

# The effects of salt, manganese, and density on life history traits in *Hesperis matronalis* L. from oldfield and roadside populations

Carl J. Rothfels, Laura L. Beaton, and Susan A. Dudley

**Abstract:** Plant communities in the verges of major roadways (roadsides) are similar to those of abandoned farm fields undergoing succession (oldfields). However, roadsides are subject to distinctive stresses. Here, we look at local adaptation in *Hesperis matronalis* L. to salinity and manganese (Mn). Plants collected from three roadside and three oldfield populations were grown in a greenhouse under controlled, high-salinity, and high-Mn conditions, and several life history traits were measured. In addition, we imposed a density treatment after vernalization. Mortality was high in both the salt (43% survival) and Mn treatments (53% survival) compared with the control (71% survival). Distinct family, population, and site variation was found in measures of plant size. There were also family-level differences in response to soil treatments. However, none of our results were consistent with local adaptation. In general, larger plants were more likely to bolt, and oldfield plants were less likely to bolt than roadside plants in all treatments (in the low-density treatment, 56% of roadside plants had bolted compared with only 16% of oldfield plants). We found that the high-density treatments resulted in earlier bolting and longer internodes, a result consistent with the shade avoidance hypothesis.

**Key words:** biennial, salinity, phenotypic plasticity, heavy metal, local adaptation.

**Résumé :** Les communautés végétales en marge des routes principales (bords de route) sont semblables à celles des terres agricoles abandonnées en succession (vieilles prairies). Cependant, les bords de route sont soumis à des stress particuliers. Les auteurs examinent l'adaptation locale de l'*Hesperis matronalis* L. à la salinité et au manganèse (Mn). Ils ont cultivé en serres, sous des conditions contrôlées impliquant de fortes teneurs en sel et en manganèse, des plantes récoltées sur les bords de trois routes et à partir de trois populations de vieilles prairies, et ont mesuré plusieurs aspects de leur cycle vital. De plus, ils ont imposé un traitement de densité après vernalisation. La mortalité est élevée dans les deux traitements avec le sel (43%) et le Mn (53%), comparativement aux témoins (71%). On observe une variation au niveau de la famille, de la population et du site, dans les mesures des dimensions des plantes. Il y a également des différences au niveau de la famille en réaction aux traitements du sol. Cependant, aucun des résultats n'est congru avec une adaptation locale. En général, les grandes plantes sont plus susceptibles de monter en graines, et les plantes de vieilles prairies sont moins susceptibles de le faire que les plantes des bords de route, quel que soit le traitement (dans le traitement à faible densité, 56% des plantes de bords de route ont monté à la graine, comparativement à seulement 16% pour les plantes de vieilles prairies). On constate que les traitements à haute densité conduisent à une montée en graines plus hâtive et des entrenœuds plus longs, un résultat qui concorde avec l'hypothèse d'intolérance à l'ombre.

**Mots clés :** bisannuel, salinité, plasticité phénotypique, métal lourd, adaptation locale.

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## Introduction

Plant populations can adapt to environmental heterogeneity on a fine scale (Van Tienderen and Van Der Toorn 1991; Sork et al. 1993; Miller and Fowler 1994; Kindell et al. 1996). The process of local adaptation depends on a balance between the influences of local selection and of gene flow (Slatkin 1987; Caprio and Tabashnik 1992). The potential for local adaptation is further constrained by the genetic variation within the subpopulations (e.g., Dudley 1996); homogenous populations

have little potential for divergence, there being insufficient differences for natural selection to act upon.

In this age of rapid anthropogenic change to the biosphere, the question of whether plants can locally adapt to new, human-created habitats can be approached from the perspectives of evolutionary biology, population biology, and conservation biology. For evolutionary ecologists, anthropogenic landscapes offer an opportunity to study rapid evolution in response to environmental change, whereas for the plants themselves, these new habitats are a

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potential ecologically important resource. In some cases of human-created environments with extreme and specific selective forces (e.g., mine tailings contaminated with heavy metals), this process has been demonstrated unequivocally (Jain and Bradshaw 1966; McNeilly 1968; Antonovics and Bradshaw 1970). However, plants inhabiting such extreme environments constitute a small percentage of the vegetation responding to anthropogenic change. Urban sprawl, industrialization, and agriculture have altered huge expanses of land, creating millions of hectares of semidegraded habitat. More ecologically important is the ability of plants to adapt to widespread anthropogenic landscapes, such as roadsides (Forman and Alexander 1998). Roadsides are characterized by specific stresses lacking in most natural environments, including the high concentrations of NaCl from deicing salt (Westing 1969; Davison 1971; Thompson et al. 1986) and manganese (Mn) from methylcyclopentadienyl manganese tricarbonyl, a deknocking additive in gasoline (Lytle et al. 1994). Salt levels along roadsides are extremely variable in both space and time. Concentrations higher than 1500 µg/g have been recorded in the spring within 1 m of the highway. Levels decline throughout the summer and with distance from the road (Thompson et al. 1986). Very few studies have examined the level of Mn found in roadside soils. Lytle et al. (1994) found levels at 1 m from the roadway of over 3000 µg/g, over 12 times the background level of 254 µg/g. Local adaptation to roadsides could thus be inferred if roadside populations show a greater ability to tolerate NaCl and Mn toxicity compared with their oldfield conspecifics. The potential for adaptation could be determined by the presence of genetic variation both within and between populations from these environments (Venable 1984).

Local adaptation to roadsides has been demonstrated for a life history trait (root length) at the seedling level; several roadside families of *Hesperis matronalis* L. displayed significantly longer radicals in Mn treatments than did their oldfield counterparts (data not shown). This study asks whether that local adaptation can be found in other life history traits at the juvenile and reproductive life stages. Three life history traits in particular are of interest: mortality, size, and bolting. Mortality is an important component of fitness and size is often correlated with fecundity, another important component of fitness (Harper and White 1974; Samson and Werk 1986). Bolting is an important life history trait for species like *Hesperis*, which are biennials or short-lived semelparous perennials (Silvertown 1984). These plants are characterized by a year or more in vegetative form followed by a "single, massive, fatal reproductive episode" (Young and Augspurger 1991). Bolting, the switch from a vegetative rosette to an upright fertile stem is thus a pivotal life history event and for facultative biennials is primarily driven by plant size rather than plant age (Evans 1982; Silvertown 1983; Kelly 1985; Couvet et al. 1990).

In this study, plants grown from seed collected from distinct roadside and oldfield populations were grown in three treatments: control, NaCl addition, and Mn addition. After vernalization, density treatments were imposed. We ask the following questions: (i) What are the genetic effects (at the

level of family, population, or site) on life history traits (mortality, size, and bolting) under the treatments? (ii) What are the environmental effects (NaCl, Mn, and density) on the life history traits? (iii) Are there any genotype × environment interactions indicating local adaptation in these performance variables?

## Materials and methods

### Study species

*Hesperis matronalis* is a short-lived semelparous crucifer, originally native to Eurasia but now common in eastern North America (Gleason and Cronquist 1991). Its showy pink, white, or purple flowers make it a favourite of gardeners, and its strong invasive capacity has resulted in it being a common component of roadsides, thickets, and open woods (Environment Canada 1999). *Hesperis matronalis* is an ideal species for this study because it is easily cultivated, and such wide-ranging species that frequently colonize disturbed habitats are believed to encounter more environmental heterogeneity than would a typical endemic or understory species (Sultan 1987).

### Sites

Seeds were collected in mid- to late June from six different populations in the vicinity of Hamilton, Ontario, Canada. Three of the populations (1–3) were from oldfield habitats on the north side of Cootes Paradise. Populations were chosen based on ease of accessibility and abundance of plants. Although *H. matronalis* is a common plant in southern Ontario, large populations are not abundant along roadsides. For this reason, it was impossible to pair the sites. The oldfield populations chosen, while geographically discrete (separated by several hundred metres), were biologically very similar: all were characterized by a full-sun environment (no canopy) and were dominated by mixed grasses and forbs (*Bromus inermis*, *Asclepias syriaca*, *Dipsacus fullorum*, and *Solidago* sp. were all common). The roadside populations (5–7) were located adjacent to Highway 403, a major local expressway, and separated by several hundred metres. Population 5 was on a shaded steep west-facing slope dominated by grasses, population 6 was in a grassy semishaded ditch, and population 7 was located in a semishaded area with limited surrounding vegetation. All the roadside sites were within 5 m of the highway edge.

### Experimental design

The seeds were stored at room temperature until May 18, 2000, when 18 seeds (six seeds per treatment) from each of five randomly selected maternal seed families from each of the six populations were planted into 7-cm pots with 178 g of 1:1 sand–perlite potting mix for a total of 540 pots. A sand–perlite mixture was used instead of either roadside or oldfield soil because unlike soil collected from either site, it is inert and it allows water to drain easily. The salt and Mn may have reacted with other compounds in the soil, making it difficult to estimate the true availability.

The plants were randomly arranged within treatment blocks to prevent contamination among pots. The plants

**Table 1.** Mixed model analysis of variance for plant height, plant diameter, and leaf number in *Hesperis matronalis* measured February 4 and 5 prior to the addition of density treatments.

Effect	df	F		
		Plant height (cm)	Plant diameter (cm)	No. of leaves
Soil treatment	2	20.73***	15.87**	0.46
Site	1	0.89	0.24	3.94
Population (site)	4	4.33*	1.64	0.53
Family (population × site)	24	1.84*	1.79*	1.24
Soil treatment × site	2	7.15*	1.58	4.72*
Soil treatment × population (site)	8	0.58	1.82 <sup>†</sup>	0.76
Soil treatment × family (population × site)	45	1.45*	1.22	1.36 <sup>†</sup>
Error	221			

**Note:** Effects were tested over an estimated term that was composed primarily of treatment × family for family effect and treatment × population for the other effects. <sup>†</sup> $P < 0.10$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

were grown in greenhouse conditions with natural light until August, when they were moved to a growth chamber where they received an 8-h light period every 24 h. The growth chamber was maintained at room temperature. Treatments were started in August. The NaCl treatment plants received 100 mL of 50 mM NaCl solution on September 11 and the Mn treatment plants received 100 mL of 750  $\mu$ M MnSO<sub>4</sub> solution on five dates (September 11, 12, 14, 22, and 25). Previous studies (data not shown) of seed germination have indicated that at these NaCl and Mn levels, plants are impacted by the treatments but are not killed.

The plants were vernalized at 8°C with an expected daylength of 8 h, starting on October 5. Unfortunately, the light control mechanism broke and the error was not corrected until November 6. Thus, the plants were likely under a 24-h daylength until November 6 and an 8-h day from then on. On December 15, the vernalization regime was altered to 1°C and 24-h night until January 30. For the period from October 5 to December 15, the plants were watered and haphazardly rearranged on a weekly basis, and for the second vernalization period (December 15 to January 30), the plants were watered and haphazardly rearranged every 2 weeks. No soil treatments were performed for the duration of the vernalization.

On January 30, the plants were relocated to the greenhouse, and on February 5, they were assigned to density treatments. Each soil treatment group (control, Mn, and NaCl) was randomly divided into high-density, low-density, and edge treatments such that the number of plants in high and low density was approximately equal. High-density plants were placed pot to pot in a square array, with the edge treatment plants forming the perimeter, while low-density pots were spaced 13 cm from their nearest neighbours. To avoid the effects of the heterogeneity of the greenhouse, the blocks were randomly reassigned positions on a weekly basis, and within each density treatment, the plants were haphazardly rearranged, also on a weekly basis. In the greenhouse, the salt treatment plants were treated twice with 100 mL of 50 mM NaCl (February 12 and March 17). The Mn plants were treated three times with 100 mL of 750  $\mu$ M MnSO<sub>4</sub> (February 12, February 15, and March 17). Throughout the experiment, the plants were fertilized weekly with 200 ppm 20:20:20 fertilizer, and dead leaves or plants were removed.

### Measurement of traits

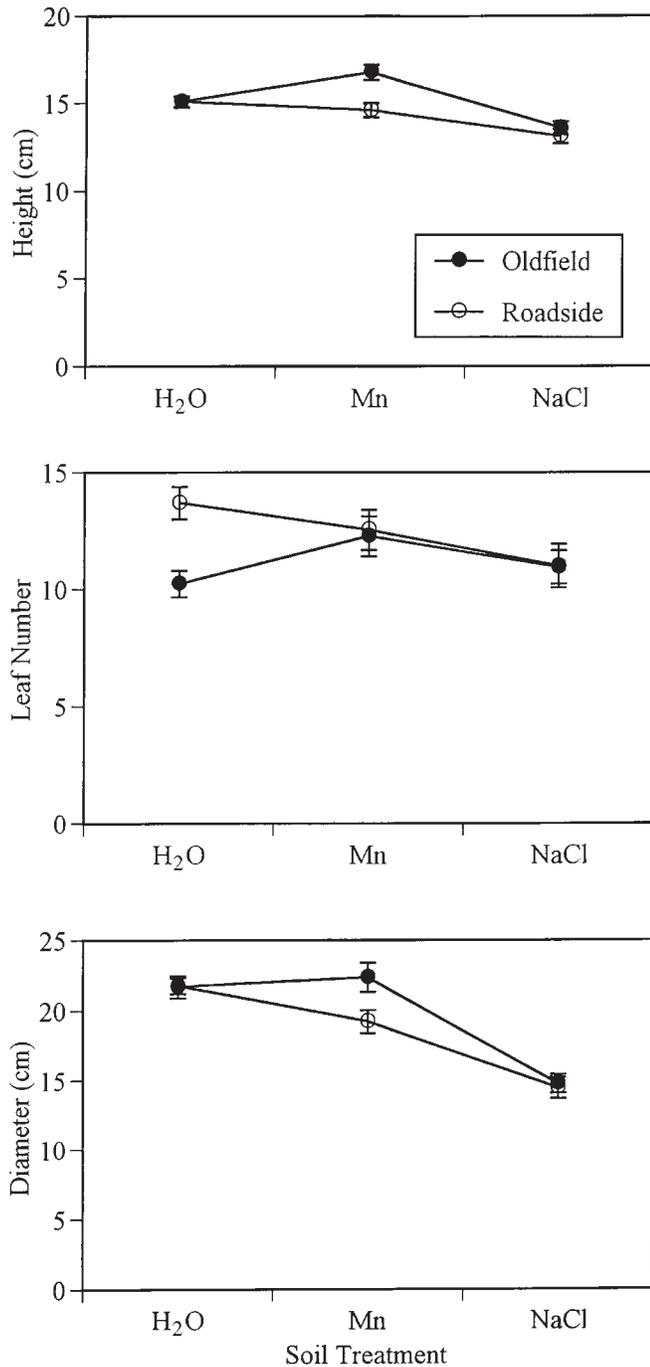
On February 4 and 5, three size measures were recorded: number of fully expanded leaves, height (in cm) of the tallest point of the plant from the soil surface, and diameter of the plant (in cm) at the widest point. Mortality was recorded throughout the study.

Bolting of the plants was recorded during five censuses: February 15, February 25, March 10, March 16, and April 1. For February 15, only the presence or absence of bolting was recorded. For all other dates, if a plant had elongated internodes, the height of the bolted stem from the soil surface to the apical meristem was recorded. Unbolted plants were assigned a bolting height of zero regardless of the height of other parts of the plant. On April 1, we also recorded the height to the first 10 nodes in bolted plants and identified the longest internode of these first 10 internodes.

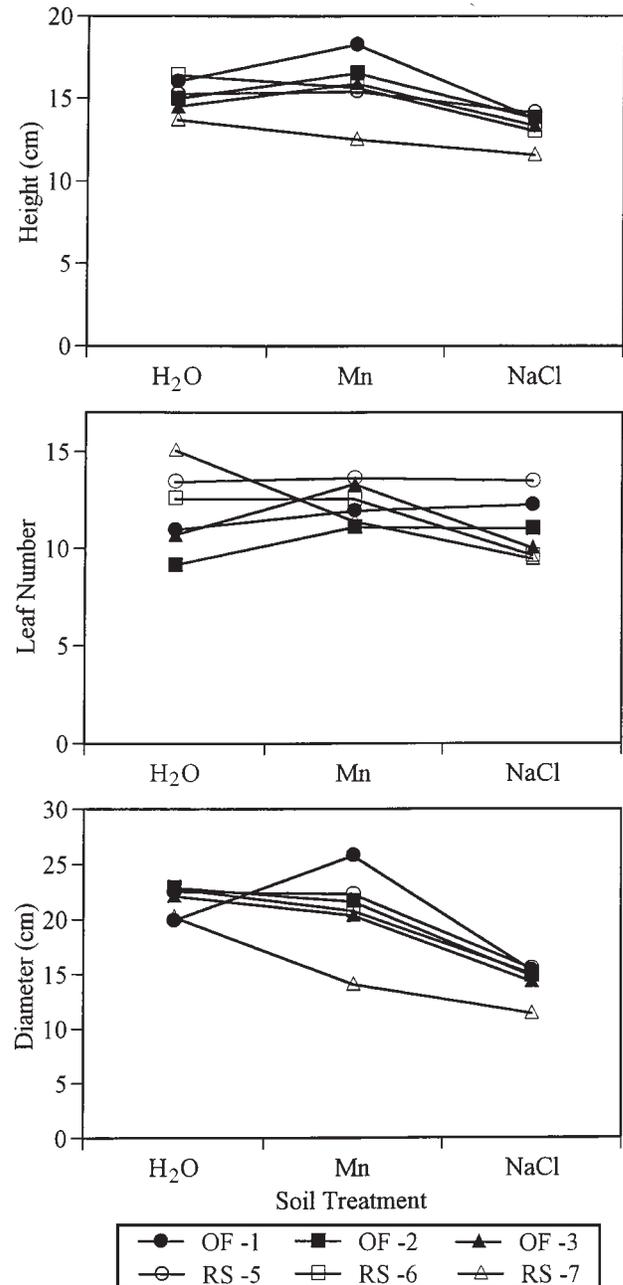
### Data analysis

The data were analyzed with PC-SAS 8.0 (SAS Institute Inc. 2000). Mixed model analyses of variance and repeated measure analyses of variance were conducted in PROC GLM for the continuous data. Analyses of variance for the categorical data (mortality and bolting proportion) were performed in PROC CATMOD. Effects included in the analysis of the size data (i.e., number of leaves, leaf length, etc.) were soil treatment, site, population nested within site, family nested within population and site, and associated interactions. Population, family nested within population and site, soil treatment × population, and soil treatment × family were considered random effects. Effects included in the bolting proportion analysis were site, soil treatment, density, and their interactions. For the analysis of the bolting height data, the soil treatment and the size variables were found to be collinear, and only size variables were included, as they were found to be better predictors. The resulting analysis of covariance (PROC GLM) included the effects of site, density, their interactions, diameter, and height in February. Effects included in the mortality analysis were treatment, site, population, and their interactions. Plant size did affect the probability of bolting (PROC LOGISTIC; logistic regression  $\chi^2 = 31.057$ ,  $P < 0.0001$ ) with positive effects of leaf number ( $b = 0.0768$ ,  $\chi^2 = 9.3742$ ,  $P < 0.0022$ ) and diameter ( $b = 0.0711$ ,  $\chi^2 = 6.8154$ ,  $P < 0.0090$ ) but no effect of plant height ( $b = -0.0042$ ,  $\chi^2 = 0.0044$ ,  $P < 0.9470$ ).

**Fig. 1.** Size means by site for *Hesperis matronalis* over the three soil treatments. Height is the distance from the soil surface to the highest point of the plant, leaf number is the number of fully expanded leaves, and diameter is the width of the plant at the widest point. Measures were taken on February 4 and 5. Bars indicate 1 SE. (Note: figures shown are norms of reaction graphs only. No inferences are being made about the response of the plants to environments intermediate between the control and the treatment levels.)



**Fig. 2.** Size means by population for *Hesperis matronalis* over the three soil treatments. Measures were taken on February 4 and 5. OF, oldfield; RS, roadside.

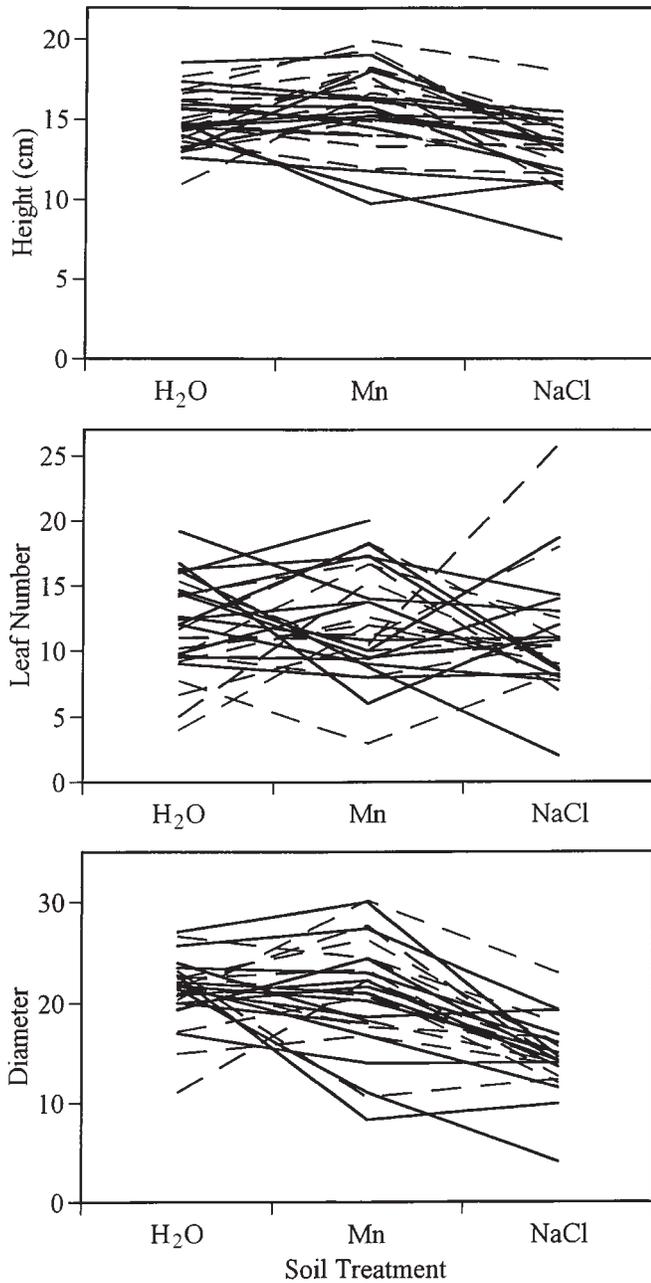


**Results**

**Size**

There was a significant effect of the soil treatments, with the plants in the NaCl treatments being shorter with smaller plant diameter, although there was no difference in leaf number (Table 1; Fig. 1). Contrary to expectation, there was no impact of Mn addition on the size of the plants. Genotypic variation in size was demonstrated at both the population (height) and the family levels (height and diameter) (Table 1; Figs. 1–3). Variation in response to the Mn treatment (as indicated by a significant genotype × environ-

**Fig. 3.** Size means by family for *Hesperis matronalis* over the three soil treatments. Measures were taken on February 4 and 5. One family died out completely in the NaCl treatment. Broken lines indicate oldfield families and solid lines indicate roadside families.



ment interaction) was found at the site level, with oldfield plants being taller than roadside plants. A significant interaction was also found for leaf number, but in this case, the difference lay in the control, with roadside plants having more leaves than oldfield plants.

**Mortality**

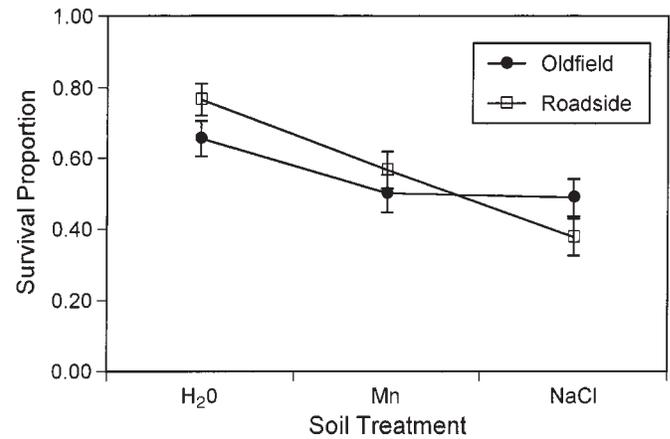
Due to inadequate sample size, analysis of mortality only considers the genotypic effects at the site and population levels (Table 2). Both Mn and NaCl had a significant impact

**Table 2.** Categorical analysis of mortality in *Hesperis matronalis*.

Effect	df	$\chi^2$
Intercept	1	354.94***
Site	1	0.23
Soil treatment	2	10.89**
Population (site)	4	11.78*
Soil treatment $\times$ site	2	1.52
Soil treatment $\times$ population (site)	8	4.65

**Note:** All deaths to March 16 scored as zero. Sample size for this analysis was 540. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

**Fig. 4.** Survival proportion to March 16 by site for *Hesperis matronalis* over the three soil treatments. Bars indicate 1 SE.



on plant survivorship, with only 53 and 43% surviving respectively compared with 71% of the control (Fig. 4). Variation in survivorship was found at the population level (Table 2). Although there was a site-level trend towards the roadside plants outsurviving the oldfield plants in both the control and Mn treatments (genotype effect), with the positions reversed under the salt treatment, this trend was not significant.

**Bolting**

Due to the addition of the density treatments, genotype effects could only be tested for at the site level. Bolting percentages were strongly influenced by both soil and density treatments (Table 3; Figs. 5 and 6). By March 16, control plants were most likely to bolt (65.4%) and NaCl plants the least (21.3%). Density treatments had a similar effect: high-density plants were most likely to bolt (65.1%) and low-density plants the least (36.6%). Site effects were also significant, with roadside plants more likely to bolt than oldfield plants under all treatments. This effect was particularly marked in the low-density treatment, where, by March 16, 56% of the roadside plants bolted compared with only 16% of the oldfield ones, but this trend towards genotype  $\times$  environment effects was not significant.

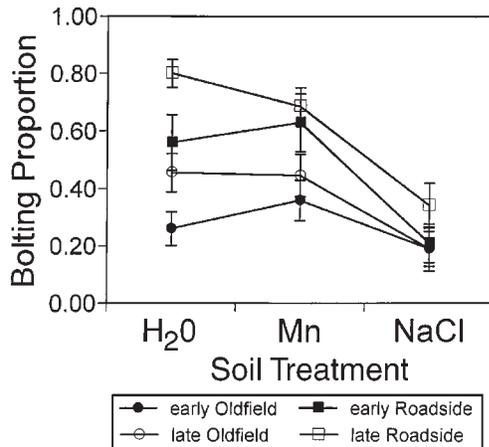
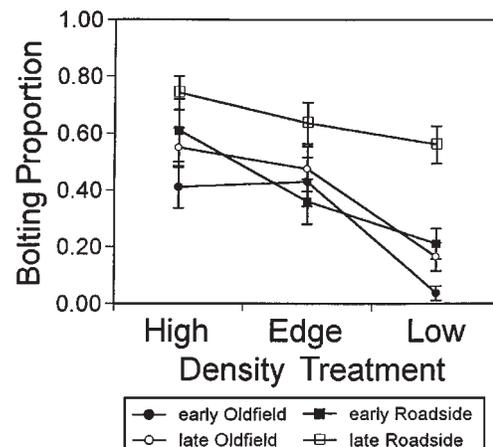
The bolting stem of high-density plants was also taller than that of either edge or low-density plants (Tables 4 and 5; Fig. 7). Roadside plants were consistently taller than the oldfield plants, and the difference was especially marked under the high-density treatment. Roadside plants differed

**Table 3.** Categorical analysis of bolting likelihood for early and late bolting in *Hesperis matronalis*.

Effect	df	$\chi^2$	
		Early bolting	Late bolting
Intercept	1	345.22***	357.90***
Site	1	17.97***	19.38***
Soil treatment	2	— <sup>a</sup>	33.10***
Density	2	36.74***	18.50***
Soil treatment × site	2	—	2.59
Density × site	2	3.61 <sup>†</sup>	3.71
Density × soil treatment	4	—	4.12
Density × soil treatment × site	4	—	1.16

**Note:** For early bolting, all plants that had bolted (clearly elongated internodes) by February 15 were scored as bolted. Only site and density treatment could be fit for this data. For late bolting, all plants that had bolted (clearly elongated internodes) by March 16 were scored as bolted. Sample size for this analysis was 305. <sup>†</sup> $P < 0.10$ ; \*\*\* $P < 0.001$ .

<sup>a</sup> Effect not estimated.

**Fig. 5.** Bolting proportion on February 15 (early) and March 16 (late) by site for *Hesperis matronalis* over the three soil treatments. Bars indicate 1 SE.**Fig. 6.** Bolting proportion on February 15 (early) and March 16 (late) by site for *Hesperis matronalis* over the three density treatments. Bars indicate 1 SE.

markedly in height from the high-density to edge treatments (27.64 to 13.57 cm) but had little difference in height between the edge and low-density treatments. Oldfield plants had a different pattern. Height for high-density and edge treatments were equivalent, but plants in low density were much shorter. This site × density effect was significant for all three census dates (Table 4). When the nonbolting class was removed from the data, the general pattern remained, although significance was lost for the site × treatment effect for the second and third censuses and from the site effect for the first census (Table 5). A repeated measures analysis of variance demonstrated that as time progressed, the site (time × site:  $F_{2,274} = 15.35$ ,  $P < 0.001$ ) and density (time × density:  $F_{4,274} = 2.96$ ,  $P < 0.01$ ) differences described above became more pronounced.

Size in February, as measured by diameter and leaf number, was also a significant determinant of bolting probability and height (Tables 4 and 5). However, even when the effect of February size was corrected for, significant density ( $F_{2,274} = 9.638$ ,  $P < 0.001$ ) and site ( $F_{1,274} = 35.43$ ,  $P < 0.001$ ) effects remained, with roadside plants taller than oldfield ones and high-density plants taller than low-density ones.

## Discussion

Density-dependent elongation has been demonstrated in both upright plants (Schmitt and Wulff 1993; Dudley and Schmitt 1995, 1996) and in the bolting phase of the obligate biennial *Alliaria petiolata* (data not shown). However, no one to our knowledge has demonstrated that biennials or semelparous perennials can be induced to bolt by increasing plant density. Many studies (Werner 1975; Gross 1981; Hirose and Kachi 1982; Couvet et al. 1990) have found that bolting in biennials is determined largely by the size of the plant. Here, we show that density is also a factor affecting bolting time.

In high density, plants bolted earlier. For upright plants, increased height has a cost of an increased allocation of resources to supportive tissues (Givnish 1982). But in high density, increased height allows a plant to grow taller than its neighbours and obtain more light (Givnish 1982), and thus, density-dependent elongation has clear adaptive value (Dudley and Schmitt 1996). However, in biennials, bolting not only increases the plant's ability to capture light in high density but also initiates the reproductive phase of the plant's life cycle. Hence, the consequences of bolting are not as clear as increasing height in an upright plant. For exam-

**Table 4.** Analysis of covariance for bolting height of *Hesperis matronalis* on four dates, with the class of unbolted plants included.

Effect	df	F			
		Feb. 25	Mar. 10	Mar. 16	Apr. 1
Site	1	18.21***	23.51***	28.72***	45.02***
Density	2	26.01***	12.80***	10.47***	5.32**
Site × density	2	9.89***	4.22*	3.55*	2.13
Feb. diameter	1	13.21***	19.37***	22.57***	32.87***
Feb. leaf number	1	101.63*	3.74 <sup>†</sup>	3.48 <sup>†</sup>	6.35*

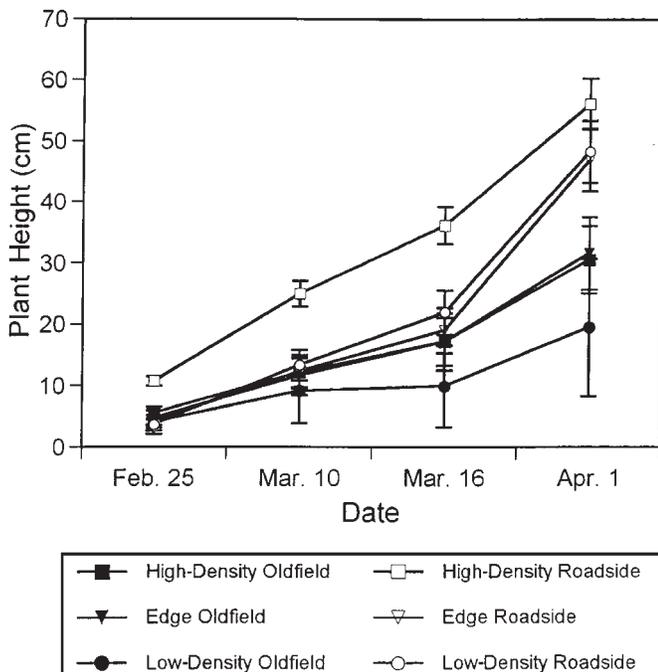
**Note:** Soil treatment has been replaced in this analysis with the size measures February diameter and February leaf number (measured February 4 and 5 prior to imposition of the density treatments). Both February size and soil treatment were strongly associated with size, but February diameter and February leaf number proved to be better predictors of bolting height. Sample size for February 25 was 286, for March 10 was 283, and for March 16 was 280. <sup>†</sup>*P* < 0.10; \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001.

**Table 5.** Analysis of covariance for bolting height of *Hesperis matronalis* on four dates, with only bolted plants included.

Effect	df	F				Internode length, Apr. 1
		Height over time				
		Feb. 25	Mar. 10	Mar. 16	Apr. 1	
Site	1	1.83	4.74*	8.90**	17.68***	1.50
Density	2	9.51***	4.08*	3.93*	0.96	4.20*
Site × density	2	5.24**	2.75 <sup>†</sup>	2.49 <sup>†</sup>	0.63	0.40
Feb. diameter	1	5.12*	9.39**	12.77***	17.22***	0.81
Feb. leaf number	1	1.43	0.25	0.30	2.85 <sup>†</sup>	0.10

**Note:** The length of the longest of the first 10 internodes on April 1 was analyzed. Soil treatment has been replaced in this analysis with the size measures February diameter and February leaf number (measured February 4 and 5 prior to imposition of the density treatments). Both February size and soil treatment were strongly associated with size, but February diameter and February leaf number proved to be better predictors of bolting height. Sample size for February 25 was 143, for March 10 was 151, for March 16 was 151, and for April 1 was 149. <sup>†</sup>*P* < 0.10; \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001.

**Fig. 7.** Bolting height by site and density treatment for *Hesperis matronalis*. The values plotted are least squares means for bolted plants only, corrected for size (February diameter and February leaf number) taken from an analysis of covariance (Table 5).



ple, *Arabidopsis* bolts earlier in high density (Halliday et al. 1994). However, unlike *H. matronalis*, the bolted plant possesses only rudimentary leaves, and therefore, changes in height are unrelated to photosynthetic advantages. Instead, low-light conditions likely indicate that the juvenile plant is being crowded by a larger plant, making it advantageous for it to complete its life cycle before the situation further declines. *Hesperis matronalis* has a leafy fertile stem, so density-dependent bolting has consequences for both increased light interception in high density and timing of reproduction.

At the level of site, roadside plants were more likely to bolt under all density treatments. Roadside plants may experience a more temporally unpredictable environment from such causes as mowing, automobile accidents, construction, and emergency stops and therefore may benefit from bolting earlier. Although delaying reproduction 1 year would allow them to gather more resources and increase their fitness, the risk of mortality in that year may outweigh any benefit. Oldfield plants appeared unlikely to bolt (in the time scale of these results) unless they were induced to by density. They may have a higher likelihood of surviving to reproduce, and therefore, delaying reproduction 1 year in order to gather more resources is likely to increase their fitness. To test this hypothesis would require a study that measures mortality levels at different life stages in both sites and the fitness of both roadside and oldfield plants when grown in both their own and the other's environment. The different phenological programs between the two sites complement the results in Pigliucci et al. (1995), which showed differences in bolting

and flowering times between populations of *Arabidopsis*. For natural populations of *Arabidopsis*, phenology is considered a significant ecological trait and is a determinant of reproductive isolation (Westerman and Lawrence 1970).

In contrast with studies conducted at the seedling stage, which demonstrated adaptation to Mn in roadside populations (data not shown), no evidence was found to indicate that the roadside populations have become locally adapted to the high NaCl and Mn levels found in roadside environments. In general, roadside plants outsurvived oldfield plants in the control and Mn treatments, but oldfield plants were more likely to survive under NaCl treatments, a result inconsistent with the local adaptation hypothesis. Similarly, oldfield plants were largest in the Mn treatment, while roadside plants did best under the control. The contrast between the apparent benefit of Mn addition for plant size and cost in mortality may indicate that Mn levels, although high enough in the early life stages of the plants to cause stress (and increase mortality), were not high enough to be toxic once the plants grew larger. Manganese is a micronutrient and is thus beneficial at low concentrations and only toxic at high levels. As the plants grew larger, the deleterious impact of the Mn may have been ameliorated and then become advantageous. Maternal effects may also have played a role in the seedling tolerance. It is not as likely that the responses of the adult plants seen here are the result of maternal effects because such effects generally do not persist beyond the juvenile stages (Roach and Wulff 1987).

It is not uncommon for diverse traits to respond differently to experimental treatments (Pigliucci et al. 1995). In this study, plant diameter, leaf number, and plant height responded differently to the treatments. In complex environments, different factors will impact the plants during different life stages and the plants will respond in different ways (i.e., increasing the number but reducing the size of leaves). The variables measured in local adaptation studies are frequently those that are easy to record, but it must not be forgotten that the morphology of plants has a function and cannot be taken as a simple indication of fitness. In addition, not all traits are genetically variable. The difference between the seedling and the adult responses to Mn illustrates the difficulty in examining local adaptation in a complex environment. We speculate that the selection pressure at the seedling stage may be much more intense than at the adult stage. Seedlings are much more sensitive to adverse environments than adults, as can be seen by the high mortality levels of juvenile plants. As they mature, their response to environmental variables changes.

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