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Evaluation of *Atriplex* lines for selenium accumulation, salt tolerance and suitability for a key agricultural insect pest

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"Capsule": *Five salt tolerant lines of the plant* Atriplex *were identified which were also resistant to beet armyworm,* Spodoptera exigua.

Abstract

Thirty *Atriplex* lines were examined for potential habitat improvement and phytoremediation of selenium (Se) contaminated sites. Studies were conducted to determine the biomass production, Se accumulation, and resistance of each line to the beet armyworm, *Spodoptera exigua*, an agriculturally important insect. Plants were tested using three salinity treatments: (1) control, no Se; (2) NaCl and $CaCl_2$ salts and 1 mg l^{-1} Se (12.7 μM) added as sodium selenate; and (3) iso-osmotic to treatment 2 containing high concentrations of sulfate and 1 mg l^{-1} Se added as sodium selenate. Insect bioassays measured survival, growth, and development. *Atriplex patula*, *A. spongiosa* 415862, *A. hortensis*, *A. hortensis* 379088 and *A. hortensis* 379092 were among the top biomass producers and Se accumulators, yet they exhibited significantly reduced insect growth, development, and survival. High background sulfate strongly reduced Se accumulation, suggesting that phytoremediation potential is greatest in saline areas having low to moderate sulfate levels. However, these lines grew well in high salinity soils, indicating possible use as a self-seeding cover crop to improve habitat. All plant lines grown in control and high sulfate salinity treatments are acceptable oviposition sites for *S. exigua*, indicating that these plants would help reduce populations of this key agricultural pest.

Keywords: *Atriplex*; Herbivory; Integrated production program; Phytoremediation; Selenium; *Spodoptera exigua*; Saline drainage

1. Introduction

Soil selenium (Se) accumulation associated with agricultural irrigation, geochemical processes, mining, and a variety of other industrial sources frequently results in significant effects on animal health (Lemly, 1997). Although Se is an essential trace nutrient important to humans and most other animals as an antioxidant (Mayland, 1994), toxicity occurs at high concentrations due to replacement of sulfur with Se in amino acids resulting in incorrect folding of the protein and consequently nonfunctional proteins and enzymes (Daniels, 1996; Lemly, 1998). Remediation strategies include removal of soil Se through microbial and plant volatilization, and by plant accumulation, harvest, and removal (Khattak et al., 1991; Nyberg, 1991; Bañuelos et al., 1996; Wu et al., 1996; Losi and Frankenberger, 1997). Use of plants in Se-remediation programs will result in the availability of Se to insect herbivores, yet relatively little is known of the response of herbivorous insects to Se in plants.

Previous studies using artificial diets for an insect herbivore indicated that even low levels of Se can increase developmental times and mortality rates of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) an agriculturally important pest insect (Trumble et al., 1998). This species demonstrated a preference for diets without sodium selenate or sodium selenite, but no preference was detected between diets with selenocystine or selenomethionine versus untreated controls (Vickerman and Trumble, 1999). Possible biotransfer of Se from plants to insects may not only have consequences for the pest insects (Trumble et al., 1998), but also for vertebrates and invertebrates that

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feed on insect herbivores (Wu et al., 1994). Unfortunately, data on effectiveness of cultivated and uncultivated plants in Se accumulation and as hosts for pest insects are minimal (Bañuelos et al., 1999).

Plants in the genus *Atriplex* (Chenopodiaceae) have been proposed as possible candidates for phytoremediation of Se. These plants could be useful both in managing the highly saline drainage water from farming operations and in the low salinity watersheds associated with point sources such as mining or sulfuric acid production plants. In the San Joaquin Valley (SJV) of California Se levels are as high as 2 mg l^{-1} Se in drainage water (Deverel et al., 1994), a concentration which potentially poses a health threat to humans and other animals (Lemly, 1997). These drainage waters are typically high in Na_2SO_4-dominant salts and are Se-laden, thus prohibiting their disposal into drainage channels and waterways.

Preliminary studies have shown that two species of *Atriplex*, *A. patula* and *A. nummularia*, can be grown in the SJV using drainage water as the only source for irrigation (Hoffman and Shannon, 1986; Watson and O'Leary, 1993). Additionally, recent studies have shown that *A. hortensis* (red orach), a salad green, also has a high salt tolerance as compared to other vegetables (Wilson et al., 2000). Because Na_2SO_4-dominant salts can reduce the uptake of Se due to competitive inhibition, it is important that plants chosen for Se remediation programs in the SJV are able to accumulate and volatilize Se in the presence of the high sulfate salinities characteristic of SJV drainage water (Grieve et al., 1999, 2001). For other point sources of Se contamination (mining, coal fly ash, oil refinery and electric utility discharge, or sulfuric acid production plants), the concerns related to elevated sulfate salinity are not important.

Atriplex spp. grow during winter and spring under dryland conditions in California, but can be grown during the summer irrigated conditions. At least 30 different species of *Atriplex* are native to California. However, some *Atriplex* species are hosts for *S. exigua* (Vickerman and Trumble, personal observation). The objectives of this research were to screen *Atriplex* lines for (1) Se uptake, (2) biomass accumulation and, (3) identification of the most favorable plant lines that would not support population development of a key insect pest.

2. Materials and methods

2.1. Plants

Atriplex germplasm was collected through both commercial sources and the Western Regional Plant Introduction Station, USDA, ARS, National Plant Germplasm System, Pullman, WA. All plants were grown in sand cultures at the USDA Salinity Laboratory outdoor facilities in Riverside CA, using cement containers (2.0×0.82×0.85 m) that were irrigated daily from 1740 L reservoirs containing treatment solutions (see Wilson et al., 2000). Sixty-two *Atriplex* lines were examined (M.C. Shannon et al. unpublished) and the most promising 30 lines were tested in insect bioassays. Assays were conducted in sets of ten plant lines and each set was consecutively numbered as test 1, test 2, and test 3. All tests were conducted from January to March 2000. The average solar radiation during this time was 315 Ly/dy with a minimum of 70 Ly/dy and a maximum of 569 Ly/dy. The average temperature was 13.3 °C with a minimum of 7.8 °C and a maximum of 21.1 °C.

Ten replicates (individual plants) of each plant line were grown in nutrient solution in three treatments. Nutrient solutions contained: 12.0 mM Ca, 7.5 mM Mg, 4.0 mM K, 40 mM SO_4, 31.5 mM Cl, 4.0 mM NO_3, 0.170 mM $P0_4$, 50 μM Fe as sodium ferric diethlyenetriamine pentaacetate, 23 μM H_3BO_3, 5 μM $MnSO_4$, 0.4 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.1 μM H_2MoO_4. Treatments included: (1) a control, containing tap water plus nutrients with no Se (EC∼9 dS/m); (2) Se + Cl, containing nutrients and added NaCl and $CaCl_2$ salts (76 mM Ca, 160mM Na; EC∼37 dS/m) and 1 mg l^{-1} Se (12.7 μM) added as sodium selenate; and (3) Se + Cl + SO_4, using simulated SJV drainage water (30 mM Mg, 260 mM, Na, 120 mM SO_4, 126 mM Cl; EC∼33 dS/m) containing high amounts of sulfate, plus 1 mg l^{-1} Se added as sodium selenate.

Plants' shoots were harvested, washed in deionized water, dried in a forced air oven at 70 °C for 72 h and weighed. Leaf samples were collected and analyzed for Se by first using acid digestion with the HNO_3/H_2O_2/HCl procedure described by Bañuelos and Akohoue (1994), and then quantified using atomic absorption with an automatic vapor accessory.

Alfalfa (*Medicago sativa* L.) was grown in sand cultures in the UC Riverside Entomology Department greenhouses and irrigated daily with modified half strength Hoagland's nutrient solution (Khattak et al., 1991). Since alfalfa is known to be a host plant for *S. exigua* (Metcalf and Flint, 1962), it was used as an absolute control for all tests to document insect survival and development on a plant known to be an acceptable host. This also indicated that the insect colony did not vary in terms of survival potential or development rate over the course of the experiments. However, the alfalfa data were excluded from statistical analyses because this plant was not grown in the outdoor sand culture tanks.

2.2. Insect development and survival bioassays

S. exigua was chosen as a model insect for this study because it is a generalist feeder and a crop pest of economic importance throughout the USA, including areas

where Se is a problem. In California, its host range includes native and introduced plants in the families Lilaceae, Fabaceae, Solanaceae, Malvaceae, Chenopodiaceae, Apiaceae, Asteraceae, and Amaranthaceae, that can be found in both cultivated and uncultivated areas (Metcalf and Flint, 1962; Peterson, 1962; Pearson et al., 1989). In addition, this species has highly mobile larvae, which select feeding sites by moving between plants (Berdegué et al., 1998).

All experiments were initiated with first instar larvae (standardized within 12 h of egg hatch) obtained from a laboratory colony maintained at 28±2 °C and 16:8 (light:dark) photoperiod. Insects were held in individually labeled containers and fed plant tissue ad libitum. Ten replicates of one insect each were tested for each of the plant line/treatment combinations in addition to the alfalfa control. Insect mortality, and the developmental stage of newly dead and all surviving larvae were recorded every other day. On day 30, the developmental stage of insects which had not yet become adults was recorded and the experiment was terminated. Few insects will achieve the adult stage if development requires over 30 days (J.T. Trumble, personal observation). Developmental stages were numbered as follows: larval instars 1–5 were stages 1–5, stage 6 was the prepupal stage, stage 7 was the pupa, and stage 8 the adult moth. Developmental stage at death is important because this information can: (1) serve as an indicator of how much plant damage has occurred; consumption by this herbivore greatly increases after molting into stage four, so ideally plant lines would be chosen for their ability to suppress insect development at or before this stage; and (2) determine if the insects potentially live long enough for beneficial parasitoids to either complete development, or to be a resource for predators.

Percent survival to pupal and adult stages, mean days to insect pupation, and mean days to adult were calculated for insects in each plant line and treatment combinations. By comparison to the data on survival to the pupal stage, survival to the adult stage can be used to determine if additional mortality occurred during the pupal stage. Pupation is a rigorous process requiring increased production of enzymes and reorganization of proteins in insects. Since Se can replace sulfur in amino acids, resulting in nonfunctional proteins and enzymes (Daniels, 1996; Lemly, 1998) it is reasonable to expect that presence of Se may slow or impede insect development.

A measure of growth, the relative growth index (RGI; Zhang et al., 1993), was calculated for day eight, the day before any of the absolute control group developed into the pupal stage. This value measures herbivore growth rate by determining relative values of the developmental stage that could have been achieved on the control (alfalfa), versus what was achieved in the treatments.

2.3. Insect oviposition bioassays

Atriplex patula, *A. spongiosa* 415862, *A. hortensis*, *A. hortensis* 379088, *A. hortensis* 379092, and *A. leucolada* 355940 plants (maintained in 10.2 cm pots) from the control and $Se+Cl+SO_4$ treatments were removed from sand culture tanks immediately after irrigation the day of the experiment. Four plants from each plant line (two from the $Se+Cl+SO_4$ treatment and two from the control) were arranged in an alternating fashion in octagonal PVC cages covered in nylon organdy (0.55 m high×1.0 m width). Pots were buried in sand so the stems were exposed in a standardized fashion. Leaves were removed as necessary to standardize leaf areas available for oviposition.

The insects used in this study were also standardized, to the greatest extent possible. All insects originated from our laboratory colony (after Vickerman and Trumble, 1999). Pupae were sexed and separated by sex until adult emergence, at which time groups of four males and four females less than 48 h old were isolated in small mating chambers for 24 h. Adults were then released into the PVC cages (four females per cage) and allowed to oviposit for 48 h (3 days and 2 nights). Each cage was a replicate. Plants were removed on day three; the total number of eggs were counted and the proportion of eggs laid on each treatment were calculated. All oviposition bioassays were conducted during June and early July 2001. The average solar radiation during this time was 644 Ly/dy with a minimum of 342 Ly/dy and a maximum of 725 Ly/dy. The average temperature was 23.3 °C with a minimum of 7.8 °C and a maximum of 23.3 °C.

2.4. Statistical analysis

Analyses included two-way analysis of variance (GLM) and the protected least significant difference (LSD) post hoc tests (SAS, 1996). For the percent survival analyses a theoretical interaction term was calculated as described in Steel and Torrie (1980) in order to test for a significant interaction. Following a two-way analysis of variance (GLM, SAS 1996), the protected LSD post hoc procedure was used and the 'no interaction' model was chosen. When interactions were present, no additional statistical analyses could be conducted (Steel and Torrie, 1980). Growth index (GI) and RGI (relative growth index) values were calculated as described by Zhang et al. (1993), where GI is calculated as:

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

where $i_{\max} = 5$, the highest attainable instar of the insect at 8 days and n = the number of insects in that instar

and N = total number of insects. RGI was determined as:

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

RGI values approaching one indicate better host plants; declining values indicate less favorable plants. Oviposition preference was analyzed using the Wilcoxon Signed Rank nonparametric procedure for paired comparisons (StatView, 1993; after Lance, 1992; Tallamy et al., 1997).

3. Results

3.1. Plant biomass and Se accumulation

Plant biomass serves as an indicator of salt and Se tolerance of the plant line. In the $Se + Cl + SO_4$ treatment most characteristic of irrigation waters from the SJV, the plant lines *A. hortensis* 379088, *A. hortensis* 379092, *A. hortensis*, *A. spongiosa* 415862, *A. patula*, *A. muelleri* 224963, *A. leptocarpa* 342565, *A. pseudocampanulata* 342567, *A. lindleyi* 415865, and *A. leucolada* 355940 were in the top 30% of biomass accumulators (Table 1).

Selenium concentrations in leaf samples are reported for each plant line in each treatment (Table 2). In the $Se + Cl + SO_4$ treatment most characteristic of irrigation waters from the SJV, plant lines *A. hortensis* 379088, *A. hortensis* 379092, *A. inflata* 330660, *A. halimus* 415863, *A. leucolada* 339807, *A. semibaccata* 299489, *A. patula*, *A. lindleyi* 415865, and *A. leucolada* 355940 were the top 30% of Se accumulators. However, the high sulfate salinity treatment resulted in substantial reductions in Se accumulation on the order of one magnitude (Table 2). Many lines had only minimal biomass and low Se accumulation, and were therefore less suitable for use in phytoremediation, or as a ground cover for habitat improvement. These lines were therefore not considered in the subsequent insect studies.

3.2. Insect development and survival

As compared to survival in test 1, insect survival to pupation was substantially reduced in all of the top-performing *Atriplex* lines (Fig. 1 a–c). In addition, the $Se + Cl$ treatment and the $Se + Cl + SO_4$ treatment had significantly reduced survival as compared to the control ($F_{2,18} = 4.26$, $P < 0.04$). In plants assigned to test 1, there was also a significant difference between plant lines ($F_{9,18} = 4.50$, $P < 0.01$). In two plant lines, the *A. hortensis* and *A. canescens* 330658, the Se containing treatments allowed no survival. A similar pattern was observed in tests 2 and 3, but significant interaction

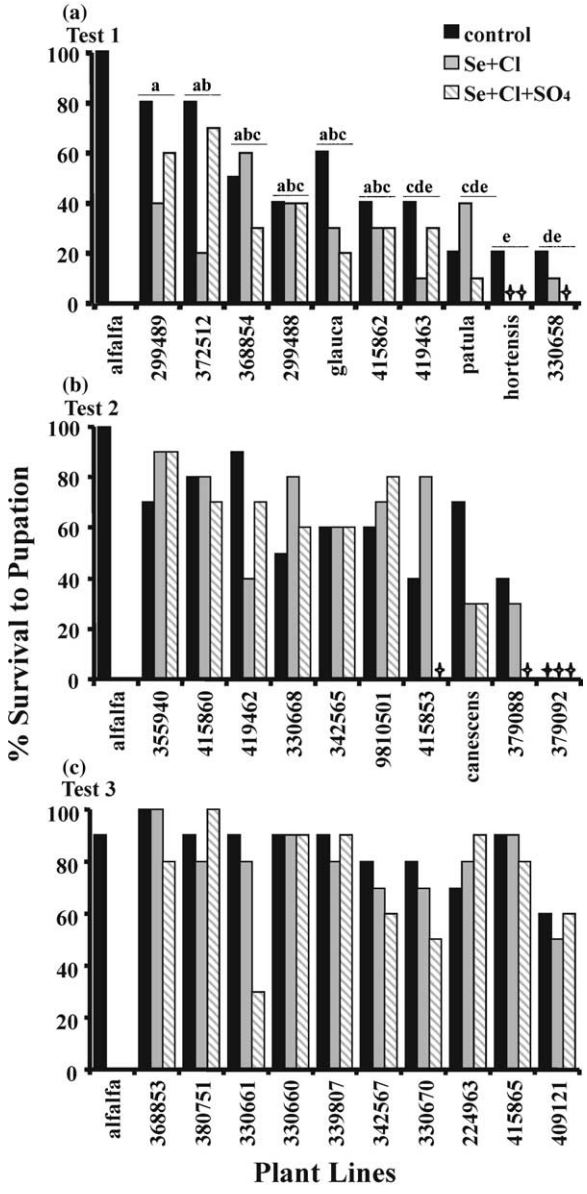

Fig. 1. Percent survival to pupation for *Spodoptera exigua* larvae fed *Atriplex* plant lines from three irrigation treatments. Significant differences between plant lines are indicated by capital letters, values with the same letters are not significantly different. In test 1, the control treatment was significantly different from both the $Se + Cl$ and the $Se + Cl + SO_4$ treatments. Tests 2 and 3 had a significant plant line ×salinity treatment interactions, so additional statistical comparisons were not possible. Plant lines which did not support survival to the pupal stage are indicated with the symbol '◆'.

terms ($P < 0.05$) precluded statistical analysis. However, line *A. hortensis* 379092 in test 2, was noteworthy for not allowing any insect to reach the pupal stage, regardless of the salinity treatment.

Survival to the adult stage was significantly different between treatments in test 1, with the $Se + Cl + SO_4$ treatment causing the most mortality ($F_{2,18} = 3.82$, $P < 0.05$; Fig. 2 a). Insect survival was significantly decreased in six of the plant lines bioassayed in test 1

Table 1
Dry plant weight of *Atriplex* plant lines tested in bioassays from three salinity treatments

Species	Line	Dry wt/plant (g)			Origin
		Control	Se + Cl	Se + Cl + SO_4	
Harvest 1					
A. hortensis	379088	42.557	11.760	16.457	WRPIS[a]
A. hortensis	379092	15.268	13.383	12.427	WRPIS
A. hortensis	hortensis	13.865	5.495	10.885	J. L. Hudson
A. spongiosa	330668	7.317	4.045	6.589	WRPIS
A. hortensis	372512	11.717	5.828	4.617	WRPIS
A. glauca	*glauca*	5.226	3.316	4.073	Carter seeds
A. inflata	330660	1.710	1.655	3.999	WRPIS
A. halimus	415853	3.123	1.221	3.433	WRPIS
A. suberecta	368854	7.494	1.407	2.911	WRPIS
A. canescens	*canescens*	0.862	0.421	0.790	Carter seeds
Harvest 2					
A. spongiosa	415862	20.248	0.572	14.298	WRPIS
A. patula	patula	13.357	7.533	9.800	S & S seeds
A. semibaccata	415860	4.463	1.220	7.953	WRPIS
A. semibaccata	368853	5.550	1.388	6.267	WRPIS
A. muelleri	380751	8.563	2.663	5.387	WRPIS
A. nummularia	419462	5.109	1.411	4.074	WRPIS
A. leucolada	339807	3.555	0.610	2.727	WRPIS
A. nummularia	419463	4.377	2.726	2.660	WRPIS
A. canescens	*canescens*	1.998	0.660	1.411	Carter seeds
A. semibaccata	299489	5.097	1.715	1.312	WRPIS
A. semibaccata	299488	4.206	1.261	0.602	WRPIS
Harvest 3					
A. muelleri	224963	25.850	25.390	42.170	WRPIS
A. leptocarpa	342565	20.620	2.660	32.490	WRPIS
A. pseudocampanulata	342567	10.220	13.510	28.285	WRPIS
A. lindleyi	415865	22.580	22.360	21.050	WRPIS
A. leucolada	355940	29.940	5.580	12.300	WRPIS
A. lentiformis	409121	7.509	1.464	5.916	WRPIS
A. canescens	*canescens*	4.094	1.287	3.478	Carter seeds
unknown sp.	330670	5.065	2.405	3.013	WRPIS
A. lentiformis	330661	2.379	1.540	2.932	WRPIS
A. hortensis	NSSL 9810501	1.613	0.712	0.717	NSSL
A. canescens	330658	8.572	0.037	0.575	WRPIS

[a] WRPIS, Western Regional Plant Introduction System, Pullman, WA, part of the USDA, ARS, National Plant Germplasm System.

($F_{9,18} = 3.87$, $P < 0.01$). The significant interaction terms in tests 2 and 3 ($P < 0.05$) precluded statistical comparisons. However, because the patterns of survival to the adult stage (Fig. 2a–c) were nearly identical to the patterns of survival to the pupal stage (Fig. 1a–c), we can conclude that most mortality occurred during the larval stage, rather than during pupation.

Development stage at death varied for insects depending on the plant line and salinity treatment (Fig. 3 a–c). In the plant lines examined in test 1, differences were significant between salinity treatments ($F_{2,270} = 4.31$, $P < 0.02$), with the Se treatments causing insects to die earlier in their life cycles. Differences in developmental stage at death were also significant between plant lines ($F_{9,270} = 7.84$, $P < 0.01$). The mean developmental stage at death was before stage four in the Se + Cl + SO_4 treatment for plant lines *A. semibaccata* 299488, *A. glauca*, *A. patula*, *A. hortensis*, and *A. canescens* 330658 from test 1. There were no significant plant line × salinity treatment interactions in test 1.

Similarly, in test 2, the between plant line differences were significant ($F_{9,270} = 10.27$, $P < 0.01$, Fig. 3b). Feeding on *A. halimus* 415853, *A. hortensis* 379088, and *A. hortensis* 379092 resulted in death before stage four. However, the most rapid mortality was seen on plant line *A. hortensis* 379092. Differences between salinity treatments were not significant. Plant × salinity treatment interactions also were not significant.

In test 3 plant line × salinity treatment interactions were significant ($F_{18,270} = 1.83$, $P < 0.03$), so additional statistical analyses were not possible. This test included few plant lines that appeared to offer substantial reductions

Table 2
Selenium concentrations of *Atriplex* plant lines tested in bioassays from three salinity treatments

Species	Line	Total Se (mg/kg)		
		Control	Se + Cl	Se + Cl + SO_4
Harvest 1				
A. hortensis	379088	0.08	40.80	3.04
A. hortensis	379092	0.14	40.00	2.70
A. inflata	330660	0.50	66.00	2.40
A. halimus	415853	0.16	82.80	2.27
A. spongiosa	330668	0.14	38.20	1.91
A. hortensis	372512	0.17	27.00	1.73
A. glauca	*glauca*	0.16	61.50	1.58
A. hortensis	hortensis	0.19	39.70	1.54
A. canescens	*canescens*	0.14	42.40	1.54
A. suberecta	368854	0.10	33.40	1.40
Harvest 2				
A. leucolada	339807	0.40	39.10	3.20
A. semibaccata	299489	0.30	33.80	2.30
A. patula	*patula*	0.10	37.60	2.00
A. spongiosa	415862	0.09	39.70	1.96
A. canescens	*canescens*	0.15	48.20	1.95
A. semibaccata	368853	0.10	34.70	1.90
A. nummularia	419462	0.14	49.80	1.82
A. semibaccata	299488	0.30	32.20	1.80
A. semibaccata	415860	0.15	35.10	1.77
A. muelleri	380751	0.40	40.10	1.70
A. nummularia	419463	0.10	33.20	1.20
Harvest 3				
A. lindleyi	415865	0.24	41.16	2.61
A. leucolada	355940	0.12	65.25	2.11
A. leptocarpa	342565	0.19	47.76	1.45
unknown sp.	330670	0.15	45.00	1.42
A. pseudocampanulata	342567	0.21	35.16	1.42
A. canescens	*canescens*	0.17	48.70	1.41
A. lentiformis	409121	0.22	47.88	1.40
A. muelleri	224963	0.20	33.24	1.38
A. hortensis	NSSL 9810501	0.19	42.24	1.25
A. lentiformis	330661	0.16	45.00	1.15
A. canescens	330658	–	–	–

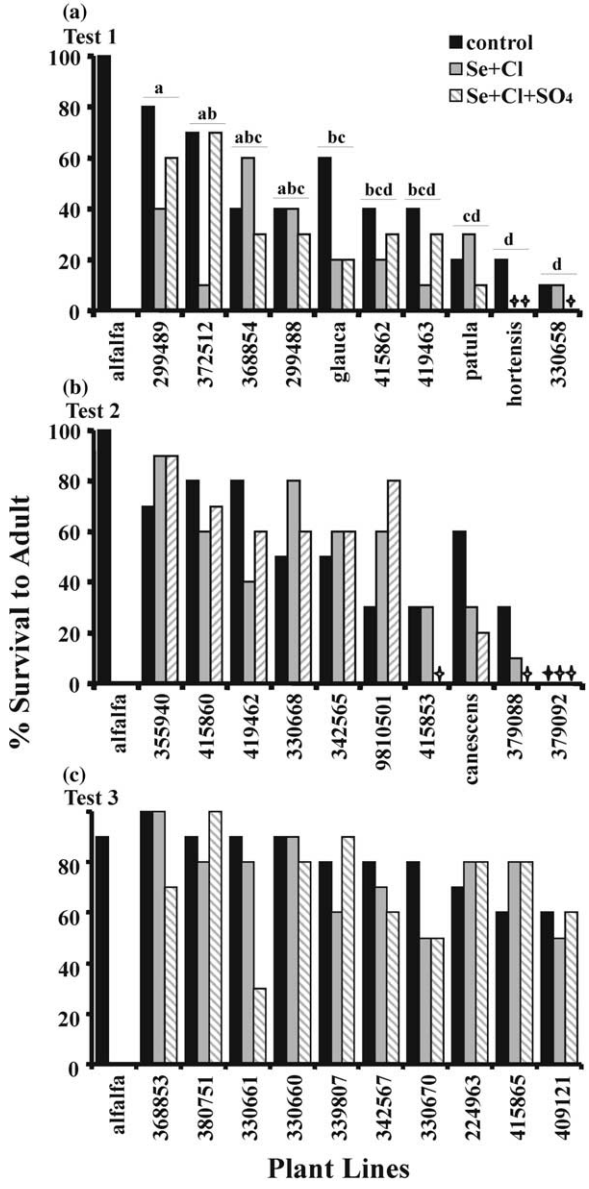

Fig. 2. Percent survival to adult for *Spodoptera exigua* larvae fed *Atriplex* plant lines from three irrigation treatments. Significant differences between plant lines are indicated by capital letters, values with the same letters are not significantly different. In test 1, the control treatment was significantly different from the Se + Cl + SO_4 treatment. Tests 2 and 3 had a significant plant line×salinity treatment interactions, so additional statistical comparisons were not possible. Plant lines which did not support survival to the adult stage are indicated with the symbol '◆'.

in developmental stage (Fig. 3c). Only *A. lentiformis* 330661 consistently caused larval death before stage four.

The relative growth index (RGI) indicated that several *Atriplex* lines that supported some insect growth did so at a greatly reduced development rate, as compared to the alfalfa control (Fig. 4 a–c). Differences in the RGI between plant lines were significant for plant lines in test 1 ($F_{9,270} = 5.78$, $P < 0.01$) and test 2 ($F_{9,270} = 7.44$, $P < 0.01$), and between salinity treatments in test 2; the no Se control plants grew significantly faster than plants in Se salinity treatments ($F_{2,270} = 5.14$, $P < 0.01$). Plant lines *A. canescens* 330658 from test 1, and *A. canescens* and *A. hortensis* 379092 from test 2 significantly reduced insect development in the Se + Cl + SO_4 treatment, as measured by RGI. Test 3 plant line×salinity treatment interactions were significant ($F_{18,270} = 1.99$, $P < 0.02$).

Day of death (Table 3) differed significantly between salinity treatments and between plant lines in test 1 ($F_{2,270} = 5.13$, $P < 0.01$; $F_{9,270} = 6.90$, $P < 0.01$), and between plant lines in test 2 ($F_{9,270} = 7.99$, $P < 0.01$) and test 3 ($F_{9,270} = 2.09$, $P < 0.04$). There were no significant plant line×salinity treatment interactions in any of the tests. Day of death on plants in both Se salinity treatments in test 1 was significantly earlier than on

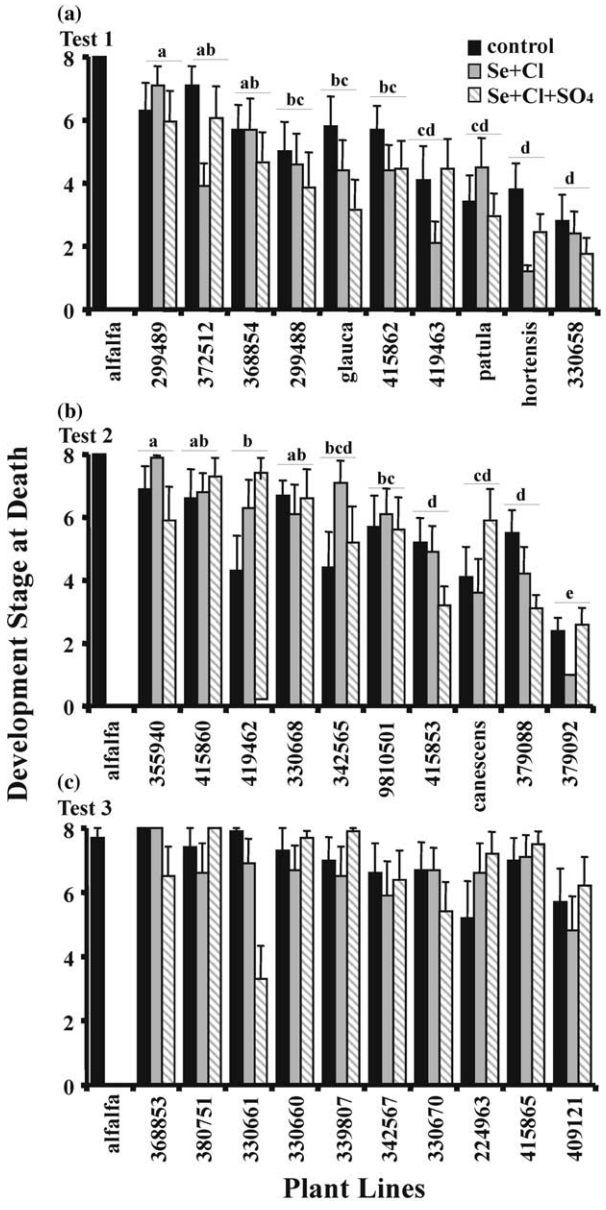

Fig. 3. Development stage at death for *Spodoptera exigua* larvae fed *Atriplex* plant lines from three irrigation treatments. Significant differences between plant lines are indicated by capital letters, values with the same letters are not significantly different. In tests 1 and 2, there were no significant treatment differences. Test 3 had a significant plant line×salinity treatment interaction, so additional statistical comparisons were not possible.

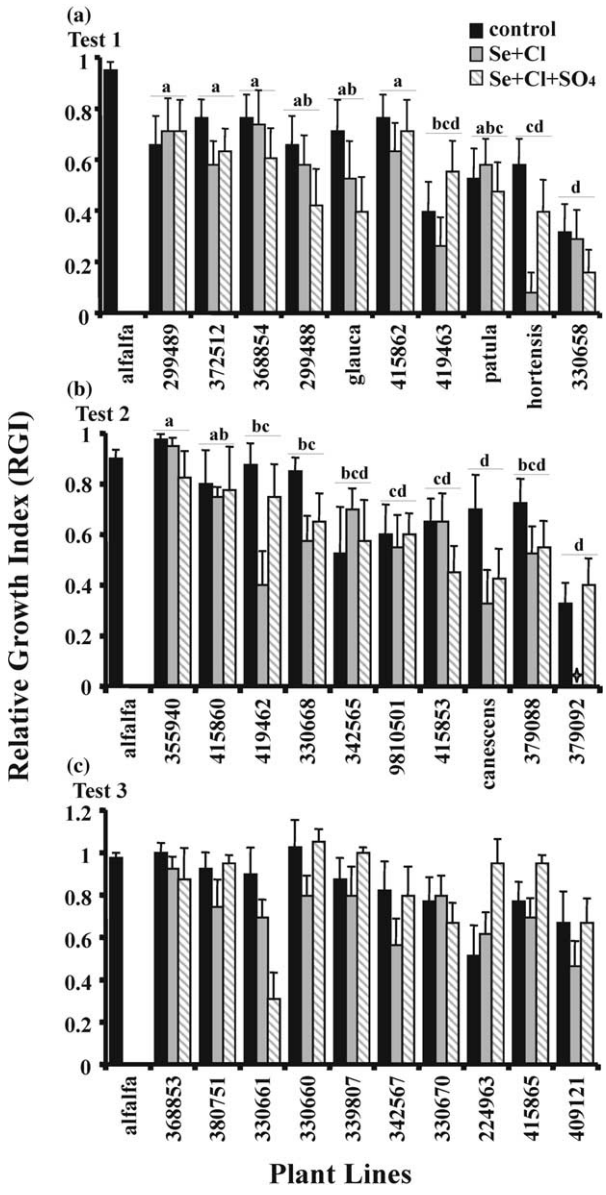

Fig. 4. Relative growth index for *Spodoptera exigua* larvae fed *Atriplex* plant lines from three irrigation treatments. Significant differences between plant lines are indicated by capital letters, values with the same letters are not significantly different. In test 1, there were no significant treatment differences. In test 2, the control treatment was significantly different from the $Se+Cl+SO_4$ treatment. Test 3 had a significant plant line×salinity treatment interaction, so additional statistical comparisons were not possible.

control plants. For plant lines *A. glauca*, *A. hortensis*, and *A. canescens* 330658 from test 1, *A. halimus* 415853, and *A. hortensis* 379092 from test 2, and *A. lentiformis* 330661 from test 3, the average day of death was before the mean day to pupation for insects in the control group that was fed alfalfa. For those insects which die, a relatively early day of death reduces the amount of time the insect is available to the next trophic level, particularly predators, and so reduces the potential for Se biotransfer.

Mean days to pupation were included specifically to establish if any plants were toxic primarily to larvae. Toxicity could result in either death or an increase in developmental time. Insects that completed development to the pupal stage varied in their mean days to pupation between plant lines and between salinity treatments (Table 4). In plants examined in test 1, these differences were significant between salinity treatments ($F_{2,76}=5.26$, $P<0.01$) and between plant lines ($F_{9,76}=4.08$, $P<0.01$). Mean days to pupation on

Table 3
Comparison of day of death for *Spodoptera exigua* larvae fed *Atriplex* plant lines from three salinity treatments

Species	Line	Mean day of death[a]			
		Control	Se + Cl	Se + Cl + SO_4	
Test 1		a	b	b	
M. sativa	(alfalfa)	30.0			
A. semibaccata	299489	22.6	27.4	22.8	a
A. hortensis	372512	27.7	18.8	23.4	ab
A. suberecta	368854	23.8	23.6	16.8	abc
A. semibaccata	299488	21.0	19.4	16.0	bcde
A. glauca	*glauca*	23.0	16.6	9.2	def
A. spongiosa	415862	21.4	19.8	19.6	abcd
A. nummularia	419463	16.4	9.2	17.6	ef
A. patula	*patula*	14.4	20.2	13.6	cdef
A. hortensis	*hortensis*	18.8	3.4	11.8	fg
A. canescens	330658	12.2	9.4	4.4	g
Test 2		a	a	a	
M. sativa	(alfalfa)	30.0			
A. leucolada	355940	24.4	29.4	27.2	a
A. semibaccata	415860	24.8	26.8	22.2	ab
A. nummularia	419462	15.4	24.4	27.6	abc
A. spongiosa	330668	23.0	23.4	24.8	ab
A. leptocarpa	342565	16.8	26.0	19.0	bcd
A. hortensis	NSSL 9810501	20.8	18.4	21.6	bcd
A. halimus	415853	21.6	17.2	13.6	ab
A. canescens	*canescens*	30.0	15.2	12.4	d
A. hortensis	379088	20.4	17.4	15.6	cd
A. hortensis	379092	8.6	4.0	11.2	e
Test 3		a	a	a	
M. sativa	(alfalfa)	28.8			
A. semibaccata	368853	30.0	30.0	25.0	a
A. muelleri	380751	27.4	24.6	30.0	ab
A. lentiformis	330661	28.4	26.8	12.2	bc
A. inflata	330660	27.4	24.6	28.6	ab
A. leucolada	339807	25.8	23.0	28.2	ab
A. pseudocampanulata	342567	25.0	21.8	24.8	abc
unknown sp.	330670	24.8	24.2	20.8	bc
A. muelleri	224963	19.4	24.4	27.2	abc
A. lindleyi	415865	24.6	26.4	27.2	ab
A. lentiformis	409121	20.0	18.0	22.2	c

Table 4
Comparison of mean days to pupation for *Spodoptera exigua* larvae fed *Atriplex* plant lines from three salinity treatments

Species	Line	Mean days to pupation[a]			
		Control	Se + Cl	Se + Cl + SO_4	
Test 1		a	b	ab	
M. sativa	(alfalfa)	12.2			
A. semibaccata	299489	13.7	14.7	12.3	a
A. hortensis	372512	15.3	21.0	16.9	c
A. suberecta	368854	14.8	12.3	16.0	ab
A. semibaccata	299488	12.5	15.0	14.5	a
A. glauca	*glauca*	13.7	14.0	13.0	a
A. spongiosa	415862	16.0	18.0	15.3	bc
A. nummularia	419463	16.5	18.0	14.0	abc
A. patula	*patula*	15.0	18.5	18.0	c
A. hortensis	*hortensis*	18.0	◆[b]	◆	c
A. canescens	330658	17.0	20.0	◆	c
Test 2					
M. sativa	(alfalfa)	12.6			
A. leucolada	355940	13	12.4	15.6	
A. semibaccata	415860	12	14.3	12.6	
A. nummularia	419462	13.8	17.0	15.4	
A. spongiosa	330668	12.9	16.3	14.3	
A. leptocarpa	342565	12.8	14.3	13.3	
A. hortensis	NSSL 9810501	15	15.3	17.3	
A. halimus	415853	18	15.3	◆	
A. canescens	*canescens*	14.3	16.0	18.0	
A. hortensis	379088	17	21.3	◆	
A. hortensis	379092	◆	◆	◆	
Test 3					
M. sativa	(alfalfa)	12.7			
A. semibaccata	368853	12.6	13.4	12.3	
A. muelleri	380751	12.7	13.5	14.2	
A. lentiformis	330661	14.7	15.8	15.3	
A. inflata	330660	13.8	14.6	14.0	
A. leucolada	339807	12.7	13.0	12.2	
A. pseudocampanulata	342567	13.0	15.0	14.0	
unknown sp.	330670	14.0	15.7	14.4	
A. muelleri	224963	14.3	13.5	12.4	
A. lindleyi	415865	13.8	15.3	12.5	
A. lentiformis	409121	14.0	14.4	13.3	

[a] Significant differences ($P<0.05$) between treatments or between plants are indicated by letters a,b,c; values with the same letters are not different (LSD, SAS, 1996). Absence of letters is due to interactions that did not allow statistical comparisons for tests 2 and 3.

[b] ◆ = Plant lines which did not support survival to the pupal stage.

control plants in test 1, was significantly shorter than that for the Se + Cl + SO_4 treatments, but there were no differences between the two Se treatments. In plants examined in tests 2 and 3, plant line×salinity treatment interactions were significant ($F_{14,131}=2.30$, $P<0.01$; $F_{18,206}=1.93$, $P<0.02$).

For those insects that made it to the adult stage, mean days to adult varied between plant lines and between salinity treatments (Table 5). Control insects fed alfalfa developed to adults in 20 days or less, while insects fed *Atriplex* lines had mean developmental times of as long as 28 days. In plants examined in test 1, differences were significant between plant lines ($F_{9,69}=4.20$, $P<0.01$), but not between treatments. In plants examined in tests 2 and 3, plant line×salinity treatment interactions were significant ($F_{14,112}=2.88$, $P<0.01$; $F_{18,191}=1.85$, and $P<0.03$), and no additional analyses were possible.

3.3. Insect oviposition bioassays

All plant lines were suitable for oviposition by *S. exigua*. No differences were found in proportion of eggs laid on control compared to Se + Cl + SO_4 treated plants in the plant lines tested (Fig. 5; *Atriplex patula*, $n=6$,

Table 5
Comparison of mean days to adult for *Spodoptera exigua* larvae fed *Atriplex* plant lines from three salinity treatments

Species	Line	Mean days to adult[a]			
		Control	Se + Cl	Se + Cl + SO_4	
Test 1		a	a	a	
M. sativa	(alfalfa)	19.0			
A. semibaccata	299489	21.1	21.0	19.7	ab
A. hortensis	372512	22.6	26.0	23.4	c
A. suberecta	368854	19.5	19.7	22.7	a
A. semibaccata	299488	19.5	22.0	19.3	a
A. glauca	*glauca*	21.0	21.0	22.0	ab
A. spongiosa	415862	22.5	24.0	22.0	bc
A. nummularia	419463	23.0	26.0	22.0	c
A. patula	*patula*	22.0	24.7	24.0	cd
A. hortensis	*hortensis*	24.0	◆[b]	◆	cd
A. canescens	330658	26.0	28.0	◆	d
Test 2					
M. sativa	(alfalfa)	19.2			
A. leucolada	355940	19.7	19.3	21.0	
A. semibaccata	415860	17.8	20.8	19.4	
A. nummularia	419462	20.0	23.5	22.0	
A. spongiosa	330668	20.7	23.0	21.0	
A. leptocarpa	342565	19.0	21.5	19.0	
A. hortensis	NSSL 9810501	22.0	22.7	24.0	
A. halimus	415853	26.7	22.0	◆	
A. canescens	*canescens*	21.0	24.0	25.0	
A. hortensis	379088	23.0	28.0	◆	
A. hortensis	379092	◆	◆	◆	
Test 3					
M. sativa	(alfalfa)	20.2			
A. semibaccata	368853	19.2	19.6	19.7	
A. muelleri	380751	19.8	20.3	21.0	
A. lentiformis	330661	21.3	23.0	22.0	
A. inflata	330660	20.9	21.1	20.8	
A. leucolada	339807	18.8	19.0	18.2	
A. pseudocampanulata	342567	19.0	22.0	19.0	
unknown sp.	330670	20.3	22.0	21.2	
A. muelleri	224963	21.0	20.3	19.0	
A. lindleyi	415865	20.0	23.0	19.5	
A. lentiformis	409121	19.3	21.6	20.3	

[a] Significant differences ($P<0.05$) between treatments or between plants are indicated by letters a,b,c; values with the same letters are not different (LSD, SAS, 1996). Absence of letters is due to interactions that did not allow statistical comparisons for tests 2 and 3.

[b] ◆ = Plant lines which did not support survival to the adult stage.

$Z=-1.15$, $P=0.25$; *A. spongiosa* 415862, $n=5$, $Z=-0.41$, $P=0.69$; *A. hortensis*, $n=5$, $Z=-1.10$, $P=0.27$; *A. hortensis* 379088, $n=2$, $Z=-1.34$, $P=0.18$; *A. hortensis* 379092, $n=5$, $Z=-0.67$, $P=0.50$; *A. leucolada* 355940, $n=5$, $Z=-0.14$, $P=0.89$).

4. Discussion

Plant lines chosen for phytoremediation programs should have high biomass, high Se accumulation and/or volatilization, and should not support the growth of agriculturally important insects into the adult stage. In our study, selenium accumulation by *Atriplex* lines was reduced in the presence of high sulfate levels. Therefore, these *Atriplex* lines have greater potential for Se-remediation of saline soils where the major anion in the substrate is not sulfate. Additional information on the amount of Se volatilized by these plants would be desirable, and it would allow a more comprehensive assessment of the phytoremediation potential of these *Atriplex* lines. Nonetheless, five *Atriplex* lines were identified that would provide a self-seeding ground cover, even in the presence of high sulfate salinity.

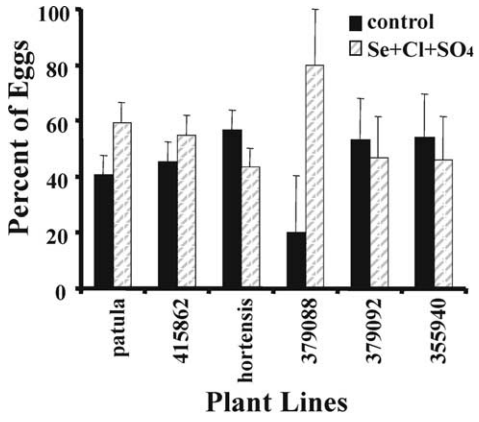

Fig. 5. Oviposition preference of *Spodoptera exigua* on selected *Atriplex* plant lines from two salinity treatments. No significant differences were found.

The presence of Se in irrigation water increased plant resistance to the insect tested in this model system, which is in agreement with the data generated for Indian mustard (Bañuelos et al., 1999). This suggests that Se may enhance plant resistance to the beet armyworm, but the literature is too limited to allow definitive generalizations.

Several plant lines with high biomass and Se accumulation in the Se + Cl + SO_4 treatment such as *A. lindleyi* 415865 and *A. leucolada* 355940 allowed 90% survival to adult eclosion, making them less suitable for either phytoremediation or for habitat improvement near agricultural regions. Although the plant lines *A. glauca*, *A. nummularia* 419463, A. canescens 330658, *A. halimus* 415853, *A. canescens*, and *A. lentiformis* 330661 suppress insect survival to 30% or less, and reduce insect development in the Se + Cl + SO_4 treatment, they were considered less desirable because of low biomass production and low Se accumulation.

Several *Atriplex* plant lines show potential for use in phytoremediation in areas with low to moderate levels of sulfate salinity. Of the five plant lines that were in the top 30% of biomass producers and Se accumulators, *A. hortensis* 379088 and *A. hortensis* 379092 allowed no larvae to survive to pupation and only 10% of the insects fed *A. patula* survived. Other plant lines that

justify further investigation include *A. hortensis* which reduced insect development and permitted no insect survival and was also in the top 60% of Se accumulators and the top 30% of plant biomass producers, and A. spongiosa 415862 which reduced insect survival by 70% and was in the top 30% of plant biomass producers and top 33% of Se accumulators. Thus, these plant lines would not be likely to host significant *S. exigua* populations, or allow substantial bioaccumulation.

Our results suggest that these *Atriplex* lines could improve grower participation in phytoremediation or habitat improvement programs by potentially reducing local populations of a key insect pest. These plant lines act as a 'sink' in which oviposition occurs, but larval development and survival are inhibited. However, the most promising plant lines should be further studied to determine their effects on other key insect pests such as *Circulifer tenellus*, an important vector of curly top virus found in many regions of the western USA having Se contamination.

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References

Bañuelos, G.S., Akohoue, S., 1994. Comparison of wet digestion and microwave digestion on selenium and boron analysis in plant tissues. Comm. Soil Sci. & Plant Anal. 25, 1655–1670.

Bañuelos, G.S., Tebbets, J.S., Johnson, J., Vail, P.V., Mackey, B., 1999. Survey of insects and biotransfer of selenium from plants used for phytoremediation. Int. J. Phytoremed. 1, 311–326.

Bañuelos, G.S., Zayed, A., Terry, N., Wu, L., Akohoue, S., Zambruzuski, S., 1996. Accumulation of selenium by different plant species grown under increasing sodium and calcium chloride salinity. Plant Soil 183, 49–59.

Berdegué, M., Reitz, S.R., Trumble, J.T., 1998. Host plant selection in *Spodoptera exigua*: do mother and offspring know best? Entomol. Exp. Appl. 89, 57–64.

Daniels, L.A., 1996. Selenium metabolism and bioavailability. Biol. Trace Element Res. 51, 185–199.

Deverel, S.J., Fio, J.L., Dubrovsky, N.M., 1994. Distribution and mobility of selenium in ground water in the Western San Joaquin Valley of California. In: Frankenberger Jr., W.T., Benson, S. (Eds.), Selenium in the Environment. Marcel Dekker, New York, pp. 157–183.

Grieve, C.M., Suarez, D.L., Shannon, M.C., 1999. Effects of saline irrigation water composition on selenium accumulation by wheat. J. Plant Nutr. 22, 1443–1450.

Grieve, C.M., Poss, J.A., Suarez, D.L., Dierig, D.A., 2001. Lesquerella growth and selenium uptake affected by saline irrigation water composition. Ind. Crops Prod. 13, 57–65.

Hoffman, G.J., Shannon, M.C., 1986. Relating plant performance and soil salinity. Reclam. Reveg. Res. 5, 211–225.

Khattak, R.A., Page, A.L., Parker, D.R., Bakhtar, D., 1991. Accumulation and interactions of arsenic, selenium, molybdenum and phosphorus in alfalfa. J. Environ. Qual. 20, 165–168.

Lance, D.R., 1992. Odors influence choice of oviposition sites by *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). J. Chem. Ecol. 18, 1227–1237.

Lemly, A.D., 1997. Environmental implications of excessive selenium: a review. Biomed. Environ. Sci. 10, 415–435.

Lemly, A.D., 1998. Pathology of selenium poisoning in fish. In: Frankenberger Jr., W.T., Engberg, R.A. (Eds.), Environmental Chemistry of Selenium. Marcel Dekker, New York , pp. 281–296.

Losi, M.E., Frankenberger Jr., W.T., 1997. Bioremediation of selenium in soil and water. Soil Sci. 162, 692–702.

Mayland, H.F., 1994. Selenium in plant and animal nutrition. In: Frankenberger Jr., W.T., Benson, S. (Eds.), Selenium in the Environment. Marcel Dekker, New York, pp. 29–45.

Metcalf, C.L., Flint, W.P., 1962. Destructive and Useful Insects, Their Habits and Control. McGraw-Hill, San Francisco.

Nyberg, S., 1991. Multiple use of plants—studies on selenium incorporation in some agricultural species for the production of organic selenium compounds. Plant Foods Hum. Nutr. 41, 69–88.

Pearson, A.C., Sevacherian, V., Ballmer, G.P., Vail, P.V., Henneberry, T.J., 1989. Spring annual hosts of five noctuid pests in the Imperial valley of California (Lepidoptera: Noctuidae). J. Kans. Entomol. Soc. 61, 464–470.

Peterson, A., 1962. Larvae of Insects, an Introduction to Nearctic Species, Part I: Lepidoptera and Plant-Infesting Hymenoptera. Edwards Brothers, Ann Arbor.

SAS, 1996. SAS/STAT 6.12 for Macintosh. SAS Institute, Cary, NC.

StatView, 1993. Albacus Concepts Inc., Berkeley, CA.

Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics: A Biometric Approach. McGraw-Hill, New York.

Tallamy, D.W., Stull, J., Ehresman, N.P., Gorski, P.M., Mason, C.E., 1997. Cucurbitacins as feeding and oviposition deterrents to insects. Environ. Entomol. 26, 678–683.

Trumble, J.T., Kund, G.S., White, K.K., 1998. Influence of form and quantity of selenium on the development and survival of an insect herbivore. Environmental Pollution 101, 175–182.

Vickerman, D.B., Trumble, J.T., 1999. Feeding preferences of *Spodoptera exigua* in response to form and concentration of selenium. Arch. Insect Biochem. Physiol. 42, 64–73.

Watson, M., O'Leary, J., 1993. Performance of *Atriplex* species in the San Joaquin Valley, California, under irrigation and with mechanical harvests. Agric. Ecosys. Environ. 43, 255–266.

Wilson, C., Lesch, S.M., Grieve, C.M., 2000. Growth stage modulates salinity tolerance of New Zealand Spinach (*Tetragonia tetragonoïides*, Pall.) and Red Orach (*Atriplex hortensis* L.). Ann. Bot. 85, 501–509.

Wu, L., Enberg, A., Biggar, J.A., 1994. Effects of elevated selenium concentration on selenium accumulation and nitrogen fixation symbiotic activity of *Melilotus indica* L. Ecotoxicol. Environ. Safety 27, 50–63.

Wu, L., Vanmantgem, P.J., Guo, X., 1996. Effects of forage plant and field legume species on soil selenium redistribution, leaching, and bioextraction in soils contaminated by agricultural drain water sediment. Arch. Environ. Contam. Toxicol. 31, 329–338.

Zhang, M., Chaduri, S.K., Kubo, I., 1993. Quantification of insect growth and its use in screening of naturally occurring insect control agents. J. Chem. Ecol. 19, 1109–1118.