

IRIS HEXAGONA HORMONAL RESPONSES TO SALINITY STRESS, LEAFMINER HERBIVORY, AND PHENOLOGY

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Abstract. We used field, common garden, and laboratory studies to investigate the influence of environmental stress on a native wetland species, *Iris hexagona* (Iridaceae), and an iris leafminer, *Cerodontha iridiphora* (Diptera: Agromyzidae). There were strong effects of salinity, herbivory, and phenology on jasmonic acid (JA) and salicylic acid (SA), two important signal phytohormones. Within 48 h, foliar JA increased an order of magnitude, and SA declined 50% in plants grown under salinity stress compared to freshwater controls. Leafminer performance declined on plants exposed to the salinity treatment. Both herbivory and salinity stress induced JA and suppressed SA, indicating that plants can respond to abiotic and biotic stress through shared hormonal pathways. Surprisingly, leafminer pupae contained JA and SA, and we discuss the implications of this novel discovery. Unrelated to salinity, there was a sharp decline in foliar JA between April and September. Despite its strong impact on hormonal signaling in plants, the abiotic environment is often ignored in ecological studies of induced plant defenses. Importantly, natural seasonal cycles and/or environmental stress can induce stronger changes in phytohormones than herbivory, and should be incorporated into the design of ecological experiments whenever possible, for a balanced understanding of phytohormonal ecology.

Key words: *abiotic stress; Cerodontha iridiphora; cross-kingdom signal sharing; Iris hexagona; jasmonic acid; leaf nitrogen; phytohormones; plant defense; plant–insect interactions; salicylic acid; signaling molecules.*

INTRODUCTION

Plants are exposed to a multitude of environmental challenges they must identify and respond to. Major advances are occurring in the biology of plant molecular signals, the pathways they initiate, and the adaptations that ensue. Much research is focused on phytohormones, which are regulatory molecules that act at very low concentrations and have numerous anti-stress functions (Hasegawa et al. 2000, Knight and Knight 2001, Weyers and Paterson 2001). The first step in a cascade of events is the production of one or more molecular signals that link environmental stimuli with the appropriate physiological responses (Karban and Baldwin 1997). These processes are not linear and specific as previously believed, but are complex and interactive networks (Stout and Bostock 1999, Knight and Knight 2001).

Jasmonic acid (JA) and salicylic acid (SA) are small, simple hormones crucial in many plant responses to ecological challenges (Agrawal et al. 1999, Stotz et al. 2002). The concentration and ratio of these and related compounds are important determinants of ecological interactions between plants, natural enemies, and the

environment (Berger 2002). JA and SA regulate separate biochemical pathways, and have distinct functions in plant development, reproduction, and physiology (Karban and Baldwin 1997). The JA- and SA-signaling pathways function independently or can interact through crosstalk, a process in which signal molecules affect each other's outcome in additive or negative ways (Chao et al. 1999, Knight and Knight 2001, Stotz et al. 2002). JA and SA have opposite effects on PR genes in tobacco (Niki et al. 1998) and tomato (Thaler et al. 1999). SA inhibits JA and its precursors in flax (Harms et al. 1998) and *Arabidopsis* (Gupta et al. 2000). The crosstalk that occurs between hormonal signals and their metabolic pathways (Moons et al. 1997, Bostock et al. 2001, Knight and Knight 2001) creates complex networks of activity that vary spatially and temporally (Hatcher et al. 2004, Voisenek et al. 2004).

Jasmonic acid (JA) and its methyl ester (JAMe) vary as a function of plant tissue type, developmental stage, and external stimuli. The highest concentrations typically occur in flowers and reproductive tissues (Creelman and Mullet 1995). JA is elicited by herbivory and mechanical wounding (McCloud and Baldwin 1997, Baldwin 1998), water deficit (Creelman and Mullet 1997), microbial activity (Blechert et al. 1995), and plant-signaling peptides (Bergey et al. 1996). The systemic induction of JA by wounding or pathogens has been attributed to damaged cell membranes that release fatty acids, which then metabolize via lipoxygenase to

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JA (Sembdner and Parthier 1993). Jasmonates and their derivatives trigger the expression of plant chemical defenses such as proteinase inhibitors, which disrupt herbivore digestive processes (Farmer et al. 1992, Karban and Baldwin 1997). Exogenous application of JA to plants induces systemic accumulation of compounds with antiherbivore properties, and elicits volatile emissions that attract herbivore natural enemies (Thaler 1999, Thaler et al. 2001).

Salicylic acid, a phenolic biosynthesized from benzoic acid and phenylalanine, is an essential constituent in the signal transduction pathways of systemic plant defenses against herbivores and pathogens (Raskin 1992, Hammerschmidt and Smith-Becker 1999). Salicylic acid initiates pathogen induced systemic acquired resistance (SAR) and triggers the expression of pathogenesis related (PR) genes, which code for defensive proteins (Klessig and Malamy 1994). SA is the precursor of methyl salicylate (MSA), a primary ingredient in pollinator-attracting floral scent emissions (Raguso and Light 1998, Pott et al. 2002). Salicylates are induced by herbivory and can reduce herbivore performance and deter feeding (Pierpoint 1994). Salicylates also occur in "green leaf" volatiles that attract herbivore natural enemies to damaged plants (Van Peoche et al. 2001).

Many new insights into the functions of phytohormones have arisen through exhaustive work on model organisms like *Arabidopsis* in controlled studies that eliminate as much environmental variation as possible (Berger 2002). Ecologists studying nonmodel, native plant species in natural settings are challenged to make sense of plant signaling processes in the face of tremendous environmental variability (Farnsworth 2004). This has produced a dichotomy in phytohormonal ecology, in which biotic factors such as herbivory are investigated separately from abiotic stress (e.g., Kessler and Baldwin 2002), despite the powerful effects and simultaneous occurrence of both. Recent ecological studies have addressed these concerns by developing new conceptual models of plant-signal responses and adaptations to diverse natural enemies and environmental conditions (Cippolini 2004, Hatcher et al. 2004, Schultz and Appel 2004, Thaler and Bostock 2004, and Voosenek et al. 2004).

To understand plant-signal responses to the environment, it is important to integrate controlled laboratory and ecological field studies. We are investigating the physiological ecology of *Iris hexagona* (Iridaceae), a native wetland perennial, and its interactions with *Cerodontha iridiphora* (Diptera: Agromyzidae), an iris leafminer. Salinity stress is a formidable and escalating environmental threat to wild and cultivated plants (Yeo 1999, Rogers and McCarty 2000). Elevated sodium weakens or kills plants by disrupting cellular, metabolic, and genetic processes (Levitt 1980, Hasegawa et al. 2000), and coastal populations are particularly vulnerable (Pezeshki et al. 1990, Howard and Mendels-

sohn 1999). One example in our study system is Hurricane Lili, which in October 2002 submerged coastal Louisiana under a 2–3-m storm surge that raised environmental salinity in freshwater wetlands more than 10-fold.

Even small increases in salinity can dramatically affect freshwater plants. For example, 2–4‰ NaCl reduced *I. hexagona* biomass by 40% and tripled seed production (Van Zandt et al. 2003), delayed flowering phenology by several days (Van Zandt and Mopper 2002), and significantly altered concentrations of JA, SA, abscisic acid (ABA), and auxin, four phytohormones with critical regulatory functions (Wang et al. 2001). The salicylates and jasmonates that are so important in plant–enemy interactions also regulate plant responses to salinity and other stresses (Iuchi et al. 1996, Tsonev et al. 1998, Chao et al. 1999, Hoyos and Zhang 2000, Wang et al. 2001), and both JA (Creelman and Mullet 1997) and SA (Yen and Yen 1999) can ameliorate the effect of salt on plant cells.

In this paper, we present laboratory, common garden, and field studies that were conducted in 1998 and 1999 with *Iris hexagona* (Iridaceae), and an iris leafminer, *Cerodontha iridiphora* (Diptera: Agromyzidae). We focused on JA and SA because both are induced by herbivory and salinity stress. The experiments presented here were designed to test independent questions; each contributes unique information, and as a whole they illustrate why long-term studies in variable environments are necessary to understand the ecological roles of plant signal hormones.

METHODS

Background on study system

Iris hexagona (Walters), in the family Iridaceae, is native to Louisiana's freshwater and brackish wetlands. The species is a long-lived perennial that reproduces sexually and clonally. The *I. hexagona* populations investigated in this study are patchily distributed throughout 300-km² Marsh Island (29°37' N, 91°54' W) that was a freshwater wetland (Orton 1959) before it separated from the Mississippi Delta mainland roughly 2000–5000 years ago (Coleman 1988, Törnqvist et al. 1996). Marsh Island salinity varies seasonally from 2–15‰ (Van Zandt and Mopper 2002) and is considerably higher than mainland *I. hexagona* habitats. Island *I. hexagona* populations are small and isolated compared with freshwater populations, which grow in large and continuous aggregations of thousands of individuals (see Plate 1).

Cerodontha iridiphora Spencer (Agromyzidae: Diptera) is an iris leafminer that establishes mines on the upper leaf surface in May through June. Mines contain multiple larvae that pupate in the basal portion of the mined leaf. Leaf damage is severe and mined leaves senesce sooner than unmined leaves (L. Schile and S. Mopper, unpublished data). Little is known about



PLATE 1. A natural population of *Iris hexagona* in a saline habitat. Populations in freshwater marsh contain thousands of individuals, but brackish marsh populations, such as this one, are small and discrete and can withstand substantially elevated salinity. Photo credit: S. Mopper.

causes of *C. iridiphora* mortality, but plant defenses are an important source of mortality in other leafminer species, particularly in the early instars (Connor and Taverner 1997, Mopper et al. 2000).

Leafminer density and pupal mass in natural I. hexagona populations

We used airboats to access three remote Marsh Island *I. hexagona* populations in 1999. Each population consisted of ~250 individual ramets, each with 5–25 leaves. We examined an average of 18 ramets per population for evidence of leafminer feeding, and maximized the distance between plants to reduce the chance of resampling the same genotypes. Density estimates were calculated as the mean number of mines per leaf. On 9 July, we collected 19, 22, and 91 leafminer pupae from three *I. hexagona* populations that were separated by at least 1 km. We also determined the interstitial soil salinity at each site (Orion 125 conductivity meter; Orion Research, Beverly, Massachusetts, USA). Pupae were returned to the laboratory and weighed.

The common garden

We established a common garden in the winter of 1997 with plants collected from 10 *I. hexagona* natural populations. Irises were grown in 30 227-L Rubbermaid mesocosms (Rubbermaid, Wooster, Ohio, USA) in a 30 × 30-m plot at the University of Louisiana Ecology Center. Each mesocosm contained ~10 *I. hexagona* plants with a total of 250 leaves. Control ($n = 10$) and salinity ($n = 10$) treatments were randomly assigned to mesocosms and a solution of Instant Ocean (Aquarium Systems, Mentor, Ohio, USA) was applied as necessary to maintain salinity. Interstitial salinity averaged 0.01 and 6‰ NaCl, in the freshwater control and salinity treatment, respectively.

Leafminer performance in the common garden

In September 1998, we collected *I. hexagona* plants from Marsh Island for an unrelated experiment and

distributed them among the established common garden experimental mesocosms. The new plants contained *C. iridiphora* leafminer pupae. When adults emerged in the spring, they oviposited on the common garden irises, and eggs hatched in May. In June, we counted the number of leaves and the number of mines, and measured leaf area. We collected a total of 164 pupae from five freshwater and six salinity mesocosms in the common garden and weighed them in the lab.

Leaf nitrogen and carbon analyses

We used a Carlo Erba NC-2500 Elemental Analyzer (CE Elantech, Lakewood, New Jersey, USA) to determine the total nitrogen (N_2) and total carbon (CO_2) content of irises growing in the control and salinity treatments. In June 1999, we removed a 5-cm portion of leaf tissue from six plants in each experimental mesocosm. We dried the tissue at 60°C for 5 d, then ground it to a fine powder in an IKA A-10 analytical mill (IKA Works, Wilmington, North Carolina, USA). We used six atropine ($N = 4.84\%$, $C = 70.6\%$) samples of 0.1, 0.5, 1.0, 2.0, 3.5, and 5.0 mg to establish a standard curve. Samples of 4 ± 0.5 mg ground plant tissue were placed into individual tin sample containers. Each leaf was analyzed at least twice and only samples with $\leq 5\%$ difference between replicates were included in the analysis. We ran atropine controls at 20-sample intervals to ensure machine calibration.

Analysis of JA and SA in iris leaves and leafminer pupae

We analyzed phytohormone concentration in plants grown under different salinity levels in the laboratory and common garden. In spring 1998, we filled 20 4-L plastic containers with deionized water and mixed in sufficient NaCl to produce 0, 100, 200, and 400 mmol/L concentrations (equivalent to 0, 5.8‰, 11.2‰, 23.6‰; see methods in Wang et al. [2001]). Four *I. hexagona* were placed in each container. Two plants each were exposed to salinity for 24 or 48 h. We col-

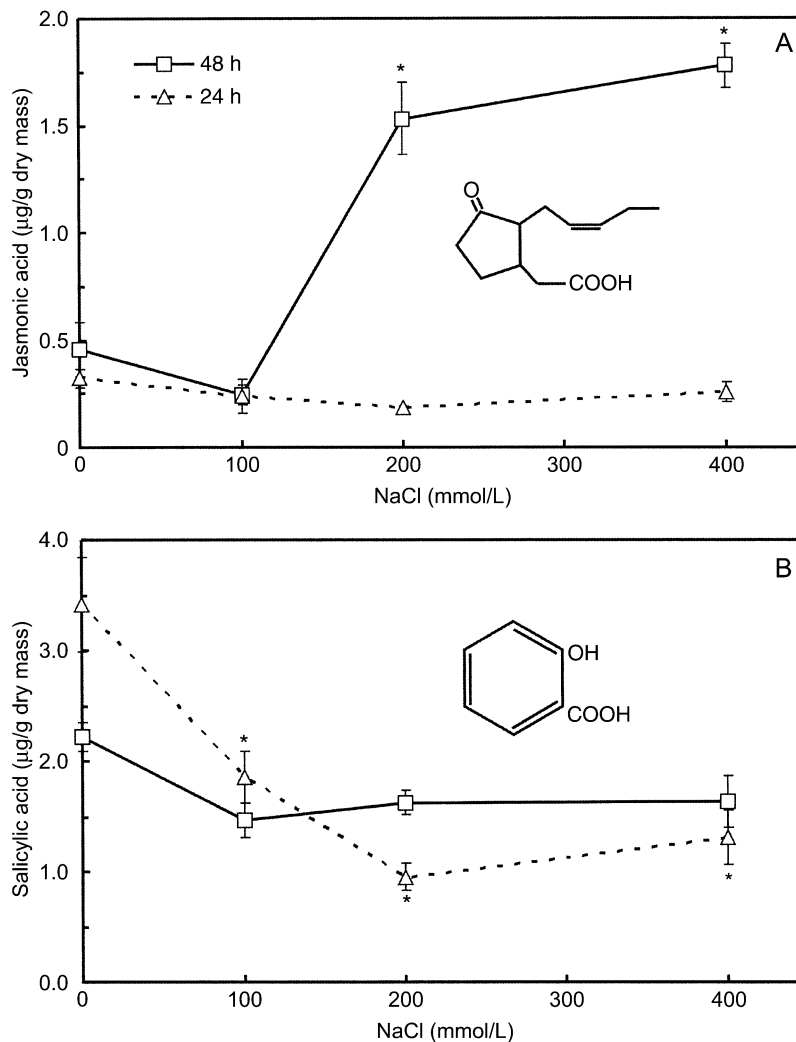


FIG. 1. Induction of JA and suppression of SA in *I. hexagona* leaves by different NaCl levels (after Wang et al. 2001). The four salinity levels are 0 (control), 100 mmol/L (5.6‰), 200 mmol/L (11.6‰), and 400 mmol/L (23.2‰).

lected foliage and measured JA and SA concentration. Monthly, from April through September, we collected leaf samples from *I. hexagona* growing in the control and salinity mesocosms in the common garden (methods in Wang et al. [2001]). In June 1999, we collected foliage from unmined and mined irises growing in three freshwater mesocosms. Plant material was placed on ice in the field and stored in the lab at -70°C until extraction. We ground ~ 1 g of plant tissue into fine powder under liquid N_2 using a mortar and pestle. We processed and analyzed undamaged portions of mined and unmined leaves, as well as 70 leafminer pupae that were collected from two freshwater mesocosms. Leafminer pupae were ground fresh in liquid nitrogen. We used high performance liquid chromatography followed by gas chromatography with mass spectroscopy in selected ion mode to extract, purify, and quantify endogenous JA and SA in plants and leafminer pupae (detailed methods in Wang et al. [2001]).

Statistical analysis

We conducted the statistical analyses with SAS Version 8 software (SAS 1999). Plant performance and leafminer data were averaged within individual mesocosms, and each was considered a replicate. It was not possible to obtain data for each comparison from all mesocosms, so sample sizes vary. We used PROC UNIVARIATE to examine data normality and variance and applied the appropriate transformations to meet parametric assumptions. All proportional data were arcsine square root transformed prior to analysis and back-transformed for presentation. We ran correlation and multivariate analyses for potentially correlated plant and leafminer variables. We used PROC GLM for analysis of variance, multivariate analysis of variance, and repeated-measures analysis of variance to assess plant characteristics, leafminer performance, and phytohormone concentrations. We used Tukey adjusted pairwise comparisons of least-squares means.

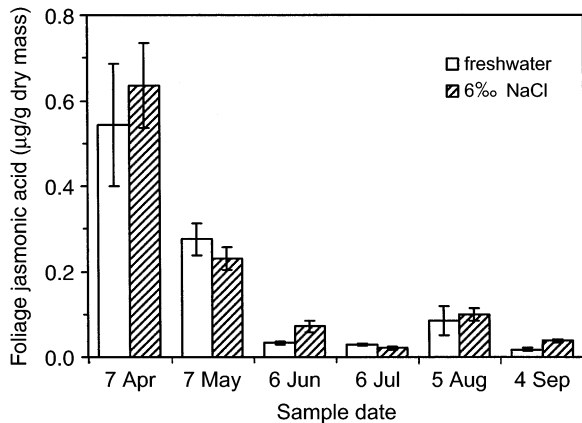


FIG. 2. Long-term pattern in foliar JA production in common garden *I. hexagona* plants growing in control (no salinity) and salinity (6‰) treatments. Bars show means \pm 1 SE (after Wang et al. 2001)

RESULTS

Short term effect of salinity on JA and SA in leaves

In the laboratory experiment, salinity rapidly altered JA and SA concentration in iris leaves (Fig. 1, from Wang et al. 2001). Salinity increased JA an order of magnitude from 0.2 to almost 2 $\mu\text{g/g}$ dry mass (Fig. 1A, $F_{3,16} = 14.0$, $P = 0.0001$). There was a significant time effect on JA production ($F_{1,16} = 77.6$, $P = 0.0001$). The 100-mmol/L NaCl treatment produced no significant change after 24 or 48 h of exposure, but the 200-mmol/L and the 400-mmol/L treatments caused significant increases after 48 h ($P = 0.0026$ and $P = 0.0008$, respectively). In contrast to the induction of JA, salinity reduced SA levels significantly ($F_{3,16} = 17.9$, $P = 0.0001$, Fig. 1B). Within 24 h of treatment with 200 mmol/L NaCl, SA had dropped by 50% ($P = 0.0001$).

Long-term changes in phytohormones

In the common garden, foliar JA declined an order of magnitude from April to September (Fig. 2, $F_{5,15} = 25.0$, $P = 0.001$), but there was no overall salinity effect on JA ($F_{1,3} = 0.0$, $P = 0.974$), and no salinity-by-month interaction ($F_{5,15} = 0.24$, $P = 0.798$). Univariate probabilities for monthly differences in foliar JA between salt-stressed and control plants are: $P_{\text{April}} = 0.971$, $P_{\text{May}} = 0.265$, $P_{\text{June}} = 0.081$, $P_{\text{July}} = 0.069$, $P_{\text{August}} = 0.764$, $P_{\text{September}} = 0.024$. We did not determine foliar SA levels, but it declined significantly in the seeds of salt-stressed irises over a 2-mo period (Wang et al. 2001).

Effect of salinity on *Iris hexagona*

Salinity reduced common garden *I. hexagona* leaf area by 10% (Fig. 3A, $F_{1,19} = 9.8$, $P = 0.01$). Salinity had no effect on carbon (Fig. 3B, $F_{1,13} = 1.9$, $P = 0.19$), but increased total leaf nitrogen 22% (Fig. 3C,

$F_{1,13} = 9.5$, $P = 0.001$). In addition, the C/N ratio was 22% lower in the salinity treatment (not shown, $F_{1,13} = 8.6$, $P = 0.01$). The number of leaves produced by control and salinity plants did not vary ($F_{1,19} = 0.41$, $P = 0.528$).

Effect of salinity on leafminer performance in the common garden and field

Experimental salinity in the common garden negatively affected leafminer performance (Fig. 4, Wilks' lambda, $F_{7,3} = 7.73$, $P = 0.06$). The four response variables were not correlated in the MANOVA analysis (Table 1). In the salinity treatment, 60% fewer larvae survived to pupation than in the control treatment (Fig. 4A, $F_{1,9} = 5.4$, $P = 0.046$), and salinity treatment plants contained 43% fewer pupae per mine than controls (Fig. 4B, $F_{1,9} = 21.0$, $P = 0.001$). Leafminer density (Fig. 4C) and pupal mass (Fig. 4D) were lower in the salinity treatment, but the differences between control and salinity were not significant ($F_{1,19} = 2.76$, $P = 0.11$, and $F_{1,15} = 1.89$, $P = 0.19$, respectively).

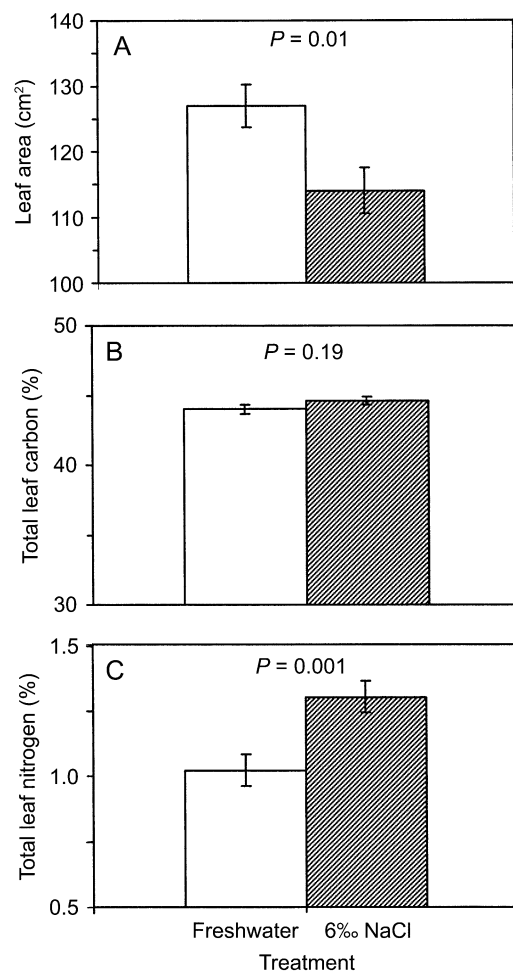


FIG. 3. Effect of freshwater and 6‰ NaCl on (A) leaf area, (B) leaf carbon, and (C) leaf nitrogen in common garden *I. hexagona* plants. Bars show means \pm 1 SE.

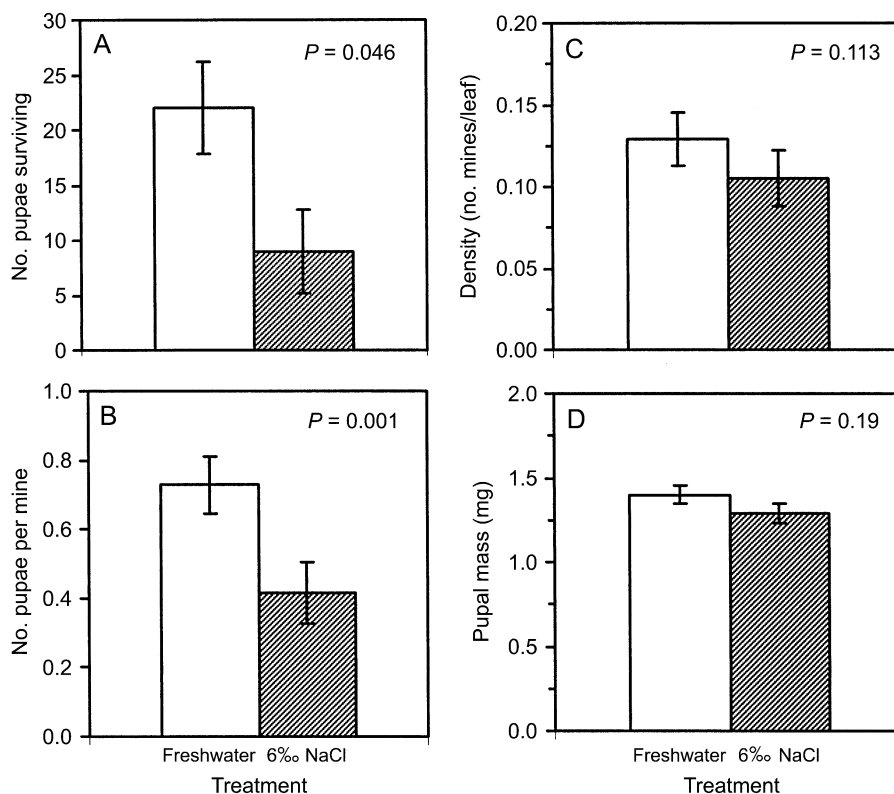


FIG. 4. Performance of iris leaf miners on plants in freshwater and 6‰ NaCl treatments. (A) Total number of pupae that survived, (B) number of mines per leaf, (C) number of pupae within individual mines, and (D) pupal mass. Bars show means \pm 1 SE.

Leafminer densities averaged $40.9 \pm 16\%$ (mean \pm 1 SE) mines per leaf in three Marsh Island *I. hexagona* populations. Pupal mass varied significantly among iris populations (Fig. 5, $F_{2,129} = 4.45$, $P = 0.014$) and was negatively correlated with interstitial salinity at the population site ($r = -0.99$, $P = 0.017$, $n = 3$). Leafminer density was not correlated with interstitial salinity ($r = 0.04$, $P = 0.97$, $n = 3$).

Effect of salinity on JA and SA in mined and unmined leaves and leafminer pupae

Jasmonic acid and salicylic acid varied significantly among mined and unmined iris leaves and leafminer pupae (Wilks lambda, $F_{4,8} = 5.24$, $P = 0.0227$). There was a nonsignificant negative correlation between JA and SA ($r = -0.412$, $P = 0.418$). Mined leaves contain almost twice as much JA as unmined leaves (Fig. 6A), but the difference was not significant ($P = 0.13$). Leaf-

miner pupae contained four times more JA than control leaves ($P = 0.0014$), and twice as much JA as mined leaves ($P = 0.006$).

Foliar SA was substantially higher than JA (Fig. 6B). The greatest amount of SA occurred in control leaves, and did not differ from mined leaves ($P = 0.536$). Leafminer pupae contained significantly less SA than control leaves ($P = 0.038$), but not mined leaves ($P = 0.116$). There are no data for mined, salt-stressed plants, but mining and salinity appear to have additive, not inhibitory, effects, and both induce JA and suppress SA production.

DISCUSSION

Jasmonic acid (JA) has been viewed as a key component of plant antiherbivore defense, whereas salicylic acid (SA) is best known for its importance in plant systemic responses to pathogen attack. These distinc-

TABLE 1. Partial correlation coefficients and probabilities (in parentheses) from the MANOVA error SSCP matrix of leafminer performance on common garden *I. hexagona*.

Parameter	No. mines/leaf	Total no. pupae	No. pupae/mine	Pupal mass (mg)
No. mines/leaf	1.000000	0.898645 (0.0004)	-0.274579 (0.4426)	0.127476 (0.7256)
Total no. pupae	0.898645 (0.0004)	1.000000	0.037525 (0.9180)	0.163474 (0.6518)
No. pupae/mine	-0.274579 (0.4426)	0.037525 (0.9180)	1.000000	0.348716 (0.3234)
Pupal mass (mg)	0.127476 (0.7256)	0.163474 (0.6518)	0.348716 (0.3234)	1.000000

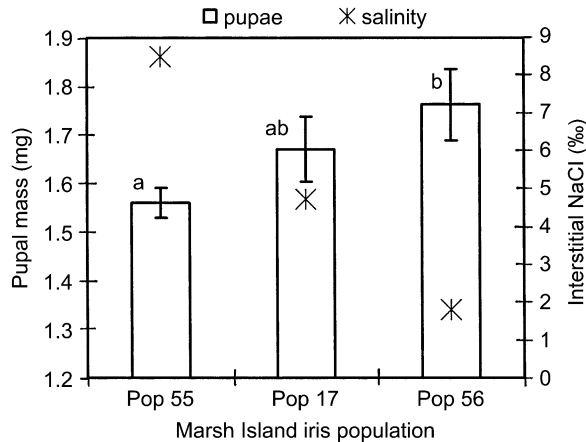


FIG. 5. Pupal mass of leafminers (bars, left axis) and interstitial salinity (✕ symbols, right axis) from three Marsh Island *I. hexagona* populations. Bars show means \pm 1 SE, letters above indicate ($P \leq 0.05$) differences based on Tukey adjusted pairwise comparisons. Correlation between pupal mass and salinity at the population site: $r = -0.99$, $P = 0.017$.

tions are increasingly blurred as we learn about their molecular biology (Walling 2000, Schultz and Appel 2004), and new models portray plant stress signals as multidimensional interactive networks (Knight and Knight 2001). Clearly, these hormones have interactive roles in plant growth, ontogeny, and in shaping and directing plant responses to abiotic and biotic challenges (Cipollini 2004, Farnsworth 2004, Hatcher et al. 2004, Voesenek et al. 2004). Our work with *I. hexagona* demonstrates that strong temporal and spatial plasticity in hormone concentrations occur, and in laboratory and common garden experiments, hormones varied significantly across brief and extended time periods (Figs. 1 and 2).

Time course studies reveal short-term JA inductions and rapid declines in *Nicotiana*, *Arabidopsis*, and other species in response to wounding or herbivory (McCloud and Baldwin 1997, Niki et al. 1998, Kessler and Baldwin 2002). We observed a sharp decline of JA from spring to fall in common garden irises that were not exposed to experimental stress and herbivory. This illustrates the inherent variation that occurs during normal phenological or developmental changes, unrelated to salinity or leafminers. Similar temporal variation in JA and SA occurred in reproductive tissues (Fig. 4 in Wang et al. 2001), but other hormonal signals produced different patterns. For example, abscisic acid (ABA) declined gradually from April to August in common garden *I. hexagona*, then abruptly increased in September (Fig. 1 in Wang et al. 2001). This natural lability exceeds herbivore- and stress-induced responses. More long-term studies are needed so we can compare hormonal signals under stressful and benign conditions, and distinguish stress responses from normal physiological cycles.

Different types of herbivory induce specific hormonal responses. For example, piercing and sucking insects inflict much less tissue damage than chewing insects, and elicit SA-triggered pathogen-like systemic acquired resistance rather than JA-induced antiherbivore defenses (Walling 2000). Leafminers elicit JA as do other leaf chewers, but since leafminer feeding is internal, the response may be localized rather than systemic. This would create a JA gradient, with the highest concentrations contained in the insects themselves, as we observed (Fig. 6A). We were surprised and intrigued to discover JA and SA in leafminer pupae (Fig. 6). To our knowledge, these plant-signaling molecules have not yet been reported in insects. Larvae evacuate their guts prior to pupation, thus the JA and SA we detected in pupae was not from undigested leaf material, but was probably sequestered or incorporated into insect cells or tissue. Interestingly, in the same GC/MS analysis (not shown), auxin (indole-3-acetic acid) and abscisic acid (ABA), were not detected in leafminer pupal tissues, although they occurred in iris

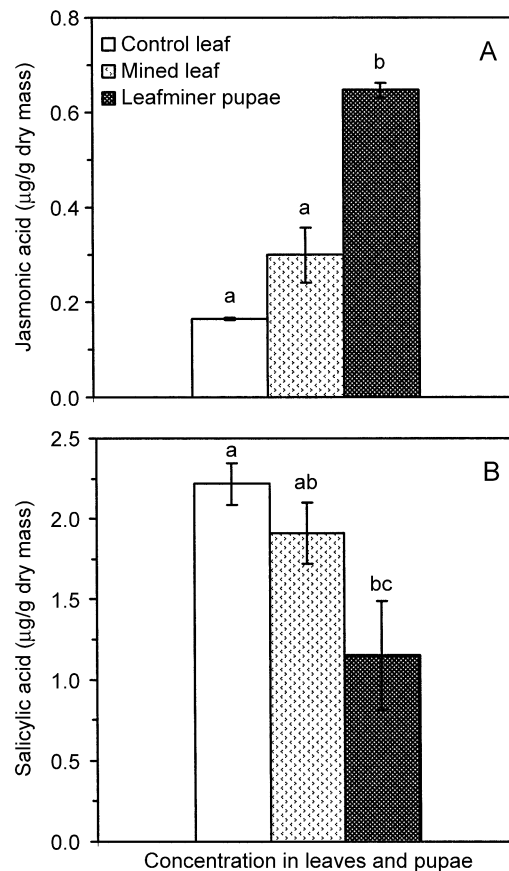


FIG. 6. Concentration of (A) jasmonic acid and (B) salicylic acid in common garden *I. hexagona* plants growing in freshwater without leafminers (control), in freshwater with leafminers (mined leaf), and in leafminer pupae. Bars show means \pm 1 SE; letters above indicate significant ($P \leq 0.05$) differences based on Tukey adjusted pairwise comparisons.

TABLE 2. Effect of salinity and leafminer herbivory on endogenous hormones in *Iris hexagona* and their presence or absence in *Cerodontha iridiphora* pupal tissue.

Parameter	Jasmonic acid	Salicylic acid	Abscisic acid	Auxin
Foliage				
Salinity stress	present	absent	present	absent
Leafminer herbivory	present	absent	unknown	unknown
Pupae	present	present	absent	absent

leaves (Y. Wang, S. Mopper, and K. Hasenstein, *unpublished data*).

The retention of phytohormones in leafminer pupae is a novel finding. It may have no adaptive significance, and merely indicate insect-specific metabolism and sequestration of plant hormones. Alternatively, leafminers may have evolved a mechanism to utilize phytohormones. Schultz and Appel (2004) discussed the evolutionary significance of such cross-kingdom sharing of (plant) signaling systems. One proposed adaptive function that may apply to leafminers is the suppression of host plant defense responses. Empirical support for this theory is presented in a recent study (Li et al. 2002). *Helicoverpa zea* feeding on celery (*Apium graveolens*) used plant hormones to activate cytochrome P450 enzymes that subsequently detoxified the plant allelochemicals induced by herbivory. In these experiments, induction of the counter-defense P450 enzymes specifically required JA and SA, and was not triggered by closely related molecules. The retention of JA and SA in leafminers may indicate a similar ability to use plant hormones. The Li et al. (2002) study did not measure JA or SA in *Helicoverpa zea* herbivores, but we can speculate that insects acquire sufficient concentrations of these hormones to trigger expression of detoxification enzymes.

Little is currently understood about these reciprocal response strategies ("eavesdropping") to counteract plant defenses (Schultz and Appel 2004), but it is an emerging and exciting field in hormonal ecology. The retention of plant hormones by insects could also enhance protection against enemies beside plants. Salicylic acid is an important component of signal transduction in antifungal defenses of both plants and mammals (Pierpoint 1994) and leafminers are highly resistant to fungal attack (Connor and Taverner 1997). The potential use of SA by insects to resist pathogens would be another example of different kingdoms using the same plant compounds for defensive purposes (Schultz and Appel 2004).

Adaptation to abiotic stress and/or natural seasonal patterns in phytohormone production can have consequences for herbivores. The relationship between SA and salinity stress has not been clearly established, but the decline of SA in salt-stressed iris is consistent with an adaptive response. In tomato (*Lycopersicon esculentum*), a decline in SA occurred in salinity-adapted plant cells compared to nonadapted cells (Molina et al.

2002), and SA can block the expression of genes associated with salinity protection (Chao et al. 1999). However, supplemental SA reduced NaCl damage to cells in the ice plant, *Mesembryanthemum crystallinum* (Yen and Yen 1999). Salinity induction of JA in iris foliage may increase resistance to herbivory, but the reduced SA could hinder plant defenses against pathogen attack. Plant natural enemies may adaptively synchronize to hormone phenological cycles to exploit the shift in concentration. For example, the greatest leaf-mining activity occurs in June, when foliage JA is low relative to April (Fig. 6). Leaves begin senescing in July (L. Schile and S. Mopper, *unpublished data*), and a narrow window exists when low foliar JA coincides with availability of leaves that are sufficiently healthy to support leaf miners.

Although they operate through separate pathways, both JA and SA were affected by salinity and herbivory, which caused similar patterns of induction and suppression (Table 2). The JA elicited by salinity stress (Fig. 1) and herbivory (Fig. 6), and the defense responses that are known to ensue (Thaler et al. 2001) may provide a mechanism for poor leafminer performance on salt-stressed *I. hexagona* (Figs. 4 and 5), despite the greater foliage nitrogen (Fig. 3). It remains to be tested if simultaneous salinity and herbivory would have additive effects (Knight and Knight 2001). When different challenges elicit similar signals (Schultz and Appel 2004), as is the case with salinity and leafminer herbivory, the cumulative effect could reduce energetic costs and enhance plant recovery from different types of stress. As the plant responds physically to one stress factor, it may increase tolerance for another. However, when signals elicit opposite responses, the strongest or earliest signal prevails and plant defense is compromised (Niki et al. 1998, Stotz 2002, Thaler and Bostock 2004). These crosstalk interactions are determined by environmental variation and must be studied in biologically realistic settings.

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