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Influence of form and quantity of selenium on the development and survival of an insect herbivore

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Abstract

Even plants classified as 'nonaccumulators' can sequester concentrations of sodium selenate, sodium selenite, selenocystine and selenomethionine that can strongly influence insect development and survival. These forms of selenium (Se), tested in diet-incorporation bioassays, proved toxic to larvae of a generalist insect herbivore at relatively low levels. Sodium selenite was the most toxic form tested against *Spodoptera exigua* (Hübner), with an LC_{50} of 9.14 $\mu g\,g^{-1}$ wet wt (21.11 $\mu g\,g^{-1}$ dry wt). Selenocystine was intermediate with an LC_{50} of 15.21 $\mu g\,g^{-1}$ wet wt. The least toxic forms, sodium selenate and selenomethionine, had LC_{50}s below 50 $\mu g\,g^{-1}$ dry wt, the upper level for tissues of plants classified as nonaccumulators. Ingestion of some forms of Se also affected growth and development. Increasing concentrations of sodium selenate and sodium selenite decreased pupal weight and added significantly to the time needed for development to the pupal and adult stages. The time required to complete the larval stage increased by over 25% and the time from egg to adult emergence was extended by 22% to nearly 30%. Selenocystine and selenomethionine did not significantly increase developmental times, even at concentrations that killed 90% or more of the test populations. Analyses of relative growth rate, relative growth index, and an analysis of covariance technique for measuring growth indicated that the form of Se affected growth rates, growth inhibition responses of the larvae, and toxicological effects. Thus, quantity and the form of Se accumulating in plants grown on Se-contaminated sites are likely to influence the population dynamics of insect herbivores. The implications of these results for the ecology of contaminated sites are discussed.

Keywords: Selenium; Herbivory; *Spodoptera*; Reclamation; Antifeedant

1. Introduction

Insects are critical components of most terrestrial and freshwater ecosystems. They serve not only as an important part of the food web for higher trophic levels, but as key herbivores and recyclers. Despite these diverse roles, little is known about how some pollutants affect insects (Heliovaara and Vaisanen, 1993). In particular, information on the effects of selenium (Se) on insect growth and survival is quite limited. This is somewhat surprising given the world-wide importance of Se contamination resulting from geochemical processes, from mining operations, and from a variety of industrial sources (Frankenberger and Benson, 1994). Considerably more information is available for Se accumulation and elimination in mammals (Daniels, 1996), avians (Heinz et al., 1990) and fish (Limly, 1996).

Previous studies where insects were fed Se focused on the peroxidation responses of houseflies to low levels of Se in their drinking water (Simmons et al., 1989a, b), or similar physiological interactions with other insects (Nakonieczny, 1993). These reports utilized insects as a model system for measuring metabolic activity, rather than examining potential population responses to Se contamination. Hogan and Cole (1989) and Hogan and Razniak (1991) reported that beetles infesting grain and other dried, stored products suffered additional mortality when exposed to diets containing 0.125–1.00% sodium selenite. These relatively high rates correspond to concentrations of 1250 to 10 000 $\mu g\,g^{-1}$. Thus, data are lacking that provide insight into individual and population level responses to Se for insects attacking living plants, most of which accumulate Se at concentrations below 100 $\mu g\,g^{-1}$ (Banuelos et al., 1997). Such information may become essential as efforts increase to produce sustainable remediation programs using plants.

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Many plant species have been studied for use in remediation programs for Se-contaminated soils, including a selection of agricultural and weed species (Nyberg, 1991; Parker and Paige, 1994; Wu et al., 1996; Banuelos et al., 1996, 1997). Generally, plants have been categorized as high, moderate or 'nonaccumulators' (Rosenfeld and Beath, 1964). The high accumulator group may sequester hundreds to thousands of $\mu g\,g^{-1}$ Se (dry wt of plant tissue), while the 'moderate accumulators' develop concentrations of 50–100 $\mu g\,g^{-1}$. Those plant species categorized as 'nonaccumulators' do not concentrate more than 50 $\mu g\,g^{-1}$ plant tissue.

Several reports have detailed the uptake and fate of Se in plants. Plants can accumulate Se as either sodium selenate, sodium selenite, or in organic forms such as selenomethionine or selenocystine, but the most common soluble form of Se in agricultural drainage water is sodium selenate (Cutter, 1982; Terry and Zayed, 1994). When accumulated as sodium selenate, plants generally convert it to sodium selenite. The Se in sodium selenite is then substituted for sulfur in certain amino acids, commonly producing selenomethionine, selenocysteine or selenocystine (Presser et al., 1994). These Se-containing amino acids may account for 10% (Wu et al., 1997) or more than 50% (Ge et al., 1996) of the total Se in the plant. All of these forms have been found in leaves, stems and roots (Terry and Zayed, 1994; Ge et al., 1996).

Measuring the effects of such materials on insect growth and development is not always a simple process. The gravimetric techniques developed by Waldbauer (1968) have been used extensively. In this approach, relative growth rate (RGR) is measured as mg biomass gained/mg larval biomass/day. However, some problems and limitations of this technique have been identified (Slansky, 1985; Schmidt and Reese, 1986; Bowers et al., 1991). Most of these concerns relate to potential magnification of errors associated with weighing and determining energy contents of the food. An additional concern is that Waldbauer's gravimetric technique relies on the use of ratios to increase the precision of measurements and remove confounding effects of body size; autocorrelations (ratios with the same measurements in both the numerator and denominator) can result (Raubenheimer and Simpson, 1993). Nonetheless, RGR is still commonly used and comparisons of RGR values are useful. However, because of the limitations of RGR, two other indices have been developed: the relative growth index (RGI) and an analysis of covariance (ANCOVA) technique.

The RGI measures growth inhibition effects of compounds in plants (Zhang et al., 1993). This index is based on a comparison of the instar insects could reach in the absence of the test material (control) versus that attained in a treatment where the material is present. In an alternative approach, Raubenheimer and Simpson (1993) and Horton and Redak (1993) suggested the use of an ANCOVA which incorporates the effects of initial weight on final weights. This technique has the advantage of decreasing the type II error rate, providing additional information from the data, and reducing the error around the dependent variable.

Although much information is available on the effects of Se on mammals (Daniels, 1996), avians (Heinz et al., 1990) and fish (Limly, 1996), little information has been published regarding insects. The primary objective of the research presented here was to evaluate the effects of various forms of Se on a generalist insect herbivore. The insect species *Spodoptera exigua* (Hübner) was chosen because of its broad host range that includes plants in the families Liliaceae, Fabaceae, Solanaceae, Malvaceae, Chenopodiaceae, Apiaceae, Asteraceae, and Amaranthaceae (Metcalf and Flint, 1962; Peterson, 1962). Many of these, including alfalfa and wild *Brassica* species, have been suggested as candidates for bioremediation of Se-contaminated sites (Banuelos et al., 1991; Khattak et al., 1991; Arthur et al., 1992; Ge et al., 1996). In addition, an artificial diet was available for this insect which allowed precise comparisons of the effects of Se without possible interactions with plant defensive compounds.

2. Materials and methods

2.1. Test insects

All experiments were initiated with first instar larvae (within 12 h of eclosion) of *S. exigua* obtained from a laboratory colony maintained at 28 ± 2°C and 16:8 (L:D) photoperiod on an artificial diet modified from Patana (1969). The colony was originally collected from Orange Co., California, USA, and has had new genetic material added every 6 to 12 months.

2.2. Diet preparation

The water-soluble forms of Se, including sodium selenate, sodium selenite, seleno-DL-methionine, and seleno-DL-cystine, were obtained from Sigma Chemical Company (St. Louis, Missouri, USA). To achieve the desired concentrations of the Se compounds, the materials were added to 300 g diet, blended for 5 min and then dispensed into 30-ml clear plastic cups (*ca* 10 ml diet/cup). Diets were left to cool at ambient temperature and one neonate *S. exigua* was allowed to develop in each cup. To determine dry weight concentrations in diets, 10 diet cups were weighed, dried at 60°C for 3 days, and then weighed again. Unless specifically noted, all concentrations in diets reported in the text and tables are in wet weight (as typically given in the literature on herbivory), but can be converted to dry weight (as used

in the soils literature) by multiplying by 2.31 for comparison. All concentrations reported are for the specific Se compounds, and not for the Se component alone.

2.3. Bioassays

All bioassays were maintained at 28±2°C, *ca* 75% RH, and 14:10 (L:D) photoperiod with fluorescent lighting. For determination of LC_{50}s (lethal concentrations that kill 50% of the test population), five to seven concentrations of each of the four forms of Se were tested individually to create the log–dose probit lines (Finney, 1971). The appropriate range of test concentrations was chosen following pilot studies. Each test concentration was replicated three to four times for a total of 350–400 larvae for each form of Se. For these experiments, larvae were placed on control or test diet as neonates (first instars) and held through adult emergence from the pupal stage. Control mortality was less than 10% in all replicates.

All surviving larvae were used for RGR and RGI analyses. Larvae were examined daily. Mortality and larval development time in days from egg hatch to pupation and adult eclosion were recorded. Larval weight at 7 (fourth instar) and 10 days (fifth instar), and pupal weight 1 day after pupation, were recorded. Recording larval weights at these days allowed larvae to reach a size adequate to minimize weighing errors, yet provided adequate time for measurable growth. RGR values were obtained from 7- and 10-day weights using the formula provided by Waldbauer (1968), where RGR = mg gained/mg larval biomass/day. GI (growth index) and RGI (relative growth index) values were calculated as described by Zhang et al. (1993), where GI is calculated as:

$$\mathrm{GI} = \frac{\sum_{i=1}^{i_{\max}}[n_{(i)} \times i] + \sum_{i=1}^{i_{\max}}[n'_{(i)} \times (i-1)]}{N \times i_{\max}},$$

where $i_{\max} = 5$, the highest attainable instar of the insect at 10 days and n is the number of insects. RGI was determined as:

$$\mathrm{RGI} = \frac{\text{GI of the test group}}{\text{GI of the control group}}.$$

If RGI values do not differ between concentrations of the same test material, then the material would not be considered a growth inhibitor (Zhang et al., 1993).

Statistical comparisons were conducted using Super-ANOVA (1989). *Post hoc* comparisons were made as appropriate with Tukey's HSD test at the $p < 0.05$ level.

For the ANCOVA analyses, larvae were reared to day 5 (third instar) on control diets, weighed (initial weights), and then transferred to either a control diet or diet containing one of the four forms of Se tested. Final weights were recorded at day 7. Recording larval weights at these days allowed larvae to reach a size adequate to minimize weighing errors, yet provided adequate time for measurable growth. ANCOVA analyses were then conducted as previously described (Horton and Redak, 1993; Raubenheimer and Simpson, 1993) by using initial weights as a covariate, and final weight as the dependent variable. The slopes of the lines resulting from the concentrations tested within each form of Se provided information: if the slopes were not significantly different but the lines were shifted downward with increasing concentration, then the rate of growth was unchanged but consumption was likely reduced. If the slopes of the lines were significantly different, then growth rate changed, and a physiological (often toxic) response would be suspected (Horton and Redak, 1993).

3. Results

All of the forms of Se tested were toxic at relatively low levels to *S. exigua* (Table 1). Slopes of the log–dose probit lines were steep (range 4.52–8.15), indicating that population responses were homogeneous. Sodium selenite was the most toxic form tested, with an LC_{50} of 9.14 $\mu g\,g^{-1}$ wet wt (21.11 $\mu g\,g^{-1}$ dry wt). The least toxic forms, sodium selenate and selenomethionine, still had LC_{50}s below 50 $\mu g\,g^{-1}$ dry wt, the upper level for non-accumulator plants.

Ingestion of dietary concentrations of 12 $\mu g\,g^{-1}$ for both sodium selenate and sodium selenite significantly reduced pupal weights (Table 2). Pupal size is of importance in *S. exigua* because body size tends to be correlated with fecundity for *Spodoptera* (Rothschild, 1969). No differences were observed for selenomethionine. Pupal weights were variable when larvae were fed selenocystine, but even at 28 $\mu g\,g^{-1}$ in diet the resulting pupae did not weigh significantly less than the controls.

The times needed from egg to pupation and from egg to adult eclosion were also affected by the form and concentration of Se ingested (Table 2). Increasing concentrations of sodium selenate and sodium selenite added significantly to the time needed for development. The time required to complete the larval stage increased by over 25% and the time from egg to adult emergence was increased by 22% to nearly 30%. Surprisingly, despite the similar LC_{50}s for sodium selenate and the two substituted amino acids, there were no statistically significant increases in developmental times when test larvae were fed selenomethionine or selenocystine as compared to control larvae (Table 2).

The form of Se fed to larvae had an important effect on RGR (Fig. 1). Even at the low concentration of 4 $\mu g\,g^{-1}$ in diet, sodium selenate significantly depressed

Table 1
Toxicity of sodium selenate, sodium selenite, selenocystine and selenomethionine to *S. exigua* when incorporated into artificial diet

Treatment	n [a]	Slope–SE	LC_{50} (95% FL) [b]
Sodium selenate	350	8.15–1.42	21.41 (20.31–22.95)
Sodium selenite	350	4.52–0.88	9.14 (7.50–10.64)
Selenocystine	400	5.59–1.02	15.21 (12.38–17.42)
Selenomethionine	350	7.03–1.50	21.18 (17.74–24.13)

[a] Number of insects used.
[b] In $\mu g\,g^{-1}$ fresh (wet) weight; convert to dry weight by multiplying by 2.31, FL = fiducial limit.

Table 2
Influence of form of Se ingested on pupal weight, days required for pupation, and days required to reach the adult stage

Form of Se	Concentration ($\mu g\,g^{-1}$ diet)	Pupal weight (mg)	Time to pupation (days)	Time to adult emergence (days)
Sodium selenate	0	112.80 ± 3.14 [a]	13.3 ± 0.5 [a]	19.9 ± 0.6 [a]
	4	106.32 ± 3.64 [ab]	15.0 ± 0.6 [a]	22.4 ± 0.7 [b]
	12	95.97 ± 3.51 [b]	19.7 ± 0.8 [b]	28.1 ± 1.0 [c]
Sodium selenite	0	108.71 ± 6.20 [a]	12.3 ± 0.5 [a]	22.6 ± 0.9 [a]
	4	104.45 ± 6.81 [a]	13.4 ± 0.5 [a]	27.9 ± 1.2 [b]
	12	83.37 ± 7.45 [b]	16.6 ± 2.1 [b]	28.8 ± 2.8 [b]
Selenomethionine	0	121.72 ± 4.35 [a]	12.9 ± 0.2 [a]	20.2 ± 0.4 [a]
	10	118.02 ± 4.89 [a]	13.5 ± 0.5 [a]	21.1 ± 0.7 [a]
	20	125.22 ± 4.19 [a]	12.0 ± 0.1 [a]	19.0 ± 0.3 [a]
	30	131.97 ± 3.02 [a]	12.8 ± 0.3 [a]	20.6 ± 0.4 [a]
Selenocystine	0	96.68 ± 2.92 [b]	16.2 ± 0.5 [a]	23.7 ± 0.5 [a]
	4	101.82 ± 4.07 [a,b]	15.9 ± 0.6 [a]	23.3 ± 0.6 [a]
	12	91.16 ± 3.33 [b]	18.7 ± 0.8 [a]	26.5 ± 0.9 [a]
	20	120.96 ± 4.77 [a]	15.3 ± .04 [a]	23.1 ± 0.5 [a]
	28	108.53 ± 9.19 [a,b]	17.0 ± 0.6 [a]	25.3 ± 0.8 [a]

Values within columns and specific form of Se are different if followed by different superscripts (Tukey's HSD test, $p \leq 0.05$).

growth rate. The decline in RGR appeared proportional to the concentration of sodium selenate in the diet. At $12\,\mu g\,g^{-1}$ in diet, the RGR had been reduced by 30%. In contrast, sodium selenite had no significant effect on RGR at $4\,\mu g\,g^{-1}$ diet, but the growth rate dropped by over 90% at a concentration of $12\,\mu g\,g^{-1}$. There were no consistent effects observed for selenocystine; the RGR of control larvae was not significantly different from that of larvae fed diets containing $32\,\mu g\,g^{-1}$. Selenomethionine had no measurable effect at any concentration up to $30\,\mu g\,g^{-1}$.

Depression of the RGI with increasing concentration indicated that three of the forms of Se acted as growth inhibitors (Fig. 2). Sodium selenate and sodium selenite were the most potent, causing reductions of about 40% at concentrations of $18\,\mu g\,g^{-1}$. Selenocystine had somewhat less growth inhibition activity, depressing the RGI by *ca* 40% at concentrations of $28\,\mu g\,g^{-1}$. No significant effects were observed for selenomethionine even at dietary concentrations of $30\,\mu g\,g^{-1}$. These results were similar to the RGR analyses for sodium selenate and selenomethionine. However, for selenocystine, the RGI indicated larval development was slowed substantially by concentrations exceeding $4\,\mu g\,g^{-1}$. The RGR, because of the statistical design which minimizes the effect of body size, could not detect this developmental reduction. In contrast, the RGR analysis for sodium selenite showed a dramatic drop in growth rate at concentrations $\geqslant 12\,\mu g\,g^{-1}$, while the RGI suggested a more gradual response to increasing concentrations.

The ANCOVA analyses for sodium selenate and sodium selenite indicated that diminished RGR and RGI values were due to reduced consumption at low concentrations, and a probable toxicity at higher concentrations (Fig. 3). The ANCOVA analysis for sodium selenate showed that the slopes of regression lines for concentrations of 0, 4, and $12\,\mu g\,g^{-1}$ were not significantly different from each other (interaction term, F concentration • initial weight = 1.198; 2,84 *df*; $p = 0.31$), indicating that larvae fed these concentrations have similar rates of growth. Because the lines were shifted downward with increasing concentration, the larvae were presumed to have consumed less diet (Horton and Redak, 1993). The slope of the line for larvae fed $24\,\mu g\,g^{-1}$ was significantly different (interaction term, F concentration • initial weight = 13.440; 3,111 *df*; $p = 0.001$), suggesting that a physiological (toxic) response had occurred. Sodium selenite produced a similar response, but the potential physiological effects were observed more rapidly; slopes of lines for concentrations of 4 and $12\,\mu g\,g^{-1}$ were significantly different from $0\,\mu g\,g^{-1}$ (interaction term, F concentration •

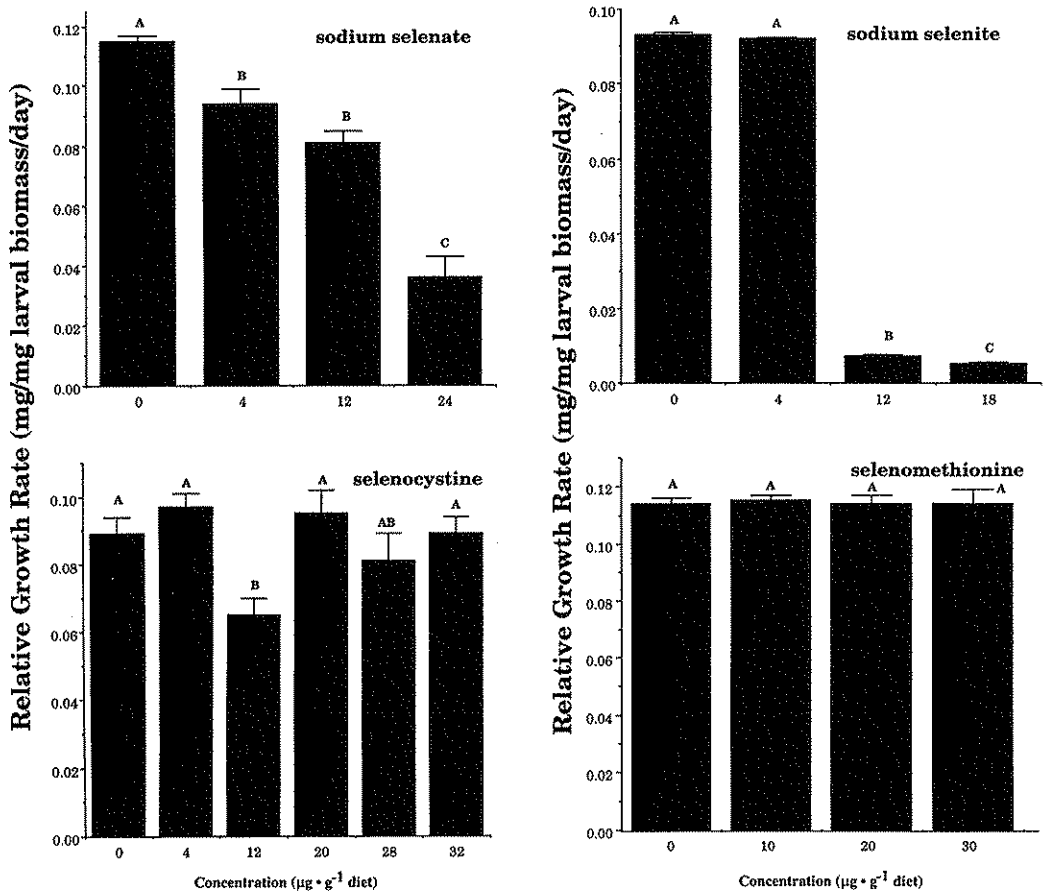

Fig. 1. Relative growth rates of *S. exigua* larvae ingesting various concentrations of sodium selenate, sodium selenite, selenocystine, and selenomethionine. Lines above bars deliniate standard errors. Within graphs, different letters above columns indicate significant differences at $p \leqslant 0.05$, Tukey's HSD test.

initial weight = 13.499; 3,111 *df*; $p = 0.001$) and from 18 $\mu g\,g^{-1}$ (interaction term, F concentration • initial weight = 10.426; 3,83 *df*; $p = 0.001$), but not from each other (interaction term, F concentration • initial weight = 0.528; 1, 55 *df*; $p = 0.471$). This suggested that differences between larvae fed 4 and 12 $\mu g\,g^{-1}$ were due to diet consumption, while at 18 $\mu g\,g^{-1}$ additional toxicity occurred. However, the slope of the line for 18 $\mu g\,g^{-1}$ was not significantly different from zero ($p = 0.72$), and, therefore, the suggestion of additional toxicity at 18 $\mu g\,g^{-1}$ is not verifiable with this data set.

Interestingly, no differences were observed between slopes of lines for either selenocystine (interaction term, F concentration • initial weight = 1.663; 5,168 *df*; $p = 0.15$) or selenomethionine (interaction term, F concentration • initial weight = 0.278; 4,140 *df*; $p = 0.89$; all lines were significantly different from zero at the $p = 0.05$ level) (Fig. 3), indicating that increasing concentration did not cause an increased physiological response. Further, values for the main effect concentration were not significantly different (e.g. regression lines were only marginally offset), suggesting that consumption was not decreased. These results were in agreement with the RGR and RGI analyses for selenomethionine which indicated that this form of Se was not reducing growth rate or acting as an antifeedant. For selenocystine, there was also agreement between the ANCOVA analysis and RGR; growth rate was not affected. However, the RGIs for selenocystine implied an antifeedant activity (albeit weaker than sodium selenate or sodium selenite). Apparently either the observed antifeedant effect was not adequate to cause a substantial reduction in consumption within the 2-day period of the ANCOVA experiments, or the older larvae are not as strongly influenced by selenocystine.

4. Discussion

Many 'nonaccumulator' plants can sequester concentrations of sodium selenate, sodium selenite, selenocystine and selenomethionine that can strongly influence insect development and survival. With LC_{50}s below 22 $\mu g\,g^{-1}$ wet wt (< 50 $\mu g\,g^{-1}$ dry wt), all the forms of Se tested were more potent against the generalist herbivore *S. exigua* than many compounds which are believed to

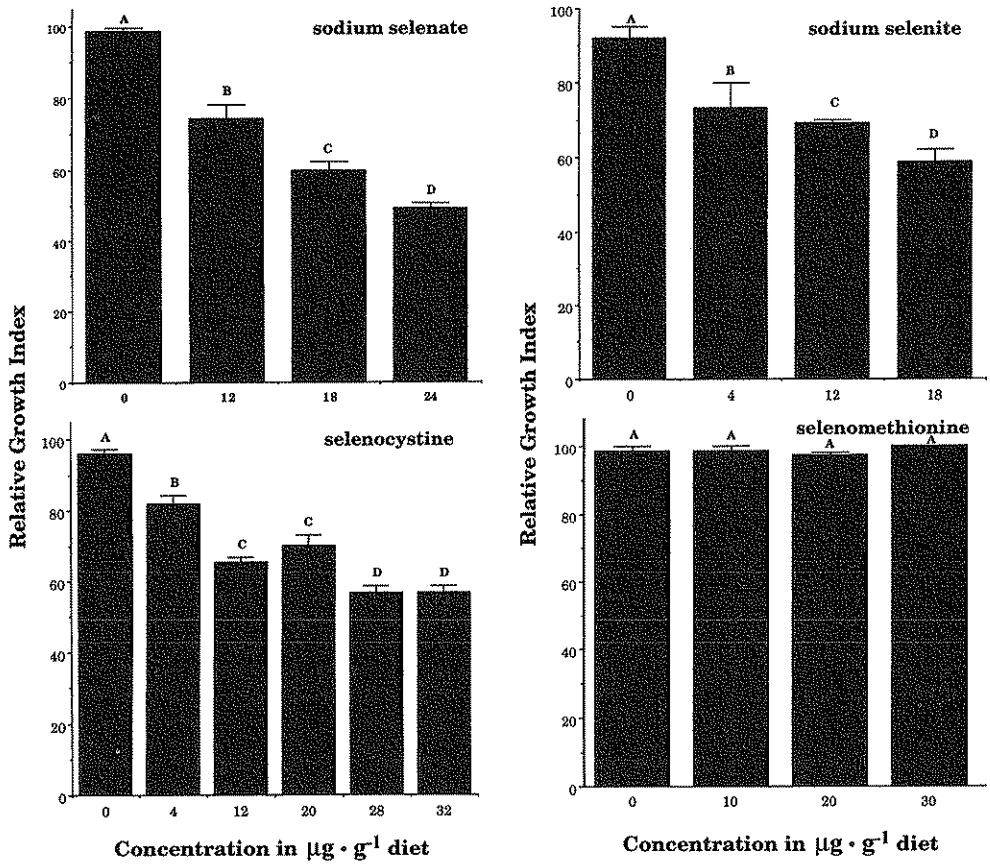

Fig. 2. Growth inhibition of *S. exigua* larvae ingesting various concentrations of sodium selenate, sodium selenite, selenocystine, and selenomethionine as measured by the relative growth index (RGI). See text for calculation of RGI. Lines above bars deliniate standard errors. Within graphs, different letters above columns indicate significant differences at $p \leqslant 0.05$, Tukey's HSD test.

have evolved for plant defense, such as the carcinogenic and mutagenic linear furanocoumarins (Diawara and Trumble, 1997; Berdegue et al., 1997). Some plant species have the ability to accumulate exceptionally high concentrations of Se on the order of 5000 $\mu g\,g^{-1}$ dry wt, a level which provides protection against even mammalian herbivores (Rosenfeld and Beath, 1964). Common herbaceous weeds occurring near the Se-contaminated Kesterson Reservoir in Merced, California, USA, had concentrations ranging from 20 to 183 $\mu g\,g^{-1}$ dry wt (Wu et al., 1993). At least in some species, Se accumulates the highest levels in leaves and seeds (Arthur et al., 1992), two plant parts that are frequently consumed by insect herbivores. Irrigation has been shown to increase Se concentrations (Wu et al., 1993), so the ecology of insect populations feeding on plants grown subject to agricultural runoff may be most affected.

Although many reports detail the accumulation of total Se in plants, relatively few provide information on the quantity of the various forms of Se in plants. Ge et al. (1996) reported that for a garlic sample containing approximately 1000 $\mu g\,g^{-1}$ Se, the bulk of the Se was in methylselenocysteine, followed by selenocystine, selenite, selenate and selenomethionine. In a broccoli sample, selenocystine, and methylselenocysteine were highest, followed by selenite and selenate. Hopefully, because of the variable nutritional and biological effects of the forms of Se, and the recent publication of techniques for detecting these forms, more reports will provide quantitative data on their occurrence and distribution in plants (Kolbl et al., 1993; Da Silva et al., 1997).

Both the quantity and the form of Se in plants are likely to influence the population dynamics of insect herbivores. Reductions in population numbers associated with direct toxicity (Table 1) and reductions in development (Figs. 1 and 2), would combine to limit population sizes. Direct toxicity minimizes the number available for reproduction, while delayed development may increase the chances for additional mortality due to abiotic (environmental) and biotic (parasitoids or predators) factors. Increasing developmental times could eliminate some generations for multivoltine species.

For some insects, such as *S. exigua*, larval behavior may also be changed by the presence of Se. Growth inhibitory activity has proven important with this insect because it has been shown to use its exceptional larval mobility (Smits et al., 1987) to selectively avoid growth inhibitors (Berdegue et al., 1996; Trumble and Millar,

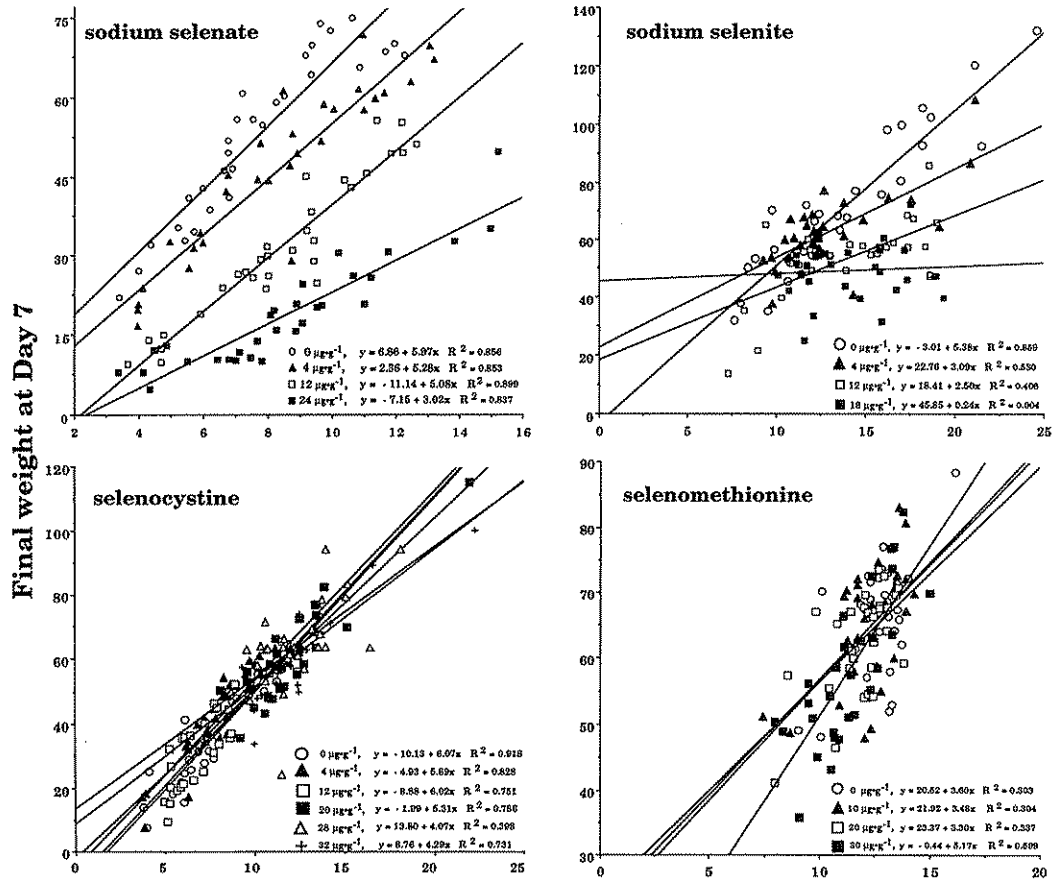

Fig. 3. Regressions of initial (5-day) versus final (7-day) weights for *S. exigua* larvae ingesting various concentrations of sodium selenate, sodium selenite, selenocystine, and selenomethionine.

1996). This suggests that the larvae would selectively feed on plants with low levels of Se, then on plants with low levels of the three growth inhibitory forms of Se, then possibly on plant parts with the lowest concentrations. Interestingly, direct toxicity may be greatest for selenomethionine which does not have an growth inhibitory activity that could serve to minimize consumption. Such selective feeding could affect plant species composition in Se-contaminated soils.

These data have been presented with three caveats. First, insects may respond to interactions between plant compounds and various forms of Se to produce different responses. Second, there may be interactions between the forms of Se that cause unanticipated antagonistic or synergistic reactions. Finally, further research may find species of herbivorous insects capable of feeding on plants with relatively high concentrations of the forms of Se we tested. Regardless, the data presented in this manuscript provide the first evidence that the development and survival of insect herbivores can be affected by low to moderate concentrations of Se contamination in plants.

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