The deleterious effects of salinity stress on leafminers and their freshwater host

L I S A   S C H I L E [1] and S U S A N   M O P P E R   Department of Biology, University of Louisiana at Lafayette, U.S.A.

Abstract. 1. Salinity is an important cause of abiotic stress in wetland communities yet little is known about its consequences for freshwater plants and their insect herbivores. The goal of this study was to test the effect of salinity on a leafmining insect, *Cerodontha iridiphora*, and its herbaceous host plant, *Iris hexagona*.

2. Leafminer performance was evaluated on irises grown in control and saline treatments, and the effects of salinity and herbivory on leaf quality and mortality was measured.

3. Leafminer density and size were significantly lower on irises grown in saline water compared with freshwater.

4. Both salinity and herbivory accelerated leaf senescence and mortality, and their combined effects increased tissue loss by an order of magnitude compared with controls.

5. Leafminers acted as nutrient sinks. The undamaged regions of mined leaves contained 40% less nitrogen than unmined leaves, providing a mechanism for the premature leaf mortality.

6. Salinity was detrimental to the performance and survival of both the iris leafminer and its host plant. We propose that glycophytic host plants and their insect herbivores will suffer more than halophytic communities from environmental salinity because they lack the adaptive mechanisms to tolerate this potent physiological stress.

Key words. *Cerodontha iridiphora*, ecological communities, environment, nitrogen, herbivory, *Iris hexagona*, insect–plant interactions, leaf mortality, wetlands.

Introduction

Salinity is a growing threat to natural ecosystems (Yeo, 1999; Rogers & McCarty, 2000) because it disrupts the physiological processes of terrestrial plants (Levitt, 1980; Yeo, 1983; Hasegawa *et al*., 2000), increases mortality of wetland populations (Krauss *et al*., 2000), and reduces the diversity of ecological communities (Flynn *et al*., 1995; Holm & Sasser, 2001). Water-deficit stress also has strong effects on plants and a rich history in the ecological literature (White, 1976; Mattson, 1980; Larsson, 1989; Waring & Cobb, 1992; Schowalter *et al*., 1999; Inbar *et al*., 2001; Awmack & Leather, 2002). By altering host-

plant physiology (Rabe, 1990; Mansour, 2000), water stress can modify insect feeding preferences (Scheirs & De Bruyn, 2005), promote population outbreaks (White, 1976; Scriber & Slansky, 1981), or reduce fitness (Koricheva *et al*., 1998). Salinity stress elicits similar physiological responses in plants as water-deficit stress, but its ecological consequences are poorly understood.

The goal of this study was to investigate the effect of salinity stress (specifically, increased Na^+) on phytophagous insects and their glycophytic (freshwater) host plants. Like water stress, salinity alters osmotic potential (Zhu, 2002), which stimulates production of organic compounds such as proline and sucrose to help restore homeostasis (Wyn Jones & Gorham, 1983; Mansour, 2000; Wang, 2002). These compounds can influence the nutritional status and performance of herbivores (Hemminga & van Soelen, 1988; Gonçalves-Alvim *et al*., 2001; Moon & Stiling, 2002a). Most ecological studies have been conducted with halophytic (salt loving) plant species that can regulate salt uptake through succulence, salt excretion, or sequestration (Hale & Orcutt, 1987).

Correspondence: Susan Mopper, Department of Biology, 300 East Saint Mary Blvd, University of Louisiana, Lafayette, LA 70504-2451, U.S.A. E-mail: mop@louisiana.edu

[1]Current address: Department of Biology, San Francisco State University, 1600 Holloway Avenue, San Francisco, CA 94132, U.S.A.

Glycophytic plants lack the adaptations of halophytes and exhibit pronounced symptoms of stress even at low salt concentrations (Greenway & Munns, 1980; Van Zandt *et al.*, 2003). For example, salinity strongly influences *Iris hexagona* growth and reproduction (Van Zandt & Mopper, 2002, 2004; Van Zandt *et al.*, 2003), concentrations of phytohormones, nitrogenous compounds, and minerals (Wang *et al.*, 2001; Wang, 2002; Mopper *et al.*, 2004), and interactions with vertebrates and invertebrates (Geddes & Mopper, 2006; Tobler *et al.*, 2006). The 2-year experiment described here tested the influence of salinity stress on a leafminer that feeds on *I. hexagona*, and investigated the dual effects of salinity and herbivory on host-plant performance. This research provides new information about how an increasingly widespread form of environmental degradation – salt stress – affects insect–plant interactions in freshwater communities, and contributes to the development of an ecological theory of salinity stress.

Natural history

Iris is a northern hemisphere genus of Iridaceae indigenous to wetland ecosystems of the US Gulf Coast. It grows best in freshwater (Van Zandt *et al.*, 2003) but salinity-tolerant populations occur in brackish habitats (Van Zandt & Mopper, 2002). This perennial species reproduces sexually and clonally, and mature plants contain 5–10 long, slender leaves that are often attacked by *Cerodontha iridiphora* leafminers (Diptera: Agromyzidae) that specialise on iris (Spencer & Steyskal, 1986). Larvae initiate linear mines at the leaf base, which expand into blotch mines towards the leaf apex. Mature mines can encompass and destroy the entire leaf. Miners feed within leaves for approximately 1 month, then return to the base of the leaf to pupate. There are two generations per year (spring and autumn), with the largest densities occurring in the spring during April to June.

Methods

This experiment was conducted for 2 years at the Center for Ecology and Environmental Technology at the University of Louisiana at Lafayette, Louisiana, U.S.A. (http://ceet.louisiana.edu/). In December 1999, 30 227-litre plastic Rubbermaid containers (Rubbermaid Products, Wooster, Ohio) were planted with *I. hexagona* in deltaic soil. The plants and associated leafminers were collected from natural brackish marsh populations growing at Marsh Island, Louisiana (29.79°N 91.78°W), a 300-km² uninhabited wildlife refuge 10 km south of the Louisiana coast. The plants and leafminers were acclimated to the common garden for approximately 18 months before the salinity treatments began. In June 2001, 30 mesocosms were randomly assigned to 10 control and 20 salinity treatments, which were maintained for 1 year prior to the beginning of the experiment. Salinity levels were established with Instant Ocean™ (Aquarium Systems, Inc., Mentor, Ohio), a synthetic sea salt, and measured using a portable YSI Salinity and Conductivity meter (YSI Incorporated, Yellow Springs, Ohio).

The control mesocosms did not receive salt and their salinity levels reflect natural variation of the soil. In the 2002 leafminer experiment, 10 control and 10 salinity mesocosms were used, and their annual salinity levels averaged (mean ± SE) 0.2 ± 0.02 p.p.t. and 7.1 ± 0.29 p.p.t. respectively. In the 2003 leafminer experiment, the same 10 control mesocosms were used (annual salinity = 0.4 ± 0.03 p.p.t.), and the second group of 10 salinity treatment mesocosms. There was a wider range of salinity levels in the 2003 experiment because of weather and equipment variability. The treatment mesocosms experienced higher salinity in August (10.2 ± 1.2 p.p.t.) when miners were in the pupal stage, but their annual average was 6.2 ± 0.5 p.p.t., slightly less than the previous year. Variation in salinity treatment levels is unavoidable in such multiyear experiments, but it reflects natural conditions in field populations (Van Zandt & Mopper, 2002). There was no significant difference between the number of leaves in fresh and saltwater mesocosms in either 2002 (fresh = 355 ± 26, saline = 317 ± 39, $P = 0.45$) or 2003 (fresh = 486 ± 22, saline = 464 ± 41, $P = 0.67$). More detailed methods for the common garden experiments are described elsewhere (Van Zandt *et al.*, 2003).

The 2002 experiment

All *C. iridiphora* mines and leaves in the control and salinity mesocosms were counted in May 2002. The density estimate 'mines per leaf' can reflect oviposition preference as well as undetected egg and early larval mortality. Leafminer densities in the natural populations were similar to common garden densities (mines per leaf: 0.10 ± 0.04 and 0.11 ± 0.01 respectively; $F_{1,16} = 0.12$, $P = 0.73$). Mines were marked individually by placing colour-coded wooden dowels adjacent to the mined leaf. In June, the amount of senescence that had occurred in all of the mined leaves in each mesocosm and in a randomly selected subset of leaves without miners was measured. Healthy tissue is bright green whereas senesced tissue is brown–black in colour and signals incipient leaf mortality. It typically begins at the apex, and spreads down towards the base of the leaf. The magnitude of senescence was ranked from one to five, representing approximately 0, 25, 50, 75, and 100% dead tissue. At least five mined leaves from each mesocosm were removed and dissected to collect pupae and assess larval fate. The number of pupae per mine were counted and weighed to the nearest 0.1 mg using a Sartorius balance, model 1713 MP8 (Sartorius AG, Goettingen, Germany). After weighing, half of the pupae were placed individually in 8-ml plastic vials and stored in a growth chamber at 70% humidity until the adults eclosed. The remaining pupae were stored at −80 °C for future molecular analysis.

The 2003 experiment

In April 2003, five mined and five unmined leaves on the same plant were monitored in each mesocosm. Weekly for 4 weeks, each leaf was digitally photographed against a grid sheet with 1-cm² demarcations. Leaf senescence was quantified

using image-analysis software (ImageJ, version 1.29 x, National Institutes of Health, Bethesda, Maryland). Every 3 days, leaf survival was documented. If the monitored leaf died, measurements on the corresponding leaves ceased. During the final 2 weeks of larval activity, 20 adjacent mined and unmined leaves in each mesocosm were monitored for senescence, as well as two leaves from unmined plants. After pupation, five mined leaves were removed from each mesocosm, digitally photographed, and total mine area analysed. Each leaf was dissected to assess larval mortality, quantify the number of pupae, and determine pupal mass. At least 25 additional mined leaves from each mesocosm were collected to determine larval fate, number of pupae per mine, and pupal mass. Each pupa was stored individually and monitored as in 2002.

Foliar nitrogen and carbon

In May 2003, leaf samples were collected from five freshwater and five saline mesocosms to quantify foliar nitrogen and carbon. In each mesocosm, roughly 10 cm of undamaged tissue was removed from the centre of one mined leaf per plant, an adjacent unmined leaf from the same plant, and one leaf from a neighbouring unmined plant. Leaf tissue was oven-dried at 60 °C for 24 h. Samples were ground to a fine powder using an IKA A-10 analytical mill (IKA Works Inc., Wilmington, North Carolina). A Carlo-Erba NC2500 Automated Elemental Analyzer (CE Instruments, Rodano, Italy) was used to determine per cent total nitrogen and carbon, with two replicates ($\approx$ 3 mg each) collected from the same tissue. Prior to each run, a standard curve was created using 0.5 mg atropine and 1, 2, 3, and 4 mg EDTA. Quality controls were run every 20 tissue samples to monitor machine accuracy. Only replicates that differed by $\leq 5\%$ were included in the analysis.

Statistical methods

All statistical procedures were conducted with SAS/STAT Version 8.2 (SAS Institute, 2001). Observations were averaged within each mesocosm and tested for normality and heteroscedasticity (PROC UNIVARIATE). When necessary, data were transformed or nonparametric procedures were used. In 2002, leafminer densities (arcsine square-root transformed), pupal mass, and leaf senescence in freshwater and saline treatments were compared using analysis of variance (PROC GLM). Larval mortality was not analysed in 2002 owing to low sample size. Numbers of pupae per mine were compared using Mann–Whitney *U* (PROC NPAR1WAY).

In 2003, regression analysis (PROC REG) was used to evaluate leafminer and host-plant performance. Regression was used instead of analysis of variance because of the greater variation among salinity levels in the experimental mesocosms. This approach was more effective at estimating the relationship between salinity and the response variables. In instances where independent variables are more continuous than factorial, regression is preferred to analysis of variance (Gotelli & Ellison, 2004).

Separate linear regressions were conducted with mesocosm salinity as the independent variable and mine density per leaf, pupal density within a mine, pupal mass, and mine area as dependent variables. Larval mortality was analysed using Mann–Whitney *U*. To assess interactive effects of salinity and herbivory on foliar nitrogen and carbon, analysis of variance was used. Leaf senescence (arcsine square-root transformed) was analysed using a multiple regression with salinity and the presence of mines as independent variables. Repeated-measures analysis of variance (REPEATED option of PROC GLM) was used to determine if salinity and leafmining activity affected the rate of leaf senescence. Multiple pairwise comparisons were conducted using adjusted Tukey tests (PDIFF ADJUST = TUKEY option in PROC GLM). Survival analysis was used to examine the combined effects of leafminer herbivory and salinity on leaf mortality (COVSANDWICH option of PROC PHREG, Lee *et al.*, 1992). The analysis corrected for autocorrelation among leaves within the same mesocosm.

Results

Salinity stress and herbivore performance

Salinity had negative effects on the performance of leafminers and iris host plants. In 2002, mine densities were lower on irises grown in saline water ($F_{1,18} = 5.78$, $P = 0.027$) and there were fewer pupae per mine ($P = 0.007$) compared with freshwater controls (Fig. 1). In 2003, salinity reduced the number of pupae per mine ($R^2 = 0.29$, $F_{1,18} = 8.88$, $P = 0.008$; Fig. 2a) and pupal mass ($R^2 = 0.23$, $F_{1,18} = 6.55$, $P = 0.02$; Fig. 2b). All other performance measures were lower in the salinity treatment but were not statistically different from the controls (mine density, $R^2 = 0.09$, $F_{1,18} = 1.39$, $P = 0.25$, Fig. 2c; mine area, $R^2 = 0.13$, $F_{1,18} = 0.86$, $P = 0.36$, Fig. 2d; late-instar larval mortality, $P = 0.31$, not shown). There was no correlation among mine area, pupal mass, and pupal density within a mine
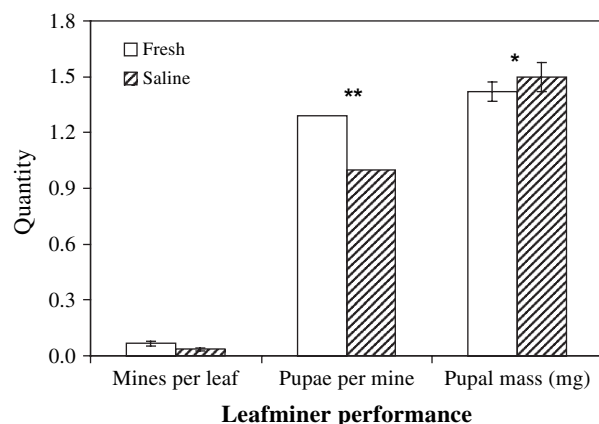
Fig. 1. Performance of leafminers on irises grown in freshwater and saline treatments in 2002. Means ($\pm$ SE) shown for mines per leaf and pupal mass, medians shown for pupae per mine (*$P \leq 0.05$, **$P \leq 0.01$).
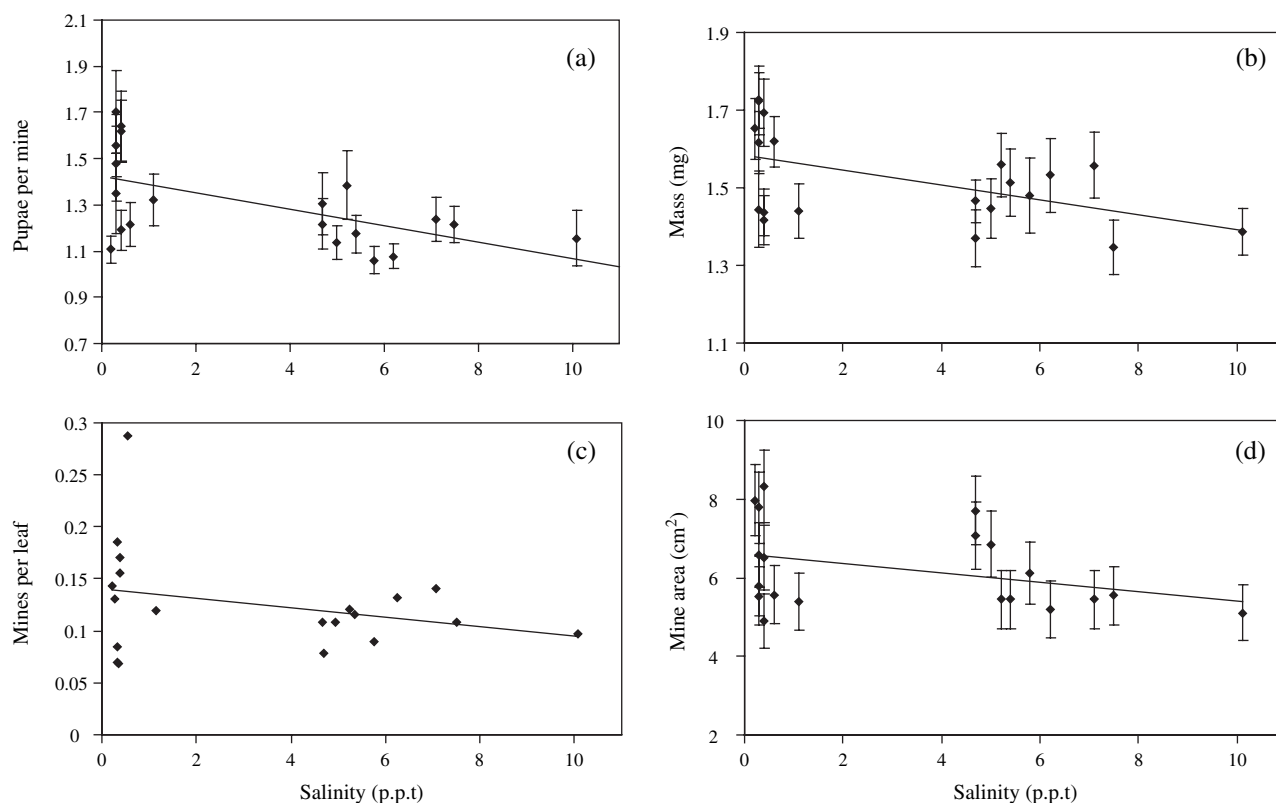
Fig. 2. Performance of leafminers on irises grown in freshwater and saline treatments in 2003. Number of pupae per mine (a), pupal mass (b), leafminer density (c), and mine area (d) ($n = 20$; ± 1 SE). Density (mines per leaf) was calculated as the total number of mines divided by the total number of leaves in each mesocosm, thus has no error bars.

(area vs. mass: $P = 0.84$; area vs. density: $P = 0.10$; mass vs. density: $P = 0.12$). Natural enemies did not influence leafminer fate in this study as no predation and only a single parasitoid wasp were observed.

Foliar nitrogen and carbon

Leafminers acted as nutrient sinks. The nitrogen levels in undamaged portions of mined leaves were 40–50% lower than in unmined leaves ($F_{2,25} = 5.34$, $P = 0.012$; Fig. 3a) and foliar carbon was reduced ($F_{2,25} = 4.44$, $P = 0.022$; Fig. 3b). Salinity did not affect foliar nitrogen or carbon ($F_{1,25} = 1.3$, $P = 0.27$ and $F_{1,25} = 0.86$, $P = 0.36$ respectively), and there was no interaction between salinity and herbivory on nitrogen or carbon content ($F_{2,25} = 0.31$, $P = 0.74$ and $F_{2,25} = 0.6$, $P = 0.56$ respectively).

Leaf senescence

Salinity and leafminers each had strong effects on leaf health and dramatically increased rates of senescence in 2002 (Fig. 4) and 2003 (Fig. 5). In 2002, mining ($F_{1,34} = 110.9$, $P < 0.0001$) and salinity ($F_{1,34} = 101.3$, $P < 0.0001$) increased leaf senescence, and there was a significant interaction between them ($F_{1,34} = 20.8$, $P < 0.0001$). Leaf senescence was greater in mined leaves than unmined leaves in both saline and freshwater mesocosms ($P < 0.0001$ and $P < 0.0004$ respectively). Salinity increased senescence in both mined and unmined leaves compared with plants grown in freshwater ($P < 0.0001$ and $P < 0.0024$ respectively).

In 2003, the combined effects of salinity and mining increased leaf senescence by roughly 50% ($R^2 = 0.8703$, $F_{3,36} = 88.24$, $P < 0.0001$), but there was no interaction between them ($F_{1,36} = 0.71$, $P = 0.68$). Mined leaves contained more senesced tissue than unmined leaves ($F_{1,36} = 192.72$, $P < 0.0001$), and leaves grown under saline conditions were more senesced than freshwater control leaves ($F_{1,36} = 71.84$, $P < 0.0001$). Leaf senescence increased with time (Wilks' lambda $F_{3,28} = 160$, $P < 0.0001$; Fig. 5b) but there was no significant interaction between time and mining, time and salinity, or time, mining, and salinity (Wilks' lambda, $F_{3,28} = 1.94$, $P = 0.14$; $F_{3,28} = 0.16$, $P = 0.92$; and $F_{3,28} = 1.17$, $P = 0.34$ respectively).

Senescence accelerated significantly in mined and unmined plants during the final 2 weeks of the experiment (Wilks' lambda, $F_{1,45} = 86.3$, $P < 0.0001$), and was greatest in mined leaves from the salinity treatment ($F_{2,45} = 2.98$, $P = 0.06$, Fig. 5b). Mined leaves always contained more senesced tissue than unmined leaves from the same plant, and mined plants always senesced more rapidly than unmined plants ($P < 0.05$ for all comparisons).
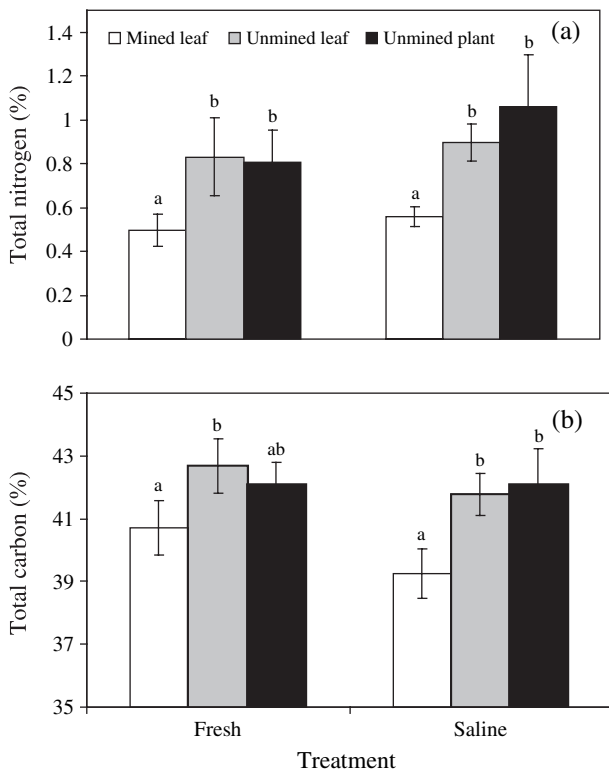
Fig. 3. Foliar nitrogen (a) and carbon (b) of mined *Iris hexagona* leaves, unmined leaves from mined plants, and leaves from unmined plants averaged within fresh and saline treatments in 2003 ($n = 5$ mesocosms per treatment; ± 1 SE; letters denote statistical differences of ≤ 0.05).

The combined effects of leafminers and salinity stress increased leaf mortality an order of magnitude compared to control leaves ($x_1 = 20.45$, $P < 0.0001$ and $x_1 = 17.79$, $P < 0.0001$ respectively; Fig. 5c). Mined leaves died sooner than unmined leaves in both the salinity ($x_1 = 13.75$, $P = 0.0002$) and freshwater ($x_1 = 5.44$, $P = 0.0197$) treatments. Additionally, mined leaves from the salinity treatment died before mined leaves from the freshwater treatment ($x_1 = 16.58$, $P < 0.0001$). Salinity did not accelerate mortality of unmined leaves ($x_1 = 0.09$, $P = 0.76$).
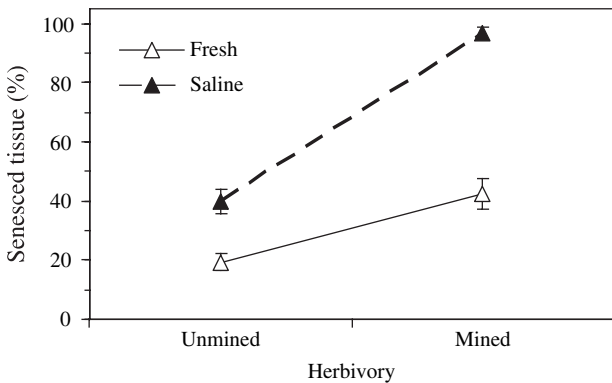
Fig. 4. Senescence of mined and unmined *Iris hexagona* leaves grown in fresh and saline treatments in 2002.
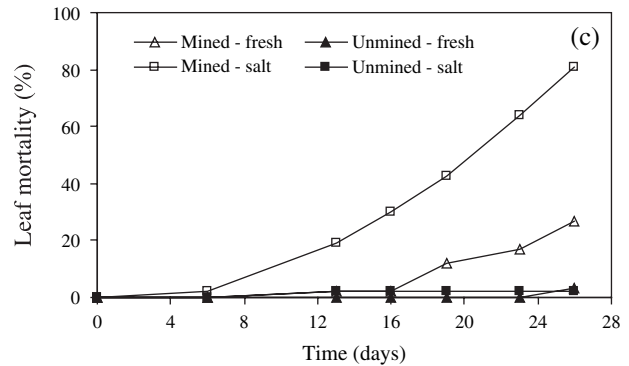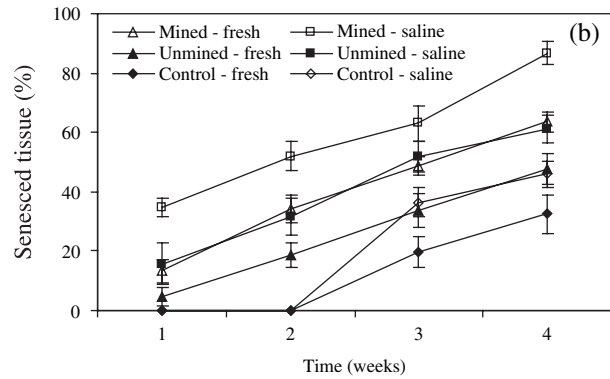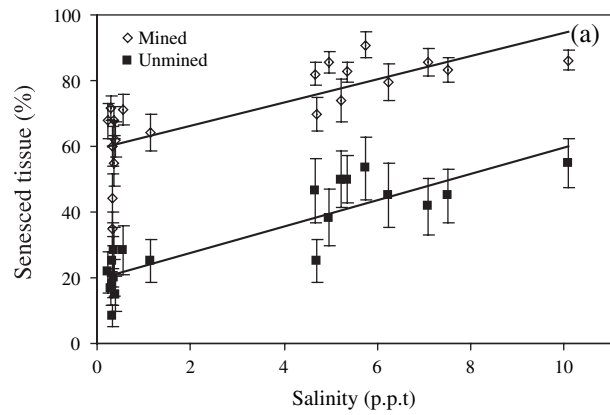
Fig. 5. Senescence of mined and unmined *Iris hexagona* leaves grown in fresh and saline treatments in 2003. Per cent leaf senescence in each mesocosm (a); senescence over time in mined and unmined leaves from the same plant and adjacent unmined 'control' plant (b); cumulative mortality (c). Data in (b) and (c) were averaged (± 1 SE) within fresh (0.4 ± 0.03 p.p.t., $n = 9$) and saline (6.2 ± 0.53 p.p.t., $n = 10$) mesocosms.

Discussion

Environmental salinity is a widespread abiotic stress (Jackson *et al.*, 1995; Yeo, 1999; Rogers & McCarty, 2000; Yokoi *et al.*, 2002), yet little is understood about how it affects ecological communities. Our study revealed that salinity can be as deleterious to leafminer performance as plant water deficits (Bultman & Faeth, 1987; Preszler & Price, 1995; Inbar *et al.*, 2001; De Bruyn *et al.*, 2002). Relatively low levels of NaCl (6–7 p.p.t.)

decreased the density and mass of iris leafminers, and markedly accelerated iris leaf senescence. When salinity was combined with leafminer herbivory, leaf mortality increased by an order of magnitude compared with controls. Leafminers acted as nutrient sinks and reduced nitrogen in adjacent uneaten foliage by as much as 50%. Nitrogen is an essential building block of compounds that plants produce to counteract abiotic stress (Rabe, 1990; White, 1993; Mansour, 2000). Disruption by leafminers of foliar nutrients could be an underlying mechanism of premature leaf mortality in salt-stressed iris, and may explain why leafminers induce abscission in woody plants (Faeth *et al.*, 1981; Preszler & Price, 1993; Mopper & Simberloff, 1995; Waddell *et al.*, 2001; Stiling *et al.*, 2002).

There is little information about the consequences of salinity on the ecological interactions between host plants and their herbivores, and the available evidence is primarily from halophytic species. Salinity had no detectable effect on lepidopteran leafminers feeding on sea aster (Hemminga & van Soelen, 1992), and improved the performance of dipteran leaf gallers on mangrove (Gonçalves-Alvim *et al.*, 2001) and homopteran planthoppers on sea ox-eye daisy (Moon & Stiling, 2002a). However, it was associated with reduced performance of coleopteran stem-borers on sea aster (Hemminga *et al.*, 1987; Hemminga & van Soelen, 1988) and lepidopteran borers and dipteran gallers on sea ox-eye daisy (Moon & Stiling, 2002a, b). In ecological studies of glycophytic associations, salinity reduced performance of dipteran leaf miners (Martel, 1998) and gall formers (Moon & Stiling, 2002b) attacking goldenrod host plants.

Until there are more studies on glycophytic plants, no general conclusions can be drawn about insect–plant interactions in salt-stressed communities. Nonetheless, we propose the hypothesis that salinity has more predictably negative effects on glycophytic herbivores and their host plants than halophytic species, because mechanisms have not evolved in freshwater plants to tolerate the physiological stress of salt (Greenway & Munns, 1980; Van Zandt *et al.*, 2003). In *I. hexagona*, salinity has a profound effect on metabolic function that could adversely affect herbivore performance (Wang *et al.*, 2001; Wang, 2002; Mopper *et al.*, 2004). For example, insects feeding on salt-stressed iris must contend with the NaCl that accumulates in leaf tissues because there are no secretory organs to remove it (Wang, 2002). The osmotic stress imposed by salinity also impedes the absorption by herbivores of essential sugars from host tissues (Foster & Treherne, 1976). This could be particularly challenging for early instar leafminers, which are particularly vulnerable to reductions in plant quality (Mopper *et al.*, 1995; Connor & Taverner, 1997; Mopper *et al.*, 2000).

Salinisation of freshwater habitat is a growing threat to natural populations in terrestrial and wetland communities. In 2005, the saltwater intrusion caused by Hurricanes Katrina and Rita caused massive environmental damage to the Gulf Coast region of the USA. Large-scale environmental disturbances are occurring ever more frequently around the globe, and it is essential to understand their physiological, ecological, and evolutionary consequences. Much more research is necessary to address these critical issues, understand the underlying biological mechanisms, and develop theory to predict future impacts.

References

Awmack, C.S. & Leather, S.R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, **47**, 817–844.

Bultman, T.L. & Faeth, S.H. (1987) Impact of irrigation and experimental drought stress on leaf-mining insects of Emory oak. *Oikos*, **48**, 5–10.

Connor, E.F. & Taverner, M.P. (1997) The evolution and adaptive significance of the leaf-mining habitat. *Oikos*, **79**, 6–25.

De Bruyn, L.J., Scheirs & Verhagen, R. (2002) Nutrient stress, host plant quality and herbivore performance of a leaf-mining fly on grass. *Oecologia*, **130**, 594–599.

Faeth, S.H., Conner, E.F. & Simberloff, D. (1981) Early leaf abscission: a neglected source of mortality for folivores. *American Naturalist*, **117**, 409–415.

Flynn, K.M., McKee, K.L. & Mendelssohn, I.A. (1995) Recovery of freshwater marsh vegetation after a saltwater intrusion event. *Oecologia*, **103**, 63–72.

Foster, W.A. & Treherne, J.E. (1976) Insects of marine saltmarshes: problems and adaptations. *Marine Insects* (ed. by L. Cheng), pp. 5–42. North Holland Publishing, Oxford.

Geddes, N. & Mopper, S. (2006) Effects of environmental salinity on vertebrate florivory and wetland communities. *Natural Areas Journal*, **26**, 31–37.

Gonçalves-Alvim, S.J., Vaz dos Santos, M.C.F. & Wilson Fernandes, G. (2001) Leaf gall abundance on *Avicennia germinans* (Avicenniaceae) along an interstitial salinity gradient. *Biotropica*, **33**, 69–77.

Gotelli, N.J. & Ellison, A.M. (2004) *A Primer of Ecological Statistics*. Sinauer Associates, Sunderland, Massachusetts.

Greenway, H. & Munns, R. (1980) Mechanisms of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology*, **31**, 149–190.

Hale, M.G. & Orcutt, D.M. (1987) *The Physiology of Plants Under Stress*. John Wiley and Sons, New York.

Hasegawa, P.M., Bressan, R.A., Zhu, J.K. & Bohnert, H.J. (2000) Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology*, **51**, 463–499.

Hemminga, M.A. & van Soelen, J. (1988) Estuarine gradients and the growth and development of *Agapanthia villosoviridescens*, (Coleoptera), a stem-borer of the salt marsh halophyte *Aster tripolium*. *Oecologia*, **77**, 307–312.

Hemminga, M.A. & van Soelen, J. (1992) The performance of the leaf mining microlepidopteran *Bucculatrix maritima* (Stt.) on the salt marsh halophyte, *Aster tripolium* (L.), exposed to different salinity conditions. *Oecologia*, **89**, 422–427.

Hemminga, M.A., van Soelen, J. & Markusse, M.M. (1987) The relation between the halophyte *Aster tripolium* and *Agapanthia villosoviridescens*, an insect invader of estuarine salt marshes. *Vegetation Between Land and Sea* (ed. by A. H. L. Huiskes, C. W. P. M. Blom and J. Rozema), pp. 214–225. Dr W. Junk, Dordrecht, the Netherlands.

Holm, G.O. & Sasser, C.E. (2001) Differential salinity response between two Mississippi River subdeltas: implications for changes in plant composition. *Estuaries*, **24**, 78–89.

Inbar, M., Doostdar, H. & Mayer, R.T. (2001) Suitability of stressed and vigorous plants to various insect herbivores. *Oikos*, **94**, 228–235.

Jackson, L.L., Foote, A.L. & Balistrieri, L.S. (1995) Hydrological, geomorphological, and chemical effects of hurricane Andrew on coastal marshes of Louisiana. *Journal of Coastal Research*, **21**, 306–323.

Koricheva, J., Larsson, S. & Haukioja, E. (1998) Insect performance on experimentally stressed woody plants: a meta-analysis. *Annual Review of Entomology*, **43**, 195–216.

Krauss, K.W., Chambers, J.L., Allen, J.A., Soileau, D.M. & Debosier, A.S. (2000) Growth and nutrition of bald cypress families planted under varying salinity regimes in Louisiana, U.S.A. *Journal of Coastal Research*, **16**, 153–163.

Larsson, S. (1989) Stressful times for the plant stress–insect performance hypothesis. *Oikos*, **56**, 77–283.

Lee, E.W., Wei, L.J. & Amato, D. (1992) Cox-type regression analysis for large number of small groups of correlated failure time observations. *Survival Analysis: State of the Art* (ed. by J. P. Klein and P. K. Goel), pp. 237–247. Kluwer Academic, Dordrecht, the Netherlands.

Levitt, J. (1980) Responses of Plants to Environmental Stress, vol. 2, 2nd edn, *Water, Radiation, Salt, and Other Stresses*. Academic Press, New York.

Mansour, M.M.F. (2000) Nitrogen containing compounds and adaptation of plants to salinity stress. *Biologia Plantarum*, **43**, 491–500.

Martel, J. (1998) Plant-mediated effects of soil salinity on a gall-inducing caterpillar *Epiblema scudderiana* (Lepidoptera: Tortricidae) and the influence of feeding guild. *European Journal of Entomology*, **95**, 545–557.

Mattson, W.J. (1980) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, **11**, 119–161.

Moon, D.C. & Stiling, P. (2002a) Top-down, bottom-up, or side to side? Within-trophic-level interactions modify trophic dynamics of a salt marsh herbivore. *Oikos*, **98**, 480–490.

Moon, D.C. & Stiling, P. (2002b) The effects of salinity and nutrients on a tritrophic salt-marsh system. *Ecology*, **83**, 2465–2476.

Mopper, S., Beck, M., Simberloff, D. & Stiling, P. (1995) Local adaptation and agents of selection in a mobile insect. *Evolution*, **49**, 810–815.

Mopper, S. & Simberloff, D. (1995) Differential herbivory in an oak population: the role of plant phenology and insect performance. *Ecology*, **76**, 1233–1241.

Mopper, S., Stiling, P., Landau, K., Simberloff, D. & van Zandt, P. (2000) Spatiotemporal variation in leafminer population structure and adaptation to individual oak trees. *Ecology*, **81**, 1577–1587.

Mopper, S., Wang, Y., Criner, C. & Hasenstein, K. (2004) *Iris hexagona* hormonal responses to salinity stress, leafminer herbivory, and phenology. *Ecology*, **85**, 38–47.

Preszler, R.W. & Price, P.W. (1993) The influence of *Salix* leaf abscission on leaf-miner survival and life history. *Ecological Entomology*, **18**, 150–154.

Preszler, R.W. & Price, P.W. (1995) A test of plant-vigor, plant-stress, and plant-genotype effects on leaf-miner oviposition and performance. *Oikos*, **74**, 485–492.

Rabe, E. (1990) Stress physiology: the functional significance of the accumulation of nitrogen-containing compounds. *Journal of Horticultural Science*, **65**, 231–243.

Rogers, C.E. & McCarty, J.P. (2000) Climate change and ecosystems of the Mid-Atlantic Region. *Climate Research*, **14**, 235–244.

SAS Institute (2001) *SAS, Version 8.2*. SAS Institute Inc., Cary, North Carolina.

Scheirs, J. & De Bruyn, L. (2005) Plant-mediated effects of drought stress on host preference and performance of a grass miner. *Oikos*, **108**, 371–385.

Schowalter, T.D., Lightfoot, D.C. & Whitford, W.G. (1999) Diversity of arthropod responses to host-plant water stress in a desert ecosystem in southern New Mexico. *American Midland Naturalist*, **142**, 281–290.

Scriber, J.M. & Slansky, F. (1981) The nutritional ecology of immature insects. *Annual Review of Entomology*, **26**, 183–211.

Spencer, K.A. & Steyskal, G.C. (1986) *Manual of the Agromyzidae (Diptera) of the United States*. Agriculture Handbook no. 638. U.S. Department of Agriculture, Springfield Virginia, U.S.A.

Stiling, P., Cattell, M., Moon, D., Rossi, A., Hungate, B., Hymus, G. et al. (2002) Elevated atmospheric CO_2 lowers herbivore abundance, but increases leaf abscission rates. *Global Change Biology*, **8**, 658–667.

Tobler, M., Van Zandt, P., Hasenstein, K. & Mopper, S. (2006) Growth and reproduction of a wetland plant in response to salinity and florivory. *Wetlands,* **26**.

Van Zandt, P.A. & Mopper, S. (2002) Delayed and carryover effects of salinity on flowering in *Iris hexagona* (Iridaceae). *American Journal of Botany*, **89**, 1847–1851.

Van Zandt, P.A. & Mopper, S. (2004) The effects of maternal salinity and seed environment on germination and growth in *Iris hexagona*. *Evolutionary Ecology Research*, **6**, 813–832.

Van Zandt, P.A., Tobler, M.A., Mouton, E., Hasenstein, K.H. & Mopper, S. (2003) Positive and negative consequences of salinity stress for the growth and reproduction of the clonal plant, *Iris hexagona*. *Journal of Ecology*, **91**, 837–846.

Waddell, K.J., Fox, C.W., White, K.D. & Mousseau, T.A. (2001) Leaf abscission phenology of a scrub oak: consequences for growth and survivorship of a leaf mining beetle. *Oecologia*, **127**, 251–258.

Wang, Y. (2002) *Ecological, physiological, and molecular responses of Iris hexagona to salinity stress*. PhD dissertation, University of Louisiana, Lafayette, U.S.A.

Wang, Y., Mopper, S. & Hasenstein, K.H. (2001) Effects of salinity on endogenous ABA, IAA, JA, and SA in *Iris hexagona*. *Journal of Chemical Ecology*, **27**, 327–342.

Waring, G.L. & Cobb, N.S. (1992) The impact of plant stress on herbivore population dynamics. *Plant–Insect Interactions*, 4 (ed. by E. Bernays), pp. 167–266. CRC, Boca Raton, Florida.

White, T.C.R. (1976) Weather, food, and plagues of locusts. *Oecologia*, **22**, 119–134.

White, T.C.R. (1993) *The Inadequate Environment – Nitrogen and the Abundance of Animals*. Springer, Berlin.

Wyn Jones, R.G. & Gorham, J. (1983) *Osmoregulation. Encyclopedia of Plant Physiology*, 12C (ed. by O. L. Lange, P. S. Nobel, C. B. Osmond and H. Ziegler), pp. 35–58. Springer, Berlin.

Yeo, A.R. (1983) Salinity resistance – physiologies and prices. *Physiologia Plantarum*, **58**, 214–222.

Yeo, A.R. (1999) Predicting the interaction between the effects of salinity and climate change on crop plants. *Scientia Horticulturae*, **78**, 159–174.

Yokoi, S., Bressan, R.A. & Hasegawa, P.M. (2002) *Salt Stress Tolerance of Plants*. Japan International, Research Center for Agricultural Sciences Working Report 25–33.

Zhu, J.K. (2002) Salt and drought stress signal transduction in plants. *Annual Review of Plant Biology*, **53**, 247–273.