The central assay chamber was not in direct view of the treatment dishes, and these were isolated from the connecting tube by a tulle fabric screen. Air was drawn by vacuum across the treatment, through the tubes, and into the central chamber at a rate of 3 cm/s. The entire assay device was located so as to obscure the observer, eliminate polarized sunlight, and diffuse the light from overhead fluorescent fixtures. Treatment locations were randomized to preclude directional preferences.

An entry was defined as movement of the parasitoid 4.5 cm into the tube. Number and duration of entries as well as percent and average time spent inside the tube per entry were calculated. Forty-five individual females were assayed. Fifteen replicates were recorded with each replicate consisting of data from three females using the same treatment dishes. The glassware in contact with the parasitoid was washed with detergent and then acetone between observations. Each bioassay was filmed for 5 min and scored later using the Observer Program by Noldus (33).

Development and Survival. S. exigua were parasitized by exposing neonate larvae to adult female C. marginiventris for 24 h. Thirty potential host larvae were randomly assigned to each treatment group with five replicates per treatment, totaling 150 individuals per treatment. Larvae were contained individually with agar covering the bottom of the container to maintain humidity (after ref 34) and fed control or Se-alfalfa, ad libitum. The number of pupae, number of adults, pupal mortality, days from oviposition to parasitoid emergence/pupation, and days to adult eclosion, number of days from emergence to eclosion, and pupal weight were recorded for each treatment group. Although the average stage of death for S. exigua larvae fed Se at this concentration is the third instar (10), in these experiments all larvae lived until parasitoid emergence.

Selenate-Fed Larvae. S. exigua larvae were fed 31 μg g wet weight sodium selenate incorporated into artificial diet (based on lethal concentration LC50 values from ref 7). These unparasitized third-instar larvae were flash frozen with the gut contents intact for collection of selenium K-edge spectroscopy data.

Statistical Analysis. StatView software (35) was used for the following statistical analyses. Differences (P < 0.05) were determined for all of factor biomod bioassay parameters, among treatments using the nonparametric Kruskal–Wallis Test with posthoc separations between treatments determined using the Mann–Whitney U test. For the survival bioassays with C. marginiventris, comparisons were made between effects of host treatment group in each statistical analysis. A one-factor analysis of variance (AOV) was used to determine differences (P < 0.05) for comparison including number of parasitoids emerging/pupating and eclosing, the number of days to parasitoid larval emergence/pupation and adult eclosion, and the number of days from pupation to adult. Differences (P < 0.05) in percent survival from pupation to adult were determined using the Mann–Whitney U test.

Selenium Quantitation. Alfalfa leaves were collected at four time points, and five subsamples of each were prepared for Se analysis (10). Selenium was determined using inductively coupled argon plasma spectrometric analysis (ICP-MS) (36) at the Division of Agriculture and Natural Resources Laboratories, U. C. Davis as previously described (10) (note that the small size of parasitoids precluded selenium measurements).

Selenium Speciation: X-ray Absorption Spectroscopy. Host larvae and parasitoids were collected the day of parasitoid emergence but after parasitoid pupation. Se-alfalfa fed third-instar larva were placed on nonnutritive agar for 12 h to eliminate gut contents, whereas selenate-fed larvae were analyzed with gut contents intact. Treatment groups were frozen (–60 °C) and later freeze-dried. Selenium K-edge spectra were collected from freeze-dried Se-fed unparasitized third-instar larvae, whole adult parasitoids, parasitoid pupae in cocoons, empty parasitoid cocoons, and fresh selenate-fed S. exigua larvae. All samples were packed into sample cells and flash frozen, and spectra were collected at 10 K.

X-ray absorption spectroscopy data were acquired at the Stanford Synchrotron Radiation Laboratory (SSRL) on the
SPEAR storage ring (55–100 mA at 3.0 GeV) on beamlines 7–3 and 9–3 each equipped with a Si(220) double-crystal monochromator. The incident X-ray intensity was monitored using an N₂-filled ionization chamber. The Se K X-ray absorption near-edge spectra were recorded as Se Kα fluorescence excitation spectra using a 30-element germanium detector. Samples were kept at between 5 and 10 K in an Oxford instruments liquid helium flow cryostat, and the X-ray energy was calibrated with reference to the lowest energy/inflection point of hexagonal elemental selenium that was assumed to be 12 658 eV. Harmonic rejection was accomplished on beamline 9–3 using an upstream vertically collimating Rh-coated mirror and downstream refocusing Rh-coated mirror and on beamline 7–3, which has no focusing optics, by detuning one monochromator crystal to approximately 50% off peak. Standards (sodium selenate, sodium selenite, seleno-DL-methionine, and trimethylselenonium iodide, obtained at the highest available purity from commercial sources and used without further purification) were measured as frozen dilute aqueous solutions (5 mM with 30% v/v glycerol) under the same conditions. Data analysis was performed using the EXAFSPAK suite of computer programs (37). Quantitative determination of the different chemical forms of selenium present was carried out by least-squares fitting of the near-edge spectra to linear combinations of the spectra of standards, as previously described (26, 38).

Results and Discussion

Alfalfa Concentrates and Partially Transforms Selenium.

Alfalfa Se concentrations, determined by ICP-MS, were 1.4 ± 0.2 μg Se/g dry weight for control plants and 327 ± 21 μg Se/g plant dry weight for plants irrigated with selenate at 3.3 mg/L or 3.3 μg Se/g water. In general, most forage and crop plants, as well as grasses, contain less than 25 μg Se/g plant dry weight and do not accumulate Se much above a ceiling of 100 μg Se/g plant dry weight when grown on seleniferous soils (39). While exceeding this ceiling, the alfalfa levels are somewhat lower than observed for Brassica juncea (Indian mustard) grown hydroponically under ideal conditions for selenium accumulation (at least 2000 μg Se/g plant dry weight) (40). Selenium hyperaccumulators such as Astragalus bisulcatus can achieve several thousand micrograms Se/g dry weight (39). X-ray absorption spectroscopy of the alfalfa tissue (see below) shows that selenium is mainly partitioned between unselenated selenate and an organic species modeled as selenomethionine. Thus, while the plant system can at least partially transform the selenate, transformation is not complete as selenate reduction is the rate-limiting step in incorporation into plants (41).

Parasitoid Females May Detect but Do Not Avoid Se-Alfalfa.

C. marginiventris females preferred tubes containing control or Se-irrigated plants to blank tubes. They entered more frequently, spent more total time, percent time, and more time per visit than in blank tubes (Table 1). For treatments offering plant material, parasitoids entered the tubes containing the Se-irrigated treatment more often than the controls, but for all other measures there were no differences found in parasitoid preference. Although significant differences were found for all preference parameters calculated among the three treatments, the differences between the individual plant treatments and the blank appear to explain the majority of this difference.

The observation that Se irrigation of alfalfa increases parasitoid approach suggests that C. marginiventris can detect selenium volatiles or volatiles related to selenium toxicity in plants. However, little or no preference was demonstrated between Se-treated and untreated alfalfa. This limited preference and lack of avoidance of Se-rich plants is also seen in ovipositing S. exigua females and larvae (10). Although C. marginiventris responds to plant volatiles produced by insect damage and frass (30, 31) and Se-alfalfa releases S volatile, our data show no Se-volatile-specific behavioral response. We conservatively conclude that C. marginiventris females will not avoid Se-rich plants when searching for potential hosts.

Selenium Slows the Development of Hosts and Parasitoids.

Parasitoid larval development and adult eclosion (emergence from the pupal case) took 2 days longer if the host was fed Se-irrigated alfalfa (Table 2). Since there was no significant change in the number of days from pupation to adult, the main increase in parasitoid development time occurred during the larval stage. However, we previously showed that similar levels of Se in alfalfa significantly
Selenium Is Biotransformed at Each Trophic Level to Less Toxic Chemical Forms. The chemical form of selenium changed as it passed through the different trophic levels from plant through host larva to pupal and adult parasitoid, as determined using X-ray absorption spectroscopy (Figure 1). In selenate-irrigated alfalfa, more than one-half (57%) of the absorbed selenium was transformed to organic forms with most of the rest (39%) remaining as selenate, together with a trace of selenite (4%) (Figure 2a, Table 3). In contrast, gut-empty larval S. exigua fed on Se-alfalfa (Figure 2c) contained only organic forms of selenium, modeled as aqueous selenomethionine. Since alfalfa contained both selenate and organic selenium, organic selenium measured in S. exigua could have been selectively absorbed from alfalfa. Therefore, we sought to determine whether S. exigua could absorb selenate and transform it to organic selenium. Spectra collected from gut-full larvae fed an artificial diet containing sodium selenate (Figure 2b) were modeled with 43% selenomethionine, 38% selenate, and 19% selenite. We conclude that S. exigua can absorb selenate from the diet and convert it to organic selenium. Some selenite is concentrated in fecal pellets (45), but the proportion of total dietary selenium that is expelled in the frass remains to be determined. The lack of selenate in Se-alfalfa fed larvae could also be explained by selective feeding on parts of the alfalfa plant that lack selenate; the hyperaccumulator A. bisulcatus shows substantial variation in the ratio of selenate to organic selenium in different ages of leaves (46, 47), but no evidence of selective feeding on alfalfa was observed.

After emergence from their Se-alfalfa fed host larva, C. marginiventris pupae contained only organic selenium, the same as the host (Figure 2d). It appears therefore that during their development, juvenile parasitoids do not biotransform selenium absorbed from the host. An unexpected finding in newly eclosed adult parasitoids (Figure 2f) and in the mature pupal cases (Figure 2e) from which the adults emerged was that high levels of a selenium species not seen in alfalfa, S. exigua larvae or juvenile parasitoids. The spectra for adult parasitoids and mature cocoons fit well to a mixture of predominantly aqueous selenomethionine and the dimethylselenonium cation (Figure 2e,f). In mammals, injected excess selenium is thought to be removed by reduction to selenide followed by either incorporation into selenoproteins or methylation to methylselenol, dimethylselenide, or dimethylselenonium with methylselenol and trimethylselenium being excreted in urine, while dimethylselenide is released by respiration (48). The pupal cases after adult emergence additionally show a component of selenite. It remains to be determined if cell turnover and autophagy during metamorphosis release selenium to the atmosphere.

Selenium Is Less Toxic to Parasitoids than to Predators. Spodoptera exigua, feeding on Se-alfalfa, consumes a mixture of selenomethionine and more toxic selenate, whereas the parasitoid is exposed only to organoselenium (in an unknown ratio of free amino acids and selenoproteins). In this way the parasitoid benefits from the detoxification mechanisms of its host such that if the host organism survives on this diet, so will the parasitoid. This would reduce the selective pressure on C. marginiventris to avoid plants releasing Se volatiles.

In contrast to this system, the predator Podisus maculiventris Say (Hemiptera: Pentatomidae) fed on selenate-fed S. exigua larvae exhibited signs of toxicity, took longer to develop, weighed 20% less, and had 30% higher mortality than controls (49). In this case, we speculated that the predator may consume more selenium, be exposed over a longer time, or be exposed to different chemical forms of selenium than the parasitoid. The XAS data indicate that the forms of selenium to which the parasitoid and the predator are exposed are different. The predator feeding on Spodoptera larvae fed a selenate-enriched artificial diet consumes roughly one-half of its total selenium as selenate (Figure 2b). In contrast, a parasitoid developing in a Se-alfalfa-fed larva is bathed in organic selenium species and never exposed to selenate. Moreover, the parasitoid, developing in the host hemolymph is exposed to a concentration of selenium that is tolerated by the host larva, whereas selenium intake by the predator would decline only when selenium toxicity decreased its appetite for larvae.

Selenium Exposure Reduces Pupal Weight and Slows Development but Does Not Influence Survival. The numbers of parasitoids surviving as pupae and adults were not reduced by exposure to selenium (Table 2). Furthermore, there was no increased mortality during the pupal stage of parasitoids from Se-fed hosts (as indicated by the percent survival from pupation to adult) (Table 2). However, parasitoid cocoons from the Se group weighed 10% less than the cocoons of parasitoids from control-fed hosts (Table 2). This delayed development on Se-containing plants would increase the time that host and parasitoid larvae are exposed to unfavorable environmental conditions and predation and would likely result in a decrease in the intrinsic rate of population increase. Additionally, because of reduced sizes and weights, parasitoids from Se-containing hosts could be expected to have slightly reduced fitness, as observed in other parasitic hymenoptera (43, 44), but this needs to be experimentally confirmed.

Increased the development time of the host (10). Thus, we could not determine if selenium was directly affecting the development time of the parasitoid or if the effect occurred indirectly through delayed growth of the host. This same difficulty was also observed for another Braconid parasitoid developing in a Lepidopteran host under metal stress (42).
At the first trophic level, alfalfa takes up selenate but biotransforms only a portion of the selenate to organic forms. It has been proposed that alfalfa reduces overall tissue methionine synthesis as a means of reducing selenium toxicity (18). The second trophic level is exposed to both organic selenium and the more toxic selenate. *S. exigua* biotransforms all absorbed selenate to organoselenium and releases unabsorbed selenium in the frass. If the third trophic level is a parasitoid, it is exposed only to organic selenium at levels controlled by the host such that if the host survives, so does the parasitoid. A predatory third trophic level would likely experience greater toxic effects since it is exposed to both organoselenium and unabsorbed selenate in the gut contents of the herbivore. There is evidence that during metamorphosis and after emergence of the adult parasite, the body burden and toxicity of selenium is further reduced by methylation and/or volatilization and some selenium is discarded with the cocoon. The methylation pathway seems to be triggered by metamorphosis since there is no evidence that the host or juvenile parasitoid methylate selenium.

**Insects Are Natural Components of Phytoremediation Efforts.** The life cycles of *S. exigua* and *C. marginiventris* are compatible with and could enhance phytoremediation programs. *C. marginiventris* emerges from and kills *S. exigua* during the third instar before the host succumbs to the toxic effects of Se. When killed by the parasitoid, third-instar larvae have lower total Se than later instars, and so less selenium is returned to the soil through microbial degradation of the carcass.

Selenium volatilization is an important route to decontaminating seleniferous soils through phytoremediation, but volatile production is limited by the chemical form of selenium in the soil and other soil conditions such as salinity. The presence of insects may improve phytoremediation by increasing biotransformation of inorganic selenium and release of volatile selenium species. The biotransformation

**FIGURE 2.** Quantitative analysis of Se near-edge spectra. Each panel shows the spectrum of an organism (filled circles) together with the best fit (—). The components of the fit are shown scaled by their relative contributions: selenate (····), selenite (·-·-), trimethylselenonium (- - - -), and selenomethionine (- - - -). See Table 3 for numerical results of the fits. (a) Se-irrigated alfalfa; (b) *S. exigua* third-instar larva fed on selenate-enriched diet; (c) *S. exigua* third-instar larva fed on Se-irrigated alfalfa; (d) *C. marginiventris*, newly pupated; (e) *C. marginiventris* empty cocoons, post-emergence; (f) *C. marginiventris*, adult.
TABLE 3. Percent Selenium Species in Trophic Level and Parasitoid Development Stage

<table>
<thead>
<tr>
<th>organism</th>
<th>selenate</th>
<th>selinite</th>
<th>seleno-methionine</th>
<th>trimethyl selenium</th>
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<tbody>
<tr>
<td>Se-irrigated alfalfa</td>
<td>39(3)</td>
<td>4(2)</td>
<td>57(3)</td>
<td></td>
</tr>
<tr>
<td>Se-alalfa-fed larvae</td>
<td>100(1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>selenate-fed larvae</td>
<td>38(2)</td>
<td>19(3)</td>
<td>43(4)</td>
<td></td>
</tr>
<tr>
<td>parasitoid pupae</td>
<td>100(1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>post-eclosion cocoons</td>
<td>7(1)</td>
<td>67(2)</td>
<td>26(2)</td>
<td>75(2)</td>
</tr>
<tr>
<td>adult parasitoids</td>
<td></td>
<td></td>
<td></td>
<td>25(2)</td>
</tr>
</tbody>
</table>

*Values derived from the percentage contributions of spectra of selenium species (all in dilute aqueous solution) to the best fit of the spectrum of the organism. Figure 2 shows plots of the fits and components. The figure in parentheses after the value is three times the estimated standard deviation, as derived from the diagonal elements of the covariance matrix.

and apparent methylation of selenium by a phytophagous insect, and its parasitoid adds an important new dimension to Se remediation.

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