EVOLUTIONARY CHANGE IN THREE SPECIES OF COMMON ROADSIDE PLANTS

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A Thesis
Submitted to the School of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree
Doctor of Philosophy

McMaster University

EVOLUTIONARY ECOLOGY OF ROADSIDE VEGETATION

DOCTOR OF PHILOSOPHY (2004) McMASTER UNIVERSITY

(Biology) Hamilton, Ontario

TITLE: Evolutionary change in three species of common roadside plants

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NUMBER OF PAGES: xxxi, 289.

ABSTRACT

Roadsides are an interesting and unusual habitat. Plants inhabiting these degraded areas are exposed to many easily identifiable and unnatural selection pressures. In this thesis, I examine the evolutionary ecology of three species (*Dipsacus sylvestris*, *Hesperis matronalis* and *Asclepias syriaca*) of common roadside plants. I collected seeds from several maternal plants from three oldfield and three roadside populations. In all six studies, I utilized a similar experimental design with analyses based on the variation between maternal families.

I first compared the salt and manganese tolerance during germination of oldfield and roadside genotypes of all three species. I found that one population of *H. matronalis* possessed a greater ability to tolerate high manganese levels than other roadside populations or oldfield populations. In addition, all three roadside populations of *D. sylvestris* had a greater ability to tolerate high salinity than oldfield populations. Some maternal families actually grew longer roots under high salinity than under control conditions. Examination of seed characteristics revealed a significant correlation among roadside maternal families in the amount of sodium leached from the seeds and the degree of salt tolerance or salt enhanced growth indicating that the salt tolerance could be a maternal effect rather than a genetic trait.

I then attempted to discern the role of seed composition, which is controlled primarily by the maternal plant, in conferring salt tolerance to roadside populations of *D. sylvestris*. I leached the soluble components of the seed prior to salt exposure and then

compared the ability of oldfield and roadside families to tolerate high salinity. I found that there were several salt tolerance mechanisms present among the roadside populations and that the maternal environment was likely involved in conferring the salt tolerance in some instances.

I established a reciprocal transplant experiment between the roadside and oldfield involving all three species. I did not observe any evidence that adaptation to roadside conditions had occurred in roadside populations of any of the three species. However, the experiment was established in the late spring after the peak salinity levels. I speculated that adaptations in *D. sylvestris* to the high salinity of roadside soils may be visible if the seeds spent the winter and early spring in the field.

I then established a second reciprocal transplant experiment involving only *D. sylvestris* between the oldfield and the roadside but planted the seeds in late fall to early winter. Again, I observed no evidence of local adaptation to the roadside environment. However, seed size, a trait controlled by the maternal plant, appeared to be very important in determining success during seedling establishment in both oldfield and roadside locations and among both oldfield and roadside genotypes.

I then attempted to determine the role of tolerance to low water potentials in conferring salt tolerance in roadside populations of *D. sylvestris.* I found that there was no relationship between the ability to tolerate low water potentials and the ability to tolerate high salinity. An additional, unintended treatment of hypoxia inflicted by one of the osmotic stress treatments, also revealed that roadside populations of *D. sylvestris* possess considerable variation for the ability to tolerate hypoxia stress. Again, there was no relationship between hypoxia tolerance traits and salt tolerance or tolerance of low water potentials.

I then questioned whether the high sodium levels present in the seeds of roadside *D. sylvestris* maternal families could be detrimental to germinating seeds. I compared the toxicity of the seed leachate of oldfield and roadside maternal families on other germinating *D. sylvestris* seeds from both the oldfield and the roadside. I found that the leachate of roadside *D. sylvestris* seeds inhibited the root growth of oldfield seeds but, the leachate of oldfield seeds had no impact on the root growth of roadside seeds.

These studies strongly indicated that high salinity is a strong selection pressure in roadside communities. However, many stresses are impacting the vegetative community of roadside simultaneously. The selection pressures created by the stresses may be pushing populations in multiple conflicting directions. Different populations and even different families within populations are responding to different selection pressures. In addition, the lack of overlap in the tolerance traits suggests that adaptation to one selection pressure may come at the cost of adaptation to a different pressure. The action of the many different selection pressures may prevent local adaptation from being observed in field studies. In conclusion, it is very clear from these studies that roadside plant populations are evolving to better tolerate the unusual environmental conditions created by roads and a unique vegetative community is developing in this habitat.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Susan A. Dudley, for her support, encouragement, and statistical and editorial advice. I would also like to thank those faculty members who have served on my supervisory and examining committees: Drs. Elizabeth Weretilnyk, Jurek Kolasa and Jon Lott.

I wish to thank David Guevera and Maria Sanchez for their assistance in establishing both field and laboratory experiments, and Paul Hoffman for his patient assistance with the sodium flame photometer.

I would like to thank my parents for their support and interest in my research and, especially, my husband, Matthew Motherwell, for his continuous encouragement, support and friendship.

TABLE OF CONTENTS

SECTION I

SECTION II

SECTION III

SECTION IV

SECTION V

SECTION VI

SECTION VII

SECTION VIII

APPENDICES

LIST OF TABLES

SECTION I

SECTION II

SECTION III

SECTION IV

SECTION V

SECTION VI

SECTION VII

LIST OF FIGURES

SECTION II

SECTION III

SECTION IV

SECTION V

SECTION VI

SECTION VII

PREFACE

The work presented in the following thesis is divided into eight chapters; a general introduction, six experimental chapters, which have been prepared as journal articles, and a general concluding chapter. Chapter 2, "*Tolerance to salt and manganese in three common roadside species*", was published in the International Journal of Plant Sciences (Beaton, L. L., and Dudley, S. A. 2004. 165(1): 37-51). Chapter 3, "*The impact of solute leaching on the salt tolerance of a common roadside plant* Dipsacus sylvestris" was prepared for publication in Functional Ecology.

SECTION I

GENERAL INTRODUCTION

1.1 Roadside Ecology

Roadside ecology has been described as the 'sleeping giant' owing to the vast expanses of land impacted by roads. In Canada, there are over 475 000 ha of land impacted by roads. In more populous countries, the extent of road damage is extremely pervasive. In the United States, 1 % of land is covered by roadside corridors with close to 20 % impacted ecologically by the presence of roads and highways. In Britain, a similar situation exists with 0.9 % of the land mass dedicated to road corridors (Forman & Alexander, 1998).

The impacts of the presence of a road extend well beyond the land dedicated to the highway itself. In Britain, the vegetation near highways was altered up to 100 – 200 m away by traffic exhaust. Large mammals have lower population densities within 100 – 200 m from major highways (Dyer *et al.,* 2002) with birds, small mammals and arthropods avoiding the land bordering roadways from tens to hundreds of meters (Fahrig *et al.,* 1995, Forman *et al.,* 2002, Kuitunen *et al.,* 1998, Reijnen *et al.,* 1996, van der Zande *et al.,* 1980). While traffic noise has been indicted as the primary source of concern related to bird behaviour, roadside lighting has been shown to interfere with the behaviour of nocturnal animals (Forman & Alexander, 1998). The major ecological impact of roads is that they act as a barrier to migration and create small isolated populations (Clarke *et al.,* 1998, Mader, 1984). For example, studies of the common frog *Rana temporaria* in Germany have shown reduced genetic diversity and population subdivision in regions with higher road density (Reh & Seitz, 1990).

For organisms that do occupy roadside habitats, the environment is severely contaminated. Roadside soils are high in a variety of metals (Table 1.1), including lead, from its use as an anti-knocking agent in gasoline, nickel and chromium from their uses in chrome plating, copper from its use in various engine parts and tires, cadmium and zinc, from their use in motor oils and the presence of zinc in tire rubber, and manganese from its use in the anti-knocking agent, methylcyclopentadienyl manganese tricarbonyl (MMT). Roadside soils in temperate regions are frequently highly saline from the use of de-icing salts, which has been shown to act as a barrier to amphibian movement. Salt spray from passing cars can injure trees as far as 120 m from the edge of highways. Roadside runoff is high in copper, lead, zinc, chromium cadmium, nickel and several polyaromatic hydrocarbons (PAH's) including phenanthrene, naphthalene, pyrene and fluoranthene (Maltby *et al.,* 1995, Maltby *et al.,* 1995). The contamination of runoff from roadsides has been shown to severely impact the diversity and composition of macroinvertebrates in the waters receiving the effluent (Maltby *et al.,* 1995, Maltby *et al.,* 1995).

In addition to depositing metals along the roadsides, traffic also causes air pollution and generates excess turbulence. Air turbulence causes increases in particulate pollution resulting in the deposition of dust on the vegetation of roadside corridors. Particulate pollution (dust) can become lodged in stomata and prevent them from closing (Eveling, 1986). The stomata are the major routes of entry for atmospheric pollutants into the leaves. For example, sulphur dioxide damage is greatest when stomata are fully opened. Thus particulate pollution heightens susceptibility to the impacts of vehicle exhaust. *Cannabis indica* grown along a highway in the Himalayas had decreased stomatal density when compared to non-roadside plants, likely as a strategy to protect themselves from noxious gases (Sharma, 1987). In addition to directly damaging vegetation, dust may also change the pH of the soil. The damage from particulate pollution can be seen up to 10 to 20 m from the edge of a highway but can extend to distances as far as 200 m if the area is either downhill or downwind from the road.

Despite the harsh abiotic conditions, roadside corridors normally support a diverse vegetative community (Forman & Alexander, 1998). However, the majority of plant species are exotic. For example, in Hawaii, roadsides are dominated by aliens, which account for ~ 65 % of species at 1 m from the highway (Wester & Juvik, 1983). The success of exotics in roadsides has been attributed both to the high frequency of disturbance and to the high nitrogen levels of roadside soils. Construction and accidents facilitate the establishment of exotic species by disturbing the existing community (Milberg & Lamont, 1995). The combustion of fossil fuels releases NO_x to the atmosphere, which is both taken up by plants through their foliage and accumulates in the surrounding soil (Port & Thompson, 1980, Spencer *et al.,* 1988). For example, NO_x concentrations have been recorded as high as 80 ppm near highways, over 16 times higher than background levels (Port & Thompson, 1980). In addition to favouring exotic species over native species, which have evolved to be most successful in environments with low nitrogen availability, high nitrogen levels create large robust plants that often support a large abundance of insect herbivores. For example, *Lolium perenne* grown in soils collected near a highway grew more rapidly than when grown in non-roadside soil and supported a much larger population of aphids (Port & Thompson, 1980, Spencer & Port, 1988, Spencer *et al.*, 1988). Many insects species are found in higher abundance along roadsides including grasshoppers, *Chorthippus bruneus* and many lepidopteran species (Port & Thompson, 1980). Roadside management practices like mowing also favour exotics.

A major concern regarding the prevalence of exotics in roadside communities is that the roadside will act as a corridor allowing exotic species to spread into previously undisturbed habitats as been demonstrated in Glacier National Park, Montana, USA (Tyser & Worley, 1992). Several studies have demonstrated that plants are able to disperse very efficiently along roadsides (eg. inland migration of the marine halophyte, *Pucinellia distans*) (Scott & Davison, 1985).

Roadsides represent a source of habitat for native communities whose habitat has been replaced for industrial, agricultural and urban activities. For example, in Great Britain, the amount of land dedicated to highway corridors was approximately equal to that in the National Nature Reserves (Ross, 1986). The restoration of roadsides to a short grass prairie ecosystem could not only provide habitat for rare plant species but could link isolated habitat fragments. In agricultural areas where roadsides support a diverse native plant community, butterfly populations are much more diverse than in agricultural areas devoid of naturalized road verges (Ouin & Burel, 2002). However, the evolutionary consequences for organisms inhabiting roadsides and facing the unusual combination of selection pressures remains relatively uninvestigated.

1.2 Microevolution

Selection pressures and ideal phenotypes differ between environments. Species may cope with this variation through either phenotypic plasticity or local adaptation. Natural selection causes better adapted genotypes to leave more offspring than more poorly adapted genotypes, eventually creating a population that is specialized to its immediate environment. Populations that have specialized to a particular environment are referred to as ecotypes. The concept of the ecotype was first proposed by Turresson (Turesson, 1923). Though substantial evidence of the high level of variation present in natural populations of plants had existed for centuries, Turresson was the first scientist to relate the variation to the action of natural selection (Antonovics, 1971). He proposed a new field, entitled genecology, which is the foundation of modern studies of local adaptation. Turresson's concept of the ecotype was based on the notion that populations adapted to specific subsets of environmental conditions. However, his research was conducted over very large geographical regions, which obscured fine scale variation in environmental conditions. Eventually, the concept of clines was introduced into ecological and environmental research (Huxley, 1938) and a conflict ensued over whether species were divided into ecotypes or would be more accurately described as ecoclines (Heslop-Harrison, 1964). The debate has since been resolved with the acknowledgement that population differentiation mimics the environment variation. Abrupt environmental changes are associated with abrupt change in population characteristics, while gradual changes in the environment are associated with gradual changes in populations (Aston & Bradshaw, 1966).

Many studies that have examined local adaptation since Turresson's time have focused on the response of populations to extreme environmental stresses. In particular, some of the most influential studies have examined adaptations to soils contaminated by metals, introduced into the environment by mining activity (Antonovics, 1968, Antonovics & Bradshaw, 1970, Antonovics *et al.,* 1971, Jain & Bradshaw, 1966, McNeilly & Antonovics, 1968). It has been suggested that the high mutation and recombination rates observed in populations experiencing environmental stress may be an adaptive response (Hoffman & Hercus, 2000). Alternatively, it may be caused because organisms under stress lack the resources to repair and protect their DNA from mutation. Related to the idea that stress causes mutation is the theory, which has largely fallen into disrepute among evolutionary biologists, of directed mutagenesis. Under this theory, mutations caused by environmental stresses are more likely to occur in genes that will confer greater tolerance to the stress. Although directed mutagenesis has been discarded from contemporary evolutionary theory because mutations are now believed to occur entirely at random, the notion that some genes are more likely to mutate under stressful conditions than others is a reasonable idea. Genes that have higher expression under stressful conditions will mutate more frequently because during transcription, when DNA is single stranded, it is more vulnerable to mutation (Hoffman & Hercus, 2000).

Many studies of adaptation and microevolutionary change focus on single characters, i.e. metal tolerance, but natural selection acts on all the characters expressed by an organism. In particular, the actions of natural selection do not differentiate between traits that are genetic in origin from those caused by the environment experienced by the organism during development (Lande & Arnold, 1983).

The response of species, or populations, to natural selection is not limitless. Selection is only able to influence the population to the degree that there is variation in the population. The limits of selection are seen most clearly in the constancy of species distribution borders and the rarity of species range expansions (Kruckeberg & Rabinowitz, 1985). One source of the inability of selection to adapt organisms to all environmental influences is the potential costs of adaptation (Chapin *et al.,* 1993). For example, metal tolerant genotypes are often competitively inferior to non-tolerant genotypes under uncontaminated conditions (Linhart & Grant, 1996). A second theory regarding the inflexibility in species distributions is related to gene flow. Peripheral populations that exist at the edge of a species' tolerance to environmental conditions experience high levels of stress. Hence, the populations are small and the number of progeny left in any generation is minimal. In contrast, populations in the centre of a species range are large and produce many migrants. Even if peripheral populations are able to adapt to the marginal conditions of the range edges, they will be swamped by gene flow from the large centrally distributed populations (Hoffman & Hercus, 2000). This theory, however, does not accurately represent the extremely limited gene flow in the majority of plant species.

Local adaptation is a balance between selection pressure and gene flow. As sessile organisms, the migration of genotypes in plants depends entirely on seed and pollen dispersal. Many studies have shown that the movement of both is extremely limited. For example, less than 1 % of *Zea mays* pollen moved further than 60 m away from the paternal plant and less than 0.3 % of *Beta vulgaris* pollen dispersed beyond 200 m (Ehrlich & Raven, 1969). Both species are wind pollinated and are expected to have long-range pollen dispersal. Insect pollinated plants also have very restricted pollen movement with turnips and radishes having outcrossing rates of < 1 % at distances > 15 m (Ehrlich & Raven, 1969). Even in the few instances where pollen has been shown to travel significant distances from the paternal plant, the distance travelled is not equitable to gene flow as it is highly unlikely that any pollen will land on a receptive stigma (Ehrlich & Raven, 1969).

Restricted seed and pollen movement results in fragmented populations, which can enhance population differentiation. However, many studies have demonstrated that isolation is not required for populations to differentiate. Many studies have demonstrated selection pressures can be sufficiently strong to counteract the effects of gene flow from neighbouring populations (McNeilly & Antonovics, 1968). In populations subject to both high selection pressures and high levels of gene flow from nearby populations, barriers to gene flow may begin to develop. For example, *Anthoxanthum odoratum* populations found growing on Zn contaminated soil in an abandoned mine site are more self-fertile than populations growing in nearby pasture populations (Antonovics, 1968). Similarly, metal tolerant populations of both *Agrostis tenuis* and *Anthoxanthum odoratum* growing on contaminated soil from an abandoned mine were not only more metal tolerant than populations in the surrounding pasture land but, also flowered earlier than pasture populations, reducing the level of influx of non-adaptive genetic material (McNeilly & Antonovics, 1968).

However, gene flow can overcome adaptive processes if population sizes are significantly different. If one population is inhabiting a much better environment than a nearby population, gene flow from the more robust population may overwhelm the adaptive process in the poorer habitat. Due to the environmental stress, populations in poor habitats may produce either fewer seeds or seeds that are of inferior quality than plants in good habitats. For example, discrepancies in seed production in terms of both quantity and quality between populations growing in a good and a poor environment prevented populations of *Ranunculus adoneus* inhabiting a less favourable site to adapt (Stanton & Galen, 1997). In marginal areas, adaptation often fails to occur because only a minority of individuals experience the poor conditions and the marginal populations act as a sink for centrally distributed populations.

Stress reduces population sizes, which leads to a reduction in the number of migrants and, hence, an increase in the probability that a population will differentiate. In addition, stress can increase the level of variability within a population. If there is a significant interaction between the genotype and the environment that changes with time, higher levels of genetic variation will be maintained (Hoffman & Hercus, 2000).

Local adaptation has been studied in many different ways beginning with common garden experiments in which individuals were collected from many different environments and then grown up in a common environment. Population differentiation was observed in differences in morphology between the genotypes. These experiments were expanded to include an examination of the response of the genotype to the environment through the completion of reciprocal transplant experiments. In this type of experiment, plants of the same species are collected from different habitats and planted back into their own and into each other's home environment. The fitness of each genotype in each environment is compared (Jordan, 1992, Kindell *et al.,* 1996, Miller & Fowler, 1994, Sork *et al.,* 1993, Van Tienderen & Van Der Toorn, 1991, Waser & Price, 1985).

In some instances, adaptations present in a population to their immediate surroundings may be obscured by the occurrence of temporal variation (Kindell *et al.,* 1996). Some adaptations are only visible during specific life history stages. For example, adaptation in *Astrida stricta* was not visible until the plants were eight weeks old (Kindell *et al.,* 1996). In addition random variation in environmental conditions can obscure the expression of adaptive variation. Harsh conditions have also been shown to interfere with the expression of adaptive variation (Kindell *et al.,* 1996). Genetically different individuals may appear the same under harsh conditions (Kindell *et al.,* 1996). Finally, studies that are conducted on seeds and seedlings may show lower levels of adaptation than studies of adult plants because maladaptive genotypes have not yet been removed at this stage of development (Jordan, 1992). There are many examples of local adaptation to both the abiotic (eg. adaptation in *Polemonium viscosum* to different elevations (Galen *et al.,* 1991), in *Anthoxanthum odoratum* to liming (Snaydon & Davies, 1982) and biotic (eg. variation in herbivory resistance in populations of *Quercus rubra* (Sork *et al.,* 1993), in competitor resistance in *Trifolium repens* (Turkington & Harper,

1979) and *Lolium perenne* (Aarssen & Turkington, 1985)) environment and to differentiation occurring on extremely small scales (Jain & Bradshaw, 1966) (eg. *Anthoxanthum odoratum* (Antonovics, 1971), *Impatiens capensis* (Schmitt & Gamble, 1990), *Agrostis stolonifera* (Aston & Bradshaw, 1966), *Delphinium nelsonii* (Waser & Price, 1985). Results of local adaptation experiments are typically demonstrated graphically in reaction norms. These figures are extremely informative because they demonstrate how the environment and the genotype interact in the formation of the phenotype. Many studies have demonstrated that a large proportion of selection occurs at the seedling stage of development, when the fate of plants is still heavily influenced by the maternal plant.

1.3 Maternal Effects

Studies of adaptation are complicated by the existence of contributions of the maternal plant beyond its nuclear genome to both the fitness and phenotype of its progeny. The genetic composition of the juvenile plant may be overwhelmed by the influences of the maternal plant, termed maternal effects, on the early success of germinating seedlings. Maternal effects are non-nuclear traits that can be transferred to offspring in three ways (Roach & Wulff, 1987). First, the maternal plant contributes non-nuclear DNA, such as that found in the mitochondria and chloroplasts, as well as organelles to the offspring. Second, the tissues of the seed outside the embryo are more genetically similar to the maternal plant than the paternal plant. The endosperm is composed of triploid cells with a diploid maternal component but only a haploid paternal component. The testa and other dispersal structures are composed entirely of maternal tissues. The testa is responsible for determining germination timing in many species and for imposing such germination requirements as cold stratification, light, rainfall and nutrient availability (Donohue & Schmitt, 1998). Finally, the maternal plant provides, and, hence, controls both the quality and quantity of resources for the embryo and endosperm. It is primarily through resource allocation, though characteristics of the testa have also been indicted as environmentally sensitive contributors to seedling phenotype (Donohue & Schmitt, 1998) that the environment experienced by the maternal plant can influence the phenotype and fitness of the offspring. The most commonly cited example of a maternal environmental effect is the influence of environmental quality on seed size. Plants growing in favorable environments are larger and have higher fitness than plants growing in poor environments. A more robust plant will produce better quality seeds that in turn develop into larger, more robust seedlings regardless of their genetic composition (Galloway, 2001, Stanton, 1984). Large seedlings are less sensitive to shade and water stress than small seedlings (Stanton, 1984).

The influence of maternal environmental effects generally diminishes as plants mature. Success experienced during germination and seedling establishment that was obtained via preparations made by the maternal plant may be unrelated to success as juvenile or adult plants (Schmitt & Antonovics, 1986). However, advantages gained by greater seed provisioning by the maternal plant may be maintained and even amplified by competition (Schmid & Dolt, 1994).

In addition to resource availability, the light quality (i.e. R:FR ratio) experienced by the maternal plant may profoundly influence the quality or quantity of light required to stimulate germination in the progeny. Exposing maternal plants to low R:FR light, which simulates canopy shade, during seed maturation may result in seeds that require light to germinate (Orozco-Segovia *et al.,* 1993) or may alter the quantity of resources allocated to individual seeds. For example, *Plantago lanceolata* grown in an unmown environment produce fewer, larger seeds than when grown in a mown environment, likely to enhance the probability of successful establishment for seeds germinating in the presence of competitors (Donohue & Schmitt, 1998).

The susceptibility of the maternal plant to the influences of its own environment during seed development may accelerate or slow the response to selection (Roach & Wulff, 1987). Progeny may continue to be influenced by the selection pressures experienced by the maternal plant even if the environment experienced by the progeny has significantly changed, creating a time lag in response to selection

Some maternal environmental effects may be adaptive. The existence of environmental stressors that are predictable between generations will favour the evolution of maternal environmental effects. If there is genetic variability for a maternal trait, selection could act to favor the persistence of the trait in a population, resulting in "transgenerational phenotypic plasticity" (Fox & Savalli, 1998). If the environment experienced by the maternal plant is predictive of the environment likely to be experienced by the offspring, then some maternal effects may be selectively advantageous (Donohue & Schmitt, 1998). The maternal plant may be able to strongly influence the environment experienced by its offspring through controls over dormancy and germination requirements.

1.4 Germination

At maturity, seeds are dormant and have negligible levels of metabolic activity. The life cycle of the plant begins when the seed germinates. Germination can be divided into three phases: imbibition, activation, and growth (Bewley & Black, 1994). Imbibition is controlled by physical processes rather than by any physiological mechanisms of the seed and depends almost entirely on the difference in water potential between the seed and its environment. As a dry seed typically contains only ~5 – 15 % water (Bewley & Black, 1994, Simon & Mills, 1983), the difference can be as high as –350 to –50 MPa (Bradford, 1995, Simon & Mills, 1983). Imbibition will occur even if the seed is dormant or not viable (Bewley & Black, 1994).

As water enters the seed, it spreads evenly from the exterior tissues to the interior with a clear distinction between areas that have been hydrated and those still in a desiccated state. As time progresses, the water content of the seed continues to increase until a plateau is reached, at which point Phase II begins (Bewley & Black, 1994, Bradford, 1995). If the environment has a very low water potential, the seed's water content will peak at a lower value. Seeds require that a critical water potential be reached or growth will not occur and the seeds will remain dormant for months or even years (Bradford, 1995). The amount of water taken up by seeds is fairly small, only two to three times the dry weight of the seed (Bewley & Black, 1994). During Phase II, there is little water movement into or out of the seed, as further water uptake is not required to initiate growth. However, additional water is required for growth to be sustained.

Though not technically a part of the germination process, growth of the embryo, Phase III, is often the only evidence that a seed has successfully germinated (Bradford, 1995). As growth begins, the osmotic potential inside the seed decreases due to the production of osmolytes from the degradation of stored reserves, which allows the seed to increase water uptake (Bradford, 1995). The accumulation of solutes allows seeds to increase the turgor of the embryo. When accompanied by the loosening of the embryonic cell walls and weakening of the external tissues, this increase in turgor may facilitate growth (Bradford, 1995). The final stage of germination is the elongation of the radical, which occurs in two phases. Phase one occurs very slowly as the barriers preventing growth are gradually overcome. Phase two occurs very rapidly and involves cell elongation followed by the formation of new cells at the root tip (Bewley & Black, 1994).

Beyond the water potential of the seed's environment, the degree of soil compaction also impacts the rate and success of germination because it affects the degree of contact between the seed and the soil (Bewley & Black, 1994). In clay soils, the seed is in contact with many soil particles, making it easier for the seed to imbibe water. In contrast, in sandy soil, there are more air pockets, lowering the degree of contact between the seed and the substrate, and causing the seed to imbibe and pass into the second and third phases of germination more slowly.

When seeds imbibe, they leak out many different substances, some of which may be reabsorbed later on in development (Givelberg *et al.,* 1984, Spaeth, 1987). Leakage is generally associated with decreased levels of seed viability (Duke *et al.,* 1986) and increased levels of pathogen growth (Simon, 1974). In particular, the loss of electrolytes has been negatively correlated with field emergence. The degree of seed leakage is associated with the age and moisture content of the seed and the temperature and water potential of the imbibing solution (Duke *et al.,* 1986). Seeds may continue to leak substances for more than 48 hours following the onset of imbibition. In peas, it is very rapid for the first two minutes then declines to a steady state after ~ 5 minutes (Larson, 1968). Once the seed reaches a water content of ~ 30 %, leakage generally ceases (Simon & Mills, 1983).

Seeds leak out a wide variety of substances, including sugars, amino acids, organic acids, proteins, phosphates and inorganic ions (Duke & Kakefadu, 1981, Givelberg *et al.,* 1984, Mullet & Considine, 1980, Simon & Mills, 1983, Spaeth, 1987). During imbibition, peas lose one third of their electrolytes and 10 % of their sugars (Simon & Mills, 1983). An interesting aspect of leakage is that some seeds leak out allelopathic substances. For example, the compounds leaked from oat hulls inhibit the germination and growth of surrounding seeds both by lowering the water potential of the surrounding solution and through the actions of allelopathic substances that are released from the hulls during germination (Haggquist *et al.,* 1984).

The causes behind leakage have remained unresolved despite extensive research. Currently, it is thought that during imbibition, membranes become transiently leaky due to a change in membrane structure from a gel phase to a liquid crystalline phase (Wolk *et al.,* 1989). Studies of *Phaseolus* have demonstrated that at low water potential, sugar substitutes for water in the plasma membrane. As water is taken up, it replaces the sugar, which is released into solution (Wolk *et al.,* 1989). Like imbibition, leakage is thought to be primarily a physical process, controlled by passive diffusion (Duke *et al.,* 1983). During the initial stages of imbibition, when the outer cells of the seed become hydrated, the rate of leakage is higher than during subsequent stages because there are more cells in the exterior of the seed than in the interior and the substances leaked from the cells do not have to travel a very long distance to reach the exterior of the seed. As the plane of water moves into the interior of the seed, the distance to the exterior of the seed increases for substances escaping the seed and there are fewer cells remaining to be hydrated, which creates lower leakage rates (McKersie & Senaratna, 1983, Powell & Matthews, 1981).

Four other theories have been proposed to explain why substances leak from seeds. First, it has been suggested that the large water potential differences between the seed and the surrounding environment cause imbibition to occur so rapidly that cell membranes are damaged during the process (Simon, 1974). The observed decline in the rate of leakage is ascribed to the impacts of membrane repair (Simon, 1974). However, this theory is unlikely because dead seeds release solutes in the same pattern as live seeds (Powell & Matthews, 1981) despite that they are unable to rearrange their membranes. In addition, cells killed by rapid imbibition would not be able to reassemble membrane fragments. Moreover, injured cells are unlikely able to repair their membranes in the face of a continuous influx of water at the same speed at which leakage has been observed to subside.

Second, it has been suggested that the desiccation of seeds causes the cell membranes to transform from a lipid bilayer to a series of hexagonal conduits, through which solutes are rapidly leaked during the initial phases of imbibition (Murphy & Noland, 1982, Simon & Mills, 1983). These conduits are rapidly changed into a lipid bilayer, which prevents further leakage. However, again, the similarity in leakage patterns between dead and live seeds implies that this theory is false (Powell & Matthews, 1981).

Third, the leakage has been attributed to cell death in the outer surface of cotyledons (Powell *et al.,* 1986). Again the similarity in leakage patterns between dead and live seeds has banished this argument (Powell & Matthews, 1981). Since all the cells of a dead seed are dead, leakage rates should remain constant.

Finally, researchers have suggested that the compounds that leak from seeds during imbibition originate entirely from the apoplast. This theory is not likely because the apoplasmic space is not likely to contain the diversity of substances found in seed leachate (Simon & Mills, 1983).

In this thesis, I investigate adaptations during germination to the roadside. I examine two aspects of the roadside environment, high salinity and high manganese levels, in particular. The following sections review the extent of contamination and toxic effects of, and the tolerance strategies of plants to, each substance.

1.5 Salinity

1.5.1 Salt in the roadside environment

In Canada, close to 5 million tonnes of de-icing salts are deposited on roads and highways annually, with Ontario accounting for a full 30 %. Although many different road salts are available, including calcium chloride ($CaCl_2$), potassium chloride, magnesium chloride and ferrocyanide salts, sodium chloride (NaCl) is used almost exclusively (Canada, 2000). For example, in Ontario, 1.45 million tonnes of NaCl are deposited on roads annually compared to only 45 500 tonnes of $CaCl_2$ (Environment Canada 2000). Salt deposited on the highway eventually reaches the soil and vegetation of the roadside through either runoff or salt spray generated from passing vehicles (Eaton *et al.,* 1999, Thompson *et al.,* 1986, Thompson *et al.,* 1986). Salt spray has been shown to impact vegetation up to 40 m away from the edge of the highway thought it range of impact is dependent on the direction of the prevailing wind and the direction, speed and density of traffic (Thompson *et al.,* 1986).

Although salts are leached from the soil throughout the spring and summer, sodium ion (Na^+) levels may remain high year round and, depending on location, may accumulate from year to year. In Britain, Na^+ levels do not appear to be increasing from one de-icing season to the next (Davison, 1971, Thompson *et al.,* 1986). However, other studies have found relationships between the salinity of the soil along the roadsides and the number of years that the highway had been salted (Davison, 1971). Many roadsides are characterized by poorly draining, clay soils that are not likely to completely leach throughout the summer. The presence of shrubs elevates the level of Na^+ found in the soils along roadsides (Thompson & Rutter, 1986). Whether Na^+ is leached from the soil will depend on many characteristics of the soil, including whether it is frozen, the amount of rainfall, the depth of the water table, the texture, structure, chemistry, organic content, permeability and cation exchange capacity of the soil and the density of the soil biota (Westing, 1969). Unlike Na^+, chloride ions (Cl^-) leach from the soil very rapidly.

The impact of the high salinity on the vegetation along roadsides has already been demonstrated in the rapid inland migration of coastal marsh species. *Puccinellia distans*, a marine plant, has migrated inland following the flow of traffic (Scott & Davison, 1985). The use of de-icing salts has also been blamed for the decline of the sugar maple along highway corridors (Westing, 1969).

1.5.2 Salt Toxicity and Tolerance

Plants exposed to highly saline soils will often have dark green, scorched leaves that are abscised earlier than under non-saline conditions (Westing, 1969). During germination and seedling establishment, plants are much more sensitive to high salinity than as adult plants (Gorham, 1992). Even halophytes, which are highly salt tolerant plants that often inhabit marine areas and salt marshes, germinate best in water with 0 % salinity (Gorham, 1992, Keiffer & Ungar, 1997, Khan & Rizvi, 1994, Khan & Ungar, 1996, Ungar, 1978).

Salt toxicity is caused by a combination of osmotic stress (Huang & Redmann, 1995), ion toxicity, nutrient deficiency (Gorham, 1992), oxidative stress (Gueta-Dahan *et al.,* 1997), and increased susceptibility to pathogens (Westing, 1969). High salinity lowers the osmotic potential of the soil, which makes it difficult for plants to take up water, resulting in osmotic stress. Low water potentials have often been cited as the main source of reduced growth in plants experiencing salt stress because cell growth is dependent on the maintenance of adequate turgor pressure (Ashraf, 1994).

Although many plants attempt to exclude Na^+ at high salinities all plants will eventually begin to accumulate high levels of Na^+ and to experience ion toxicity. Although the mechanism of Na^+ toxicity is not clearly known, it has been demonstrated that Na^+ competes with K^+ at membrane binding sites and interferes with enzymes that require K^+. K^+ is involved in over 50 enzymatic processes and, in most circumstances, Na^+ cannot perform the functions of K^+. Na^+ is transported passively into the cytoplasm down an electrochemical gradient (Rengel, 1992). Once inside, excess ions may be compartmentalized in the vacuole. Na^+ is actively pumped across the tonoplast via a Na^+/H^+ antiporter (Apse *et al.,* 1999). Once concentrations inside the vacuole get significantly higher than in the surrounding cytoplasm, Na^+ will begin to leak from the vacuole due to the difference in concentration (Glenn *et al.,* 1999). Once the vacuole becomes full or if Na^+ is entering the cell faster than it can be pumped into the vacuole, in order to protect its enzymatic functions, the cell may begin to export Na^+ to the apoplasm where it builds up on the cell walls. The osmotic potential of the apoplasm rapidly drops, which causes the cell to dehydrate (Gorham, 1992). Hence, the cell will succumb to either high internal Na^+ levels interfering with K^+ functions or to osmotic stress (Munns, 1993, 2002).

Na^+ is transported to the shoot in the transpiration stream through the xylem. Hence, the stomata control the rate of Na^+ movement. Stomata are opened and closed by the movement of K^+ in and out of the guard cells. The flow of K^+ out of the guard cell closes the stomata and flow into the cell opens it. In glycophytes, excess Na^+ interferes with this process. For example, in *Commelina communis* growing at high salinity, Na^+ accumulates in and around the guard cells instead of K^+ (Jarvis & Mansfield, 1980). An influx of Na^+ into the guard cell can open the stomata, but the cells are unable to close in response to darkness or high carbon dioxide or abscisic acid levels. Some halophytes are able to utilize Na^+ in the place of K^+ in guard cell function. Two species, *Cakile maritime* (Eshel *et al.,* 1974) and *Suaeda maritima* (Robinson *et al.,* 1997) are able to close their stomates with either Na^+ or K^+, while *Aster tripolium* (Kerstiens *et al.,* 2002) excludes Na^+ from the guard cells and closes its stomata in response to high internal salt concentrations (Robinson *et al.,* 1997).

The ability of the root to bind cations other than Na^+ is harmed at high salinities, which results in a deficiency of several nutrients (Epstein, 1998). In particular, Na^+ displaces both calcium (Ca^{++}), which has been shown to alleviate salt toxicity, and K^+ on the plasma membrane (Liu & Zhu, 1998). High internal Na^+ levels also alter the ability of plants to exchange gases through the stomata causing a reduction in nitrate and nitrite uptake (Fuhrer & Erismann, 1980).

The importance of the disruption of the plant's K^+ nutrition in causing salt toxicity has been demonstrated in studies of mutants of *Arabidopsis* that are overly sensitive to salt (*SOS* mutants) (Zhu *et al.,* 1998). Plants have two different K^+ uptake systems: low affinity and high affinity (Liu & Zhu, 1997). If K^+ levels are low, plants employ the high affinity system. Impairment of the high affinity uptake system appears to result in elevated salt sensitivity (Liu & Zhu, 1997). In fact all of the 42 *SOS* mutants studied had reduced function in one of three genes that are involved in K^+ nutrition (Zhu *et al.,* 1998).

Exposure to high salinity has been linked to oxidative stress. There is some evidence that salt tolerant genotypes have increased anti-oxidant levels. In citrus, salt exposure resulted in increased levels of phospholipid hydroperoxide, glutathione peroxidase, superoxide dismutase and ascorbate peroxidase (Gueta-Dahan *et al.,* 1997).

Salt tolerance varies considerably both within and between species. For example, *Spartina patens* possesses considerable variation in salt tolerance (Hester *et al.,* 1996). The degree of salt tolerance and the amount of variation in the degree of salt tolerance present in a population is related to the level of salinity stress. For example, *Festuca rubra* individuals growing in a less saline part of a salt marsh were less salt tolerant than individuals growing in a more saline part of the marsh (Ashraf, 1994). The strong directional selection imposed by high salinity often eliminates most of the additive genetic variation for salt tolerance in impacted populations. For example, salt tolerance has been fixed in *Festuca rubra* and *Agrostis stolonifera* populations collected from highly saline environments while there is considerable variability in salt tolerance in populations collected from low salinity habitats (Ashraf, 1994).

In order to achieve salt tolerance, plants must possess three different traits (Glenn *et al.,* 1999, Winicov, 1998). First, the plant must be able to control the rate of uptake and the rate and destination of transport of Na^+. Second, plants must be able to change the allocation of resources between shoots and roots and alter the rate of transpiration and stomatal closure. Third, plants must able to respond to high salinity appropriately at the cellular level (i.e. prevent Na^+ accumulation in the cytoplasm).

Tolerance to high salinity appears to come at the cost of reduced competitive ability under non-saline conditions. Halophytes, which are highly salt tolerant plants found growing in marine environments and salt marshes, are frequently competitively inferior to glycophytes, which are salt sensitive species, under non-saline conditions (Gorham, 1992). For example, comparisons of *Sonneratia alba* and *S. lanceolata* have revealed that the more salt tolerant species, *S. alba* has obtained its tolerance at the expense of growth rate and competitive ability under low salinity (Ball & Pidsley, 1995).

Plants have developed many different mechanisms to tolerate high salinity. Many species accumulate excess Na^+ in old leaves while relying on young leaves for photosynthesis. Many species produce osmotic compounds, including proline, glycinebetaine, polyols, cyclitols, organic acids, sugars, malate and oxalate, in order to maintain a low internal osmotic potential (Ashraf, 1994, Gorham, 1992). It remains unclear if these compounds are a symptom of salt stress or actually confer tolerance (Munns, 1993). For example, the *SOS* mutants of *A. thaliana* previously described produce more proline than the wild types (Zhu *et al.,* 1998). Other studies have indicated that proline accumulates in response to drought stress in addition to salinity stress and may, therefore, be a general response to low osmotic potential (Ashraf, 1994). Proline may actually be negatively associated with salt tolerance because it is important in the temporary endurance of extremely stressful conditions and may not be related to overall tolerance (Ashraf, 1994). *Lycopersicon exculentum*, a salt sensitive species, produces more compatible solutes than the salt tolerant species *L. penelli* (Santa-Cruz *et al.,* 1998).

The use of organic compatible solutes to adjust osmotic potential is costly (Robinson *et al.,* 1997). In contrast, species that accumulate Na^+ as an osmotica, are able to maintain low osmotic potentials at very low energetic cost. Despite the potential toxicity, many salt tolerant species accumulate high Na^+ levels (Gorham, 1992, Reimann & Breckel, 1995). For example, *Atriplex canescens* accumulates K^+ under drought stress but Na^+ under salt stress (Glenn *et al.,* 1996), while *Holosarcia pergranulata* accumulates both Na^+ and Ca^{++} in response to high salinity (Short & Colmer, 1999).

Halophytes have a wide variety of salt tolerance mechanisms ranging from salt bladders and glands to succulent tissues (Gorham, 1992). There are 5000 to 6000 species of halophytes, accounting for ~ 2 % of angiosperms (Glenn *et al.,* 1999). 44 % of known halophytes are in the Chenopodiaceae family (Short & Colmer, 1999). In most halophytes, Na^+ is actively accumulated in the cytoplasm to maintain a low osmotic potential.

1.6 Manganese

Manganese (Mn) is the twelfth most abundant element in the earth's crust and the fifth most abundant metal (Bhuie & Roy, 2001). It is plentiful in most soils, ranging in concentration from 500 to 850 ppm. Plants require a soil environment with 20 to 500 ppm Mn. Below 20 ppm, vegetation will suffer from Mn deficiency, while above 500 ppm Mn becomes toxic (Brault *et al.,* 1994). The concentration of Mn in the soil does not adequately portray the level of Mn accessible to the vegetation. Mn availability is affected by the concentration of other cations in the soil and the soil's cation exchange capacity, drainage, organic matter content, temperature, compactness, microbial activity and, especially, pH (Brault *et al.,* 1994, Chacon *et al.,* 1998, Kitoa *et al.,* 1997). Acidic soils have substantially higher available Mn levels than non-acidic soils. A one unit decrease in pH leads to a 100 fold increase in Mn solubility, which can increase Mn toxicity.

Mn is required for many anthropogenic activities. Worldwide, anthropogenic sources account for ~ 1/3 of the 900 000 tonnes of Mn that enter the atmosphere annually (Loranger & Zayed, 1994). Since the 1970's, when the anti-knocking agent methylcyclopentadienyl manganese tricarbonyl (MMT) in gasoline was first introduced, there has been an increase in Mn pollution (Brault *et al.,* 1994, Loranger & Zayed, 1994). Currently, MMT is used in Canada, USA, Europe, Asia and South and Central America (Corp, 2000). In Canada, MMT began to replace lead in gasoline in 1976 and by 1992 was the only anti-knocking agent in Canadian gasoline (Bhuie & Roy, 2001). In addition to reducing lead levels in the environment, MMT reduces nitrate emissions and crude oil consumption and makes cars run more smoothly (Bhuie & Roy, 2001). One litre of gasoline contains 0.72 g of MMT (Brault *et al.,* 1994). Following combustion, 30 % of the Mn contained in the gasoline is released to the environment, primarily as Mn oxides, the most common of which is hausmannite (Mn_3O_4) (Brault *et al.,* 1994, Loranger & Zayed, 1994).

The use of MMT in gasoline has resulted in the accumulation of Mn in roadside soils in some areas, but not others. In Utah, USA, roadside soil was found to have Mn levels of 3046 μg/g at 1 m from the highway compared to only 254 μg/g at 15 m from the highway (Lytle *et al.,* 1994). In contrast, examination of the soil in the greater Toronto area of Ontario, Canada did not reveal elevated Mn levels (Bhuie & Roy, 2001). Roadside vegetation in Utah was also shown to have elevated Mn levels, especially in aquatic plants, which experience anoxic environments that are more favourable for Mn uptake. For example, *Ranunculus aquatilis* had Mn levels of 13680 μg/g (Lytle *et al.,* 1994).

Though toxic at high levels, Mn is a micronutrient involved in many plant processes including the water splitting system in the second stage of photosynthesis and its role as a cofactor of different enzymes involved in redox reactions and protection against oxidative stress (Kitoa *et al.,* 1997). Mn activities are also required for the phytoene synthetase activity, ent-kaurene synthetase activity and mevalonic kinase (Wilkinson & Ohki, 1988). Finally, it is also either required or at least highly beneficial for enzymes involved with the synthesis of gibberellic acid and chloroplast pigments (Wilkinson & Ohki, 1988).

Above 500 ppm, Mn becomes toxic to plants. The symptoms of Mn toxicity resemble iron deficiency, with the leaves developing brown speckles, which are deposits of Mn and phenols in the cellular wall of the peripheral cells of the petiole and palisade parenchyma (Chacon *et al.,* 1998), regions of necrosis and/or chlorosis and becoming wrinkled. Before the foliar symptoms of Mn toxicity develop, growth has already been reduced (Subrahmanyam & Rathore, 2000). The response of the plant is affected by the age of the leaf, the temperature, soil nutrient balance, genotype and light intensity (Kitoa *et al.,* 1997). Unlike other metals, Mn does not have a specific cellular target. There are several theories regarding the source of Mn toxicity, relating to both auxin destruction and oxidative stress. Excess Mn may result in the destruction of auxin through activation of indoleacetic acid (IAA) oxidase. In *Vigna unguiculata*, increases in IAA oxidase activity brought about by excess Mn causes a loss of apical dominance, which induces the formation of auxillary shoots and reduces grain yield (Horst, 1982).

There is considerable evidence that Mn toxicity is a result of oxidative stress (Kitoa *et al.,* 1997, Wissemeier & Horst, 1990). In the apoplast, MnII may be oxidized to MnIII, which is a very powerful oxidant of proteins and lipids (Fecht-Christoffers *et al.,* 2003). In *V. unguiculata* varieties with enhanced Mn tolerance, excess Mn induces an increase in the anti-oxidant abscorbic acid, which is not seen in Mn sensitive varieties (Fecht-Christoffers *et al.,* 2003). Other anti-oxidant enzymes including GPX, ASPX and SOD, have reduced activity levels under excess Mn, which causes the plant to experience oxidative stress (Santandrea *et al.,* 2000).

Oxidative stress brought about by excess Mn appears to be particularly detrimental to the chloroplasts of young leaves. Excess Mn rapidly degrades chlorophyll through lipid peroxidation resulting in decreased carbon dioxide assimilation caused by the inhibition of electron transport (Kitoa *et al.,* 1997, Wissemeier & Horst, 1990). The chloroplasts of immature leaves are more sensitive to excess Mn than mature leaves. Unlike mature leaves, immature leaves are actively building thykaloids and require Mn for the establishment of photosynthesis system II. Mn is actively imported into the chloroplasts of immature leaves. In contrast, mature leaves no longer require Mn as their chloroplasts are fully formed and sequester Mn in vacuoles. The excess Mn in the chloroplasts causes chlorosis. Bean genotypes that were tolerant of high Mn had lower levels of Mn in their chloroplasts than did sensitive varieties, suggesting that tolerance to high Mn may be the result of a Mn exclusion mechanism in the chloroplast (Gonzalez & Lynch, 1999).

Many species have been identified that possess variation in Mn tolerance, including *Clusia multiflora* (Chacon *et al.,* 1998), *Triticale* (Mugwira *et al.,* 1981), *Triticum aestivum* (MacFie *et al.,* 1989), rice (Lidon, 2001), *Nicotiana tobacum* (Petolino & Collins, 1985, Santandrea *et al.,* 2000), *Vigna unguiculata* (Horst, 1982) and soybean (Heenan *et al.,* 1981, Ohki *et al.,* 1980). Two different tolerance mechanisms have been observed in plants. First many species appear to exclude Mn from the roots and to minimize its translocation to the shoots (Lidon, 2001). This type of tolerance mechanism has been seen in *N. tobacum*, soybean and rice (Lidon, 2001, Wang *et al.,* 1992). Second, the absence of brown spots and the even distribution of Mn throughout the leaves in tolerant varieties of *C. multiflora* suggests that tolerance may involve increased binding of Mn to cell walls (Chacon *et al.,* 1998, Rogalla & Romheld, 2002).

Table 1.1 Reports of metal contamination of roadside soil

Metal	Background level (> 50 m distance from highway) (µg/g)	Roadside level (1-2 m from highway) (µg/g)	Study location	Reference
Lead	4.1	24.3	Nigeria	(Ndiokwere, 1984)
	124.4	1769.1	USA	(Warren & Birch, 1987)
Cadmium	0.4	1.7	Nigeria	(Ndiokwere, 1984)
	0.2	6	USA	(Warren & Birch, 1987)
Chromium	0.73	2.6	Nigeria	(Ndiokwere, 1984)
Nickel	3.3	1.01	Nigeria	(Ndiokwere, 1984)
Zn	11.7	47.8	Nigeria	(Ndiokwere, 1984)
	93.9	971.9	USA	(Warren & Birch, 1987)
Copper	31.0	345.8	USA	(Warren & Birch, 1987)

SECTION II

SALT AND MANGANESE TOLERANCE IN THREE COMMON ROADSIDE SPECIES

2

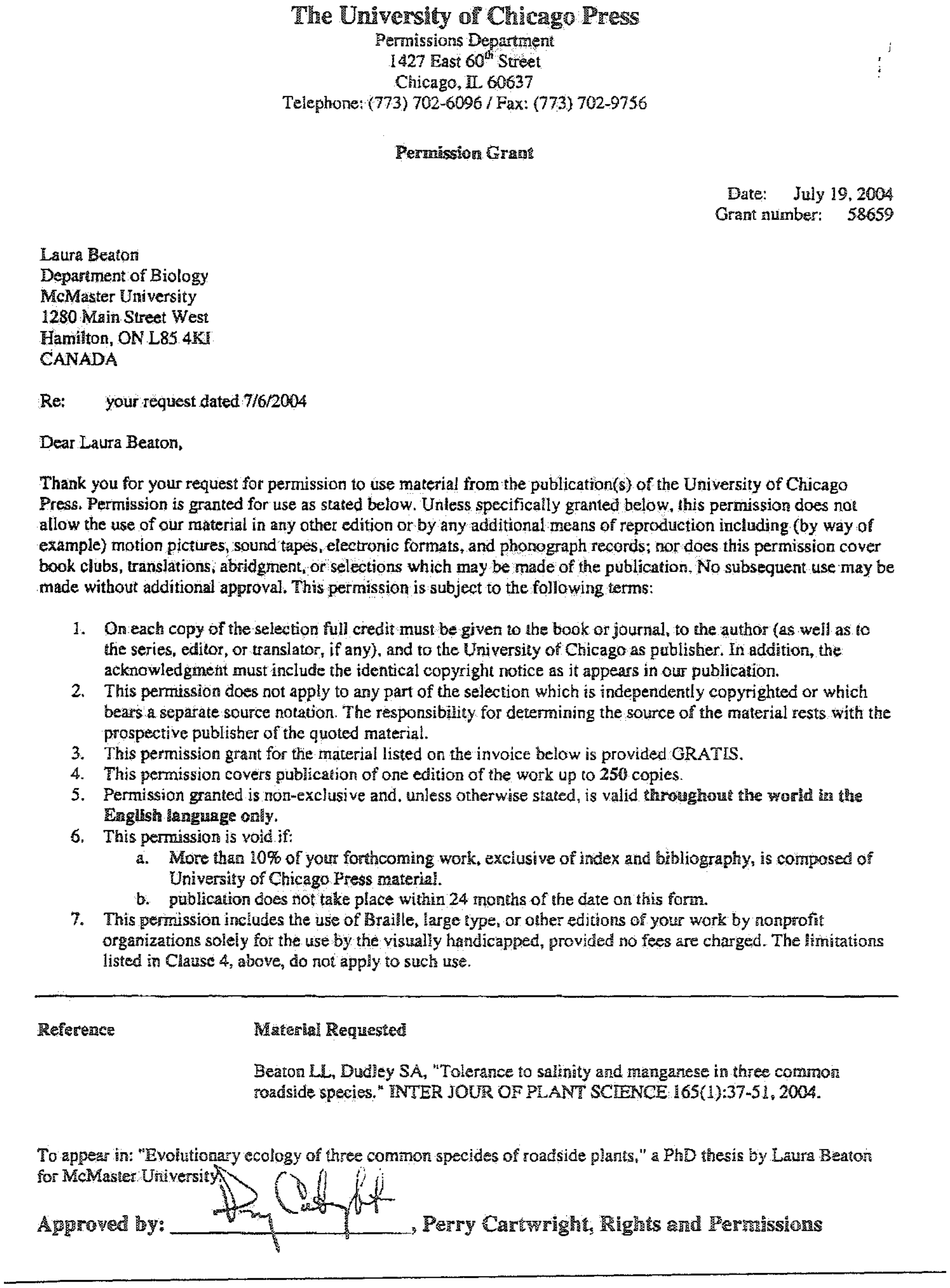
Salt and manganese tolerance in three common roadside species.

(Beaton, L. L. and Dudley, S. A. 2004. International Journal of Plant Sciences. 165(1): 37-51)

2.1 CLARIFICATION OF CONTRIBUTIONS for Beaton and Dudley (2004)

In the following study, I was responsible for proposing the idea, designing and conducting the experiments, analyzing the data and writing the manuscript. Throughout the process, I consulted S.A. Dudley on methodology and statistical analysis and received considerable editorial commentary, which significantly improved the manuscript.

2.2 *Letter of consent from the University of Chicago Press granting permission to include the published journal article (Beaton and Dudley 2004) as part of the present thesis.*



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	Beaton LL, Dudley SA, "Tolerance to salinity and manganese in three common roadside species." INTER JOUR OF PLANT SCIENCE 165(1):37-51, 2004.

To appear in: "Evolutionary ecology of three common specides of roadside plants," a PhD thesis by Laura Beaton for McMaster University.

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2.3

Tolerance to Salinity and Manganese in Three Common Roadside Species.

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International Journal of Plant Science 165(1): 37-51.

Beaton, L. L. and Dudley, S. A. 2004. *Salt and manganese tolerance in three common roadside species.* International Journal of Plant Sciences. 165(1): 37-51.

2.4 ABSTRACT

Roadside soils are contaminated with a variety of substances toxic to plants including manganese (Mn^{++}) from the use of the anti-knocking agent methylcyclopentadienyl manganese tricarbonyl in gasoline and salt from the use of de-icing salts. We investigated whether roadside populations of three species (*Asclepias syriaca* L., *Dipsacus sylvestris* Huds. and *Hesperis matronalis* L.) were more tolerant of these contaminants during germination than populations located in a more benign environment; i.e. oldfields. We used field collected maternal families because the life history of the three species, two monocarpic perennials and one perennial, prevented us from growing the plants for one generation in a common environment. Family responses to the treatments are therefore a combination of genetic traits and maternal environmental effects. Consequently, we measured several traits likely to be affected by the maternal environment, including seed size and the quantity of sodium ions ($Na^{+)}$ leached from seeds during germination. Roadside populations of neither *A. syriaca* nor *D. sylvestris* demonstrated any adaptation to elevated Mn^{++} levels during germination. However, one roadside population of *H. matronalis* did display a low level of Mn^{++} tolerance. There was no relationship between the seed characteristics and Mn^{++} tolerance in *H. matronalis*. Roadside populations of neither *A. syriaca* nor *H. matronalis* demonstrated any adaptation to high soil salinity during germination. However, roadside populations of *D.*

sylvestris did display tolerance to elevated salinity levels. Salt tolerance in roadside populations of *D. sylvestris* appeared to be highly related to both seed size and Na^+ concentration of the seed leachate. The observed salt tolerance of roadside *D. sylvestris* seeds may be a maternal environmental effect rather than a genetic trait.

2.5 INTRODUCTION

Numerous studies have demonstrated that plant populations can become adapted to harsh environments (Galen *et al.* 1991, Nellessen and Ungar 1993). Many species of plants have adapted to the high metal concentrations of the soil of abandoned mines (e.g., *Agrostis tenuis* (Antonovics and Bradshaw 1970, Karataglis 1982, McNeilly and Antonovics 1968), *Mimulus guttatus*, (Macnair *et al.* 1993). Like mine tailings, roadside soils are also contaminated by metals. However, in other ways, roadsides are very different from mine tailings. In studies of local adaptations to mines, focal populations are limited to the relatively small, isolated area impacted by the mine, while roads impact hundreds of thousands of hectares of land. Although the vegetation occupying mine sites faces several selection pressures beyond the metal contamination of the soil (i.e. low nutrient levels, exposed environment (Antonovics*et al.* 1971)), the high metal levels are undoubtedly the most important factor determining species composition. In contrast, plants living near a road are impacted by a variety of anthropogenic stresses (Forman and Alexander 1998) any one of which could be imposing strong selection pressure; smog, air turbulence, particulate pollution (Thompson*et al.* 1984), contamination of the soil by moderate levels of a variety of metals (cadmium, zinc, copper, lead, and manganese (Mn^{++})(Harrison *et al.* 1985, Yassoglou *et al.* 1987); (Johnston and Harrison 1984)) and high salinity (Thompson *et al.* 1986).

Relatively little is known about whether roadside plants have evolved to be able to better tolerate the various soil contaminants. Bryson and Barker (2002) found species that differed in their ability to tolerate salt in roadsides in Massachusettes. Lead tolerance has been found in roadside populations of *Plantago lanceolata* (Wu and Antonovics 1976) in one study, but not in another (Tonsor 1990). These studies focused on roadside and non-roadside populations in very close proximity (<80 m (Wu and Antonovics 1976) and < 30 m (Tonsor 1990)). Rothfels *et al.* (2002) have shown that when comparing roadside populations with isolated oldfield populations (i.e. not located in close proximity) that tolerance to neither salt nor Mn^{++} has evolved at the adult stage. However, it is unknown whether tolerance to either contaminant has evolved during germination and seedling establishment. In this study we examine the evolutionary impact of two potential selection agents in the roadside, high soil salinity from the use of de-icing salts, and Mn^{++} contamination from the use of the anti-knocking agent methylcyclopentadienyl Manganese tricarbonyl (MMT) in gasoline, on seedling growth of three common roadside species.

The juvenile stages of a plant's life history are likely of considerable consequence in terms of evolutionary change, owing to the high mortality levels during germination and seedling establishment (Schmitt and Antonovics 1986). In the spring, when roadside salt concentrations are at their peak (Thompson *et al.* 1986), seeds germinate and seedlings become established. Early on in development, plants are much more sensitive to high salinity than they are as adults (Gorham 1992). High salt levels can cause osmotic stress, ion toxicity, oxidative stress (Gueta-Dahan *et al.* 1997) and/or nutrient deficiency (Gorham 1992, Liu and Zhu 1997). Huang and Redmann (1995) demonstrated that germinating barley seeds are most impacted by the effects of salt on the osmotic potential of the soil. High salt levels in the soil increase the osmotic potential, inhibiting the ability of plants to take up water, which, consequently, leads to osmotic stress (Gorham 1992).

High salinity has been an important selection agent in roadside plant communities since the 1960's, when the use of de-icing salts became common. Since that time, increased soil salinity has had an impact on the composition of the roadside plant community. In coastal areas, highly salt tolerant marine plants (halophytes) have invaded road verges (Scott and Davison 1985). In non-marine areas, however, salt sensitive species (glycophytes) still dominate roadside vegetation. Since both agricultural and natural glycophyte species have been shown to possess high levels of variation in salt tolerance (Hester *et al.* 1996, Hester *et al.* 1998), we hypothesize that glycophyte species have evolved higher salt tolerance in roadside sites.

Mn^{++} became a potential contaminant of roadside soils in 1976 when MMT began to replace lead as the anti-knocking agent used in gasoline in Canada (Loranger and Zayed 1994). The combustion of gasoline containing MMT releases low levels of manganese oxides into the atmosphere (Abbott 1987). Their half-life in the atmosphere is very short and the metal is quickly deposited on the ground (Abbott 1987). A recent study in Utah found that Mn^{++} was accumulating in the soil along roadsides. At 1 m from the highway's edge, levels were as high as 3046 µg/g; over 12 times higher than levels 250 m from the highway (Lytle *et al.* 1994). The potential for Mn^{++} to act as a selection agent is complicated by its role as a micro-nutrient involved in many enzymatic processes including photosynthesis (Marschner 1995). Its presence is beneficial at levels under 500 μg/g (Brault *et al.* 1994), but becomes toxic above this level. Consequently, it has the potential to be acting as a selection agent in the roadside environment. Although the levels found in Utah were six times higher than the minimum levels known to cause toxicity, the availability of soil Mn^{++} to plants is impacted by the pH, drainage, organic matter content, temperature, compactness and microbial activity of the soil (Brault *et al.* 1994). Most local adaptation studies of metal tolerance examine populations inhabiting extremely toxic environments. Few, if any, studies have demonstrated adaptation to a metal at low toxicity levels (Rothfels *et al.* 2002).

In this study we collected seeds from several maternal families from three oldfield and three roadside populations of *Dipsacus sylvestris* Huds., *Hesperis matronalis* L. and *Asclepias syriaca* L. All the seeds from a single plant (the maternal parent) comprise one maternal family and consequently the seeds are half sibs. Oldfields, which are successional communities commonly arising from abandoned farm land, were used as a control habitat because they support a vegetative community similar to roadsides. Seeds were exposed to solutions high in salt or in Mn^{++} or to distilled water and germinated. We ask the following questions: 1) Is there variation among families in the ability of seedlings to tolerate either salt or Mn^{++}?; 2) Do roadside populations demonstrate a greater tolerance for high salinity or high Mn^{++} levels than do oldfield populations?; 3) What is the impact of different characteristics of the seed on the performance of germinating seedlings?

2.6 METHODS

2.6.1 *Study Species*

Dipsacus sylvestris (common teasel, Dipsaceae family), *Hesperis matronalis* (dames rocket, Cruciferae family), and *Asclepias syriaca* (milkweed, Asclepiadaceae family) were chosen as the study species because they are abundant in both oldfields and roadsides, they have large inflorescence allowing for easy identification on the roadside, and they produce large numbers of seeds. *Dipsacus sylvestris*, a native of Europe, is common in roadsides. It is a monocarpic perennial that grows vegetatively as a rosette for at least one season. In the spring, if the plant has reached a critical size, it will bolt to its reproductive form of an upright flowering stem. Flowering occurs from July to September and seeds are shed throughout the fall. A single teasel plant can produce upwards of 3000 seeds (Werner 1975).

Hesperis matronalis, a native of Asia, grows mainly in semi-shaded habitats including roadsides. Also a monocarpic perennial, it grows vegetatively as a rosette for at least one season. In the spring, if the plant has reached a critical size, it bolts to form an upright flowering stem. Flowering occurs from May to August and seeds are shed throughout the summer. Each plant can produce several hundred seeds (Neilsen *et al.* 1995).

Asclepias syriaca, a native of northeastern North America is commonly found in roadsides. It is a perennial that reproduces from seed and by adventitious root buds. Flowering occurs from June to August and seeds, which are produced in pods, are released from September to October. Each plant can produce upwards of 2000 seeds, which are dispersed easily by the wind (Bhowmik and Bandeen 1976).

2.6.2 *Seed Collection*

For each species, we selected three roadside and three oldfield populations. Roadside populations were located in full sunlight, 2 - 10 m from the pavement's edge (of Highway 403 between the cities of Hamilton and Burlington, Ontario, Canada). Highway 403 is a six lane highway with a speed limit of 90 – 100 km/h. Traffic volume ranges between 44 000 to 72 000 vehicles daily (two way traffic) (Ontario Ministry of Transportation, 1994). At all the roadside sites, the surrounding vegetation was primarily various species of grass and/or crown vetch (*Coronilla varia*)(which is planted following highway construction to stabilize the soil) with a few common oldfield plants (e.g. *Solidago* sp., *Daucus carota*). Oldfield populations were located on Royal Botanical Gardens (cities of Burlington and Hamilton, Ontario, Canada) conservation lands in full sunlight. All oldfield sites were found > 100 m from the nearest road (in all cases, a two lane road with a 50 - 70 km/h speed limit) and several km from the nearest major highway. The surrounding vegetation of these sites was dominated by a variety of grass species with a wide variety of forbs. Seeds were collected from 15 - 20 plants from each population. To ensure that an adequate number of seeds were collected from each plant, the largest plants from each population were chosen for seed collection. Following collection, seeds were processed and stored in paper envelopes at room temperature.

2.6.3 **D. sylvestris** *Salt and Mn^{++} Tolerance at Multiple Levels*

A pilot trial with several roadside and oldfield families of *D. sylvestris* was carried out to determine the appropriate NaCl and $MnSO_4$ levels. We determined that root length was a good measure of performance; it was easily measured, highly correlated with seedling mass (root $r = 0.84098$, $p<0.0001$), and not correlated with the date of germination. We chose stress levels at which seedlings suffered a decline in root growth but could still form roots. Based on this information, 70 mM NaCl solution was chosen as the treatment for the focal experiment. Mn^{++} tolerance trials indicated that Mn^{++} did not affect seedling root length at levels under 0.5 mM $MnSO_4$. However, unlike the salinity trial, no level was found in the initial test at which seedlings suffered a decline in root growth but still formed roots. A second, smaller trial of Mn^{++} levels examined the impact of $MnSO_4$ levels of 1, 2, 3 and 4 mM $MnSO_4$. From the subsequent test, 3 mM $MnSO_4$ solution was chosen as the treatment for the future experiments.

2.6.4 *Salt and Mn^{++} Tolerance*

Seeds from each of five maternal families of the six populations (i.e. fifteen maternal families from the roadside and fifteen maternal families from the oldfield) from each of the three species were placed in petri-dishes lined with filter paper (10 seeds from one maternal family per dish) and containing one of the two test solutions or a control (distilled water). Each treatment/seed combination was replicated twice for a total of twenty seeds from each family per treatment. The two replicates were completed at two different times. *A. syriaca* seeds displayed a strong dormancy that was overcome by soaking the seeds for one week in the test solutions and then scarifying the seeds by scraping away the seed coat with a razor blade. Seeds were incubated at room temperature under artificial lighting composed of both incandescent and fluorescent sources with an irradiance level of 160 μmoles/m^2/s. On the third, fifth and seventh day, 2 ml of distilled water was added to each tray to replenish moisture lost to evaporation. After 10 days, the root length of the emerging seedlings was measured. For *A. syriaca* seedlings, we found that root length was not a reliable measure of size, because of a lack of clear differentiation between the hypocotyl and the root. Consequently, a second experiment was done with only *A. syriaca*, measuring the total seedling length from the root tip to the end of the hypocotyl. Both replicates were completed simultaneously.

2.6.5 *Seed Characteristics*

One hundred seeds from each maternal family from each population from each species were weighed in ten groups of ten seeds. A lack of seeds precluded this measurement in several *H. matronalis* maternal families. The sodium ion (Na$^+$) content of the seeds was determined by measuring the Na$^+$ concentration of the seed leachate. Ten seeds from each maternal family from each population (due to their size, only five *A. syriaca* seeds that had been scarified) were placed in centrifuge tubes in 1 ml of distilled water and soaked for 72 hours at room temperature. The Na$^+$ concentration of the resultant leachate was measured using a sodium flame photometer (Evans Electroselenium Limited).

2.6.6 *Statistical Analysis*

Statistical analysis was conducted using SAS version 7.0 for Windows.

2.6.6.1 *Salt and Mn^{++} Tolerance*

The *H. matronalis* and *A. syriaca* data collected from the local adaptation experiment were log transformed to satisfy the assumptions of normality and homoscedasticity. *Dipsacus sylvestris* data did not require transformation. Nested mixed model ANOVA's (PROC GLM) were conducted to examine the effect of the treatment on the germinating seedlings and to identify local adaptation to salt and Mn^{++} toxicity in the roadside populations. We had three treatments; distilled water, salt solution, and Mn^{++} solution. However, for clarity, we have included the salt and distilled water data in one analysis to measure genetic variance for salt tolerance, and then the Mn^{++} and distilled water data in a separate analysis to measure genetic variance for Mn^{++} tolerance. The experimental design is composed of a hierarchical structure of maternal families within populations and populations within sites, so nested mixed model analyses were done. Population, maternal family and their interactions are considered random variables, while site and the treatment are considered fixed. The block was not included in these analyses, because we found no effect of block within the control treatment of *D. sylvestris*. Though there was a significant effect of block in the Mn^{++} and salt treatments, it is likely the result of slight variation in the solutions and so does not provide any additional meaningful information.

To aid in interpreting the mixed model ANOVAs, we carried out within treatment ANOVAs with family nested in population, and population nested within site (PROC GLM). By excluding the interactions in the model, we are able to examine variation in root growth within the treatments. The correlation between the family means for root length in NaCl solution and in $MnSO_4$ solution was used as an estimate of the genetic correlations for tolerance to salt and to Mn^{++} (Via 1984) (PROC CORR).

2.6.6.2 ***Seed Characteristics***

Nested mixed model analyses of variance were conducted for each species to examine differences in seed mass and Na^+ concentration of the seed leachate between sites, populations and maternal families (PROC GLM). Correlations between the seed mass and salt tolerance, Mn^{++} tolerance and root length in the control solution were conducted (PROC CORR). To determine if the roadside and oldfield populations differed in the relationship between seed mass and salt tolerance, Mn^{++} tolerance and root length in the control solution an ANCOVA was conducted (PROC GLM). Correlations between the Na^+ concentration of the seed leachate and salt tolerance were conducted (PROC CORR). To determine if the roadside and oldfield populations differed in the relationship between Na^+ concentration of the leachate and salt tolerance, an ANCOVA was conducted (PROC GLM).

2.7 RESULTS

2.7.1 *Salt and Mn^{++} Tolerance*

2.7.1.1 **Dipsacus sylvestris**

Root length was reduced in the salt treatment compared to the control (Table 2.1, Figure 2.1a). On average, oldfield populations performed better than roadside populations over both treatments (Table 2.1). There was also significant variation in performance averaged over both treatments among maternal families but not among populations (Table 2.1).

However, there were significant interactions between the site, population and maternal family and the salt treatment (Table 2.1). In the control treatments, seeds collected from roadside populations had significantly shorter roots than seeds collected from oldfield populations (Figure 2.1a and b). Within the control treatment, there was also significant variation in root length among populations and among maternal families within populations (Table 2.2). In the salt treatment, root length did not vary between sites or among populations, though there was among family variation (Table 2.2). Seeds of all 15 maternal families from the oldfield populations experienced a decline in root growth with the addition of the salt treatment (Figure 2.1a). In contrast, seeds from five of the maternal families from the three roadside populations had longer roots in the salt treatment than in the control treatment (Figure 2.1a). The roadside families that did have shorter roots in the salt treatment did not suffer the same degree of decline as the oldfield families (Figure 2.1a).

Seeds collected from both roadside and oldfield populations had shorter roots when grown in the Mn^{++} treatment (Table 2.1; Figure 2.1b). Seeds from the roadside population performed better overall, but there was no overall variance in performance among populations or among families within populations.

However, there was genotype×environment interaction at the levels of the site and the maternal family (Table 2.1, Figure 2.1b). Within the Mn^{++} treatment, root length did not differ between sites or among populations, though there were significant differences among maternal families (Table 2.2). The significant Mn^{++}×site interaction can be attributed to the significantly greater root length of oldfield seedlings in the control treatment and the lack of site differences for root length in the Mn^{++} treatment (Table 2.2; Figure 2.1b). Though root length declined in Mn^{++} for all families, the significant Mn^{++}×maternal family interaction suggests potential genetic variance for tolerance to Mn^{++}.

2.7.1.2 Hesperis matronalis

Seeds from both the oldfield and roadside populations had shorter roots when grown in the salt treatment (Table 2.1; Figure 2.2a). There was no difference between the performance of oldfield and roadside sites averaged over both treatments (Table 2.1). There was significant variation in average performance among populations but no difference in average performance among maternal families (Table 2.1) across both treatments.

There were no genotype×environment interactions at any level with salt, indicating a lack of genetic variance for salt tolerance (Table 2.1; Figure 2.2a). Within the control treatment, there were no significant differences between sites, among populations, or among families in root length (Table 2.2, Figure 2.2a), though oldfield families tended to have longer average root lengths than roadside families. Within the salt treatment, there was significant variation in root length between sites and among maternal families, though not among populations (Table 2.2; Figure 2.2a). Seeds collected from both the oldfield and roadside populations had highly reduced growth in the salt treatment (Figure 2.2a), with the exception of one oldfield family with exceptionally high root length in the salt treatment.

Seeds from both oldfield and roadside populations had shorter roots in the Mn^{++} treatment than in the control (Figure 2.2b). On average, there was no difference in the performance of oldfield and roadside populations over both treatments (Table 2.1). Nor was there any significant variation in overall performance among maternal families or populations (Table 2.1).

Though there was no site×Mn^{++} interaction, there were significant interactions of Mn^{++} with the population and the maternal families (Table 2.1). Within the Mn^{++} treatment, there was significant variation in root length at the population and maternal family levels, but not at the site level (Table 2.2). Seeds from two maternal families collected from roadsides did not have the same large reduction in root length as the rest of the families (Figure 2.2b), indicating variation in Mn^{++} tolerance during germination.

2.7.1.3 Asclepias syriaca

Seeds from both roadside and oldfield populations had shorter roots in the salt treatment than in the control treatment (Figure 2.3a). On average, there was no difference in the performance of oldfield and roadside populations over both treatments (Table 2.1). Nor was there any significant variation in overall performance among maternal families or populations (Table 2.1).

There was a significant interaction between the maternal family and the salt treatment (Table 2.1), but no interactions between salt and population or site. Within the control treatment, significant variation in root length existed at the maternal family level but not at the site or population levels (Table 2.2). Within the salt treatment, the only significant variation was also among maternal families. Though all families had declining root length in salt, the salt×family variation indicates that there was variation in how much families were impacted by the addition of salt. However, there was no indication that roadside populations were more adapted to high salt levels.

Seeds from both roadside and oldfield populations had shorter roots in the Mn^{++} treatment than in the control treatment (Table 2.1; Figure 2.3b). There was no overall variation in root length at the level of the site or population (Table 2.1), though there was among maternal family variation.

There was a genotype×Mn^{++} interaction only at the level of the maternal family (Table 2.1, Figure 2.3b). Within the Mn^{++} treatment, there was no difference between oldfield and roadside sites or among populations in root length (Table 2.2). However, there was significant variation in root length among maternal families within the Mn^{++} treatment (Table 2.2). All families had reduced growth in Mn^{++} . These results indicate that there was apparent genetic variation within both the oldfield and roadside populations and that this variation included differences in tolerance to Mn^{++}. However, there was no indication that roadside populations were more tolerant of high Mn^{++} levels.

2.7.2 *Seed Characteristics*

2.7.2.1 **Dipsacus sylvestris**

There was variability in the mass of *D. sylvestris* seeds at the population and maternal family level, but no difference between sites (Table 2.3; Figure 2.4). However, seeds collected from roadside populations had significantly higher Na^+ concentrations in their leachate than seeds collected from oldfields (Table 2.3; Figure 2.5). There was no variability in Na^+ content at the population or maternal family level. There were striking differences between sites in the relations between family means for seed mass and salt tolerance, Mn^{++} tolerance and root length in the control treatment (Table 2.4; Figure 2.4). In the control treatment, the relationship between seed mass and root length differed between sites (Table 2.4); seeds collected from roadside populations displayed a negative relationship between seed mass and root length (i.e. larger seeds grew shorter roots than smaller seeds (Figure 2.4a), while there was a slight positive relationship between seed size and root length in seeds collected from oldfield populations. The relationship between seed mass and salt tolerance was significantly different between sites (Table 2.4); seed mass was more positively associated with salt tolerance in seeds collected from roadsides than from oldfields (Figure 2.4b). Though seed mass was positively associated with Mn^{++} tolerance in families collected from both sites (Figure 2.4c), the relationship was stronger in families collected from roadside populations (Figure 2.4c; Table 2.4). The relationship between family means for Na^+ concentration of the seed leachate and salt tolerance differed between sites (Table 2.5) with families from roadsides showing a strong positive correlation contrasted with the lack of relationship in the familes collected from oldfield populations.

2.7.2.2 Hesperis matronalis

There was variability in the seed mass of *H. matronalis* seeds at the population and maternal family level, but no difference between sites (Table 2.3). Nor was there any variability at the site, population, or maternal family level in the Na^+ concentration of the seed leachate (Table 2.3). Seed mass was not significantly correlated with root length in the control ($r = 0.1039$, $p = 0.6920$). Nor were there significant correlations between seed mass and salt tolerance ($r = 0.04672$, $p = 0.7385$) or Mn^{++} tolerance ($r = 0.28666$, $p = 0.1500$). Seeds collected from the two sites did not differ in the relationship between seed mass and salt tolerance ($F = 0.0$, $p = 0.9969$), Mn^{++} tolerance ($F = 4.15$, $p = 0.0609$), and root length in the control (F = 0.99, p = 0.3363)(Table 4). The Na^+ concentration of the seeds was not related to salt tolerance (r = -0.32876, p = 0.2511) (Table 2.5).

2.7.2.3 Asclepias syriaca

There was a high level of variability in seed mass and Na^+ concentration of the seed leachate at the maternal family level but not at the level of the population or site (Table 2.3). Seed mass was not significantly correlated with root length in the control (r = 0.32077, p = 0.1039). Nor were there significant correlations between seed mass and salt tolerance (r = 0.04672, p = 0.8063) or Mn^{++} tolerance (r = 0.28666, p = 0.1246). Seeds collected from the two sites also did not differ in the relationship between seed mass and salt tolerance (F = 1.98, p = 0.1714), Mn^{++} tolerance (F = 2.71, p = 0.1117) and root length in the control (F = 0.23, p = 0.6351) (Table 4). The Na^+ concentration of the seeds was not related to salt tolerance (r = -0.08352, p = 0.6608) (Table 5).

2.8 DISCUSSION

Here we asked whether evolutionary responses for tolerance to high salinity and tolerance to high Mn^{++} could be found in three common roadside species. Though we did find evidence of the evolution of tolerance, we found irregular patterns both between species and between stresses. *A. syriaca* displayed variation for tolerance (i.e. genotype × environment interaction) to both stresses at the maternal family level, but roadside populations showed no greater adaptation to either stress than oldfield populations. *Hesperis matronalis* displayed variability for tolerance to Mn^{++} at the maternal family and population level, though there was no evidence of variance in tolerance to salinity. For this species, roadside populations did exhibit greater tolerance to high Mn^{++} levels than did oldfield populations. *Dipsacus sylvestris* displayed variation for tolerance to both stresses at the maternal family level and the population level. However, while roadside populations showed greater tolerance to high salinity, neither roadside nor oldfield plants grew well in high Mn^{++} levels. We speculate that maternal environmental effects, the ecology and life history of the species, the genetic structure of roadside populations and the complex nature of the roadside environment have each played a significant role in the observed tolerances. The extent to which a population has adapted to the roadside environment has implications for the potential for roadsides to act as a refuge for native prairie plants or as a dispersal corridor between isolated habitat fragments.

It is striking that it was only the exotic species, *D. sylvestris* and *H. matronalis*, that demonstrated evolutionary change. Successful exotic species tend to possess several common life history characteristics (i.e. short generation time, large number of seeds, self-compatibility, etc. (Baker, 1965)). Such characters may also increase the rate of evolution. Our results are consistent with the argument of (Lee 2002) that successful exotic species are those that evolve rapidly in response to novel selection pressures.

Asclepias syriaca did not display adaptation to either stress despite the presence of significant among family variation in root length in each of the three treatments. This species differs from the other two species in two critical ways beyond that it is native to North America; it is a perennial, and it is not restricted to reproduction by seed (Bhowmik and Bandeen 1976). Perennial species have a longer generation time than annuals or monocarpic perennials like *H. matronalis* and *D. sylvestris*. *Asclepias syriaca* is able to reproduce by adventitious root buds, and we have observed that in the field seedling recruitment is low (Chapter 4). Consequently, the majority of plants found in roadside communities may be part of long-lived clones. These life history characteristics, though they buffer species against variation in the environment, may limit the rate at which a population is able to adapt to new environmental conditions.

Macnair hypothesized that adaptations to soil contamination resulting from anthropogenic activity are the result primarily of major gene changes (Macnair 1991). The restricted distribution of the Mn^{++} tolerance in roadside families of *H. matronalis* implies that the adaptation may be due to a gene of major effect. Two families of one roadside population are clearly adapted to high Mn^{++} levels in the soil. Within families, individuals showed either high tolerance or low tolerance with an absence of intermediate values. If the adaptation was polygenic, a gradient of genotypes from highly tolerant to non-tolerant is expected. If the selection pressure for Mn^{++} tolerance is high enough, this trait should spread fairly rapidly both within and between roadside populations.

Because we worked with field collected seeds, we observed tolerances expected to be expressed in the maternal environment but these tolerances comprise both genetic traits of the seedlings and traits imposed by the maternal environment. The best known maternal environmental effects result from resource partitioning. A plant in a favorable environment can allocate more resources to its offspring, increasing their chances of survival, while a plant inhabiting a poor environment will produce seeds of inferior quality (Roach and Wulff 1987). Despite the high nitrogen levels of roadside soils, the many other environmental stresses of the roadside environment should render roadsides a poor environment. As expected, both *H. matronalis* and *D. sylvestris* seeds collected from roadside populations had shorter roots in the control treatment than did seeds collected from oldfield populations, though this difference was only significant for *D. sylvestris*. However, *A. syriaca* showed no difference in performance between roadside and oldfield populations in distilled water. These results are consistent with deleterious effects of the maternal environment lowering the fitness of the roadside seeds for the two exotic species.

Dipsacus sylvestris was the only species that had high levels of Na^+ in seeds from plants growing in the roadside. Despite the shorter root lengths of *D. sylvestris* seedlings from roadside populations in the control treatment, some maternal families from roadside populations of *D. sylvestris* are clearly adapted to better tolerate high salinity. All oldfield families had drastically reduced growth in the salt treatment. However, some roadside families were not affected by the presence of salt and others actually benefited from the presence of salt. This pattern of increased growth in mild salt is typical of halophytes (Greenway and Munns 1980), though *D. sylvestris* is considered a glycophyte (Ellenberg *et al.* 1991).

We speculate that maternal environment effects may have conferred salt tolerance in roadside populations of *D. sylvestris*, the only species to have accumulated more salt in seeds from the roadside. Seed size (Roach and Wulff 1987) is commonly affected by the maternal environment. Larger *D. sylvestris* seeds collected from the roadside were more salt tolerant than smaller seeds. In the roadside, these larger seeds also contained significantly higher levels of Na^+ than did the smaller seeds. All roadside seeds contained higher levels of Na^+ than did seeds of oldfield populations, indicating a likely maternal environment effect for Na^+ content. It has been suggested that the uptake and storage of Na^+ can enhance germination by creating an osmotic gradient that allows the developing embryo to more easily take up water during germination under saline conditions (Eddleman and Romo 1987). Because salinity tolerance was so highly correlated with both seed size and Na^+ content, traits potentially affected by the maternal environment, the salinity tolerance of roadside populations of *D. sylvestris* may be derived from the environment experienced by the maternal plant. Leaching out salt from roadside seeds eliminated salt tolerance in some families (Chapter 3), a result also consistent with the hypothesis of maternal environment induced adaptation.

In *D. sylvestris,* the significant interaction between the site and Mn^{++} treatment appears to be the result of better performance by oldfield populations in distilled water, rather than increased Mn^{++} tolerance in roadside populations. Although there was among family variation in the Mn^{++} treatment, there was no difference between roadside and oldfield populations. Moreover, we did not see any families perform better in Mn^{++} than distilled water, though some families were not as greatly affected by Mn^{++} as were others.

Like others who have used this method (Shu *et al.* 2002, Zalecka and Wierzbicka 2002), we use root length as a measure of seedling performance. However, it has been suggested that lower relative growth rates are an adaptation to poor quality habitats (Chapin *et al.* 1993). Growing more slowly reduces carbon demands allowing resources to be diverted to other activities (Chapin *et al* 1993) (i.e. salinity tolerance). A study of *Sonneratia alba* and *S. lanceolata* (Ball and Pidsley 1995) indicated that salt tolerance is acquired at the cost of reduced growth. The more salt tolerant species, *S. alba* was 50 % smaller under ideal (i.e. low salinity) growth conditions than the less salt tolerant species *S. lanceolata*. Consequently, it could be argued that the lower root lengths of seedlings from roadside populations of *D. sylvestris* may itself be an adaptation. However, the vulnerability of seedlings to drought and other stresses would argue that rapid growth in the seedling stage must be strongly selected.

Previous studies of the effects of Mn^{++} and salt on growth during the adult and reproductive phases of life of *H. matronalis* showed that though salt affected the size of the plants, high Mn^{++} levels did not. This supports the idea that the seedling stage of development is the most sensitive to environmental stresses. High Mn^{++} levels severely reduced the root length of all three species. Seeds collected from roadside populations had reduced growth in control solutions in both *D. sylvestris* and *H. matronalis*. *Hesperis matronalis* grown from seeds collected in the roadside were bigger as rosettes than plants grown from seeds collected from oldfields (Rothfels *et al.* 2002). Selection pressures and the effects of the maternal environment are likely different during germination and seedling establishment than during the adult life of plants growing in the roadside.

Several studies of local adaptation to mine tailings and other severe stresses demonstrate that strong selection is a barrier to successful migration of less adapted genotypes (Antonovics and Bradshaw 1970, McNeilly and Antonovics 1968). In those studies, metal tolerant populations showed superior performance to metal intolerant populations for both the stressful and favourable environments. Such selection pressures would act as a 'selection filter', allowing only those genotypes capable of adapting to roadside conditions to successfully disperse through roadside corridors. In this study, however, for the exotics, though oldfield seeds performed better than roadside in the favourable environment, both performed equally well in the stressful environments. This argues for a one-way selection filter, such that oldfield genotypes can move readily into roadsides, but roadside genotypes will be excluded from the oldfield. However, for *D. sylvestris*, the fate of oldfield genotypes in the next generation depends on whether the salinity tolerance and seed quality (i.e. performance in favorable conditions) are maternal environment or genetic, e.g. if tolerance is genetic, and maternal environment results in poor quality seeds, then descendants of oldfield plants will not persist. However, if tolerance is induced by the environment, then descendants of oldfield plants could be well adapted to the novel roadside environment.

If local adaptation does not evolve, as in *A. syriaca*, dispersal and gene flow between roadside populations and oldfield populations should occur unimpeded. Moreover, populations dispersing through roadside corridors would not be impacted by any selection pressures as they move between habitat fragments. However, we speculate that an inability to adapt to the roadside environment could limit the future abundance of a species in the roadside. As other species continue to adapt to roadside environmental conditions, they could become competitively superior to those species that did not adapt to the novel selection pressures, eventually resulting in competitive exclusion. If this does occur, long-lived native species that reproduce asexually may be found less often in roadsides, while rapidly evolving exotics become more predominant. While roadsides could potentially act as both habitat and corridor for native species, highly adaptable exotic species will likely remain the dominant vegetation along roadsides unless extensive management in undertaken.

ACKNOWLEDGEMENTS

We would like to thank the Royal Botanical Gardens in Hamilton, Ontario for allowing seed collection on their conservation lands, Sid Lang at the Ontario Ministry of Transportation for help in obtaining permission to work on highway verges, Mary DeGuzman for assistance in seed collection and David Guevera and Maria Sanchez for field and laboratory assistance.

Table 2.1: Nested mixed model analyses of variance of the effects of maternal family nested within population and site, population nested within site, and site on the root length of *D. sylvestris* seeds, the log of the root length of *H. matronalis* and the log of the seedling length of *A. syriaca* seeds germinated in salt, Mn^{++} and control treatments. Twenty seeds from each maternal family were exposed to each treatment. Separate analyses were done for salt compared to the control and Mn^{++} compared to the control. Seeds that did not germinate are not included in the analysis. Treatment and site are considered fixed effects, while population and maternal family and their interactions are considered random effects. The degrees of freedom for maternal family, maternal family×treatment and error are indicated in parentheses below the mean square. (T = treatment; S = site; P = population; M = maternal family).

			D. sylvestris			
			Salt vs. Control		Mn vs. Control	
Source	df	Mixed- model Denominator	Mean Square	*F*	Mean Square	*F*
T	1	$MS_{T\times P}$	100.47	26.69 **	685.11	166.17 ***
S × T	1	$MS_{T\times P}$	105.52	28.03 **	119.70	29.03 **
S	1	MS_{P}	135.04	18.03 *	120.55	23.89 **
P	4	$MS_{T\times P} + MS_{M} - MS_{T\times M}$	7.49	1.27	5.05	1.25
P × T	4	$MS_{T\times M}$	3.77	2.81 *	4.12	2.74 †
M		$MS_{T\times M}$	3.50 (24)	2.60 *	1.41 (24)	0.93

M × T	MS_E	1.34 (24)	2.57 ***	1.51 (24)	3.78 ***
Error		0.52 (1091)		0.40 (1094)	

*† P < 0.1; * P < .05; ** P < 0.01; *** P < 0.001.*

Table 2.1 Continued

			H. matronalis			
			Salt vs. Control		Mn vs. Control	
Source	df	Mixed- model Denominator	Mean Square	*F*	Mean Square	*F*
T	1	$MS_{T\times P}$	180.68	1714.15 ***	141.49	85.73 ***
S × T	1	$MS_{T\times P}$	0.02	0.21	3.49	2.11
S	1	MS_P	7.76	6.62 †	1.82	5.30 †
P	4	$MS_{T\times P} + MS_M - MS_{T\times M}$	1.22	5.18 *	0.34	0.21
P × T	4	$MS_{T\times M}$	0.07	0.26	1.90	3.44 *
M		$MS_{T\times M}$	0.52	1.78 †	0.49	0.79
			(24)		(24)	
M × T		MS_E	0.29	1.03	0.63	1.68 *
			(19)		(22)	
Error			0.28		0.38	
			(287)		(320)	

*† $P < 0.1$; * $P < .05$; *** $P < 0.001$.*

Table 2.1 Continued

			A. syriaca			
			Salt vs. Control		Mn vs. Control	
Source	df	Mixed- model Denominator	Mean Square	*F*	Mean Square	*F*
T	1	$MS_{T\times P}$	1002.31	690.38 ***	189.91	583.30 ***
S × T	1	$MS_{T\times P}$	0.39	0.27	0.76	2.34
S	1	MS_P	0.71	0.14	0.41	0.14
P	4	$MS_{T\times P} + MS_M - MS_{T\times M}$	5.22	2.24	2.97	1.52
P × T	4	$MS_{T\times M}$	1.46	0.78	0.32	0.52
M		$MS_{T\times M}$	2.80 (24)	1.47	2.29 (24)	3.66 **
M × T		MS_E	1.90 (24)	3.25 ***	0.63 (24)	1.56 *
Error			0.58 (703)		0.40 (808)	

*$P < .05$; ** $P < 0.01$; *** $P < 0.001$.*

Table 2.2: Nested analyses of variance of the effects of maternal family nested within population and site, population nested within site and site on the root length of *D. sylvestris* seeds, the log of the root length of *H. matronalis* seeds, and the log of the seedling length of *A. syriaca* seeds germinated in70 Mm NaCl (NaCl), 3 Mm $MnSO_4$ (Mn), and distilled water (Control) treatments. Twenty seeds from each maternal family were exposed to each treatment. Seeds that did not germinate are not included in the analysis. Site is considered a fixed effect, while population and maternal family are considered random effects. The degrees of freedom for the error and the maternal family are indicated in parentheses below the mean square. (P = population; M = maternal family).

			D. sylvestris					
			Control		NaCl		Mn	
Source	df	Denominator Mean square	Mean Square	*F*	Mean Square	*F*	Mean Square	*F*
Site	1	MS_P	236.44	29.02 **	0.922	0.28	0.00	0.00
Population	4	MS_M	8.16	3.67 *	3.24	1.25	0.99	1.46
Maternal Family		MS_E	2.25 (24)	3.63 ***	2.59 (24)	6.08 ***	0.68 (24)	3.69 ***
Error			0.62 (542)		0.43 (549		0.18 (552)	

† P < .05; ** P < 0.01; *** P < 0.001.*

Table 2.2: Continued

			H. matronalis					
			Control		NaCl		Mn	
Source	df	Denominator Mean square	Mean Square	*F*	Mean Square	*F*	Mean Square	*F*
Site	1	MS_P	2.02	6.18 †	0.29	12.47 **	0.32	0.56
Population	4	MS_M	0.34	1.85	0.02	0.53	0.77	5.59 **
Maternal Family		MS_E	0.21 (23)	1.44	0.05 (20)	2.33 **	0.15 (23)	2.03 **
Error			0.15 (136)		0.02 (184)		0.07 (184)	

*† $P < 0.1$; ** $P < 0.01$.*

Table 2.2: Continued

			A. syriaca					
			Control		NaCl		Mn	
Source	df	Denominator Mean square	Mean Square	*F*	Mean Square	*F*	Mean Square	*F*
Site	1	MS_P	0.00	0.00	0.12	0.18	0.64	0.82
Population	4	MS_M	1.37	0.97	0.65	1.63	0.78	1.44
Maternal Family		MS_E	0.723 (24)	3.02 ***	0.41 (24)	5.03 ***	0.55 (24)	4.67 ***
Error			0.24 (386)		0.12 (422)		0.08 (317)	

*** $P < 0.001$.

Table 2.3: Nested analyses of variance of the effects of maternal family nested within population and site, population nested within site, and site on the mass and the Na^+ concentration of the leachate of *D. sylvestris*, *H. matronalis* and *A. syriaca* seeds. Site is considered a fixed effect, while population and maternal family are considered random effects. The degrees of freedom for the error and the maternal family are indicated in parentheses below the mean square. (S= site; P = population; M = maternal family; Mass = seed mass; Na^+ = Na^+ concentration of the seed leachate).

			D. sylvestris				*H. matronalis*			
			Mass		Na^+		Mass		Na^+	
Source	Mixed – model Denominator	df	Mean Square	*F*	Mean Square	*F*	Mean Square	*F*	Mean Square	*F*
S	MS_P	1	1290.4	1.02	367.78	18.50 *	426.7	1.48	14.51	0.42
P	MS_M	4	1261.0	5.69 **	19.85	0.68	298.8	7.40 ***	34.14	1.36
M	MS_E		221.6 (18)	35.63 ***	29.25 (15)	1.71 †	54.0 (13)	15.15 ***	25.16 (11)	1.95 †
Error			6.2 (216)		17.13 (42)		3.6 (136)		12.90 (31)	

† $P < 0.1$; * $P < .05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2.3 Continued

			A. syriaca			
			Mass		Na^+	
Source	Mixed – model Denominator	df	Mean Square	*F*	Mean Square	*F*
S	MS_P	1	1171.8	1.10	1081.60	1.46
P	MS_M	4	1065.0	1.01	741.82	0.91
M	MS_E		1053.0 (24)	130.28 ***	815.48 (24)	152.59 ***
Error			8.1 (270)		5.34 (60)	

*† $P < 0.1$; *** $P < 0.001$.*

Table 2.4: Analyses of covariance of the effects of site and seed mass on the salt tolerance, Mn^{++} tolerance, and root length (or seedling length for *A. syriaca*) after 10 days in the control treatment of germinating *D. sylvestris*, *H. matronalis* and *A. syriaca* seeds. Salt and Mn^{++} tolerance are calculated by dividing the average root length in the treatment solution by the average root length in the control solution. The degrees of freedom for the error is indicated in parentheses below the mean square. (S = site; SM = seed mass)

		D. sylvestris						*H. matronalis*					
		Salt Tolerance		Mn Tolerance		Control Root Length		Salt Tolerance		Mn Tolerance		Control Root Length	
Source	df	Mean Square	*F*	Mean Square	*F*	Mean Square	*F*	Mean Square	*F*	Mean Square	*F*	Mean Square	*F*
S	1	0.48	16.24 ***	0.21	21.56 ***	0.20	2.32	0.00	0	0	0.03	2.52	0.06
SM	1	0.04	1.40	0.02	2.40	0.20	2.32	0.00	0	0	0.03	2.10	0.46
S×SM	1	0.13	4.36 *	0.05	4.95 *	0.98	11.18 ***	0.00	0	0	0.06	4.15 †	0.94
Error		0.03 (23)		0.01 (23)		0.09 (20)		0.00 (12)			0.01 (14)		0.94 (14)

*† P < 0.1; * P < .05; *** P < 0.001.*

Table 2.4: Continued

		A. syriaca					
		Salt Tolerance		Mn Tolerance		Control Root Length	
Source	df	Mean Square	*F*	Mean Square	*F*	Mean Square	*F*
S	1	0.00	0.52	0.01	0.78	4.92	2.92
SM	1	0.01	1.69	0.02	2.05	0.32	0.19
S×SM	1	0.01	1.98	0.03	2.71	0.39	0.23
Error		0.01 (26)		0.01 (26)		1.68 (26)	

Table 2.5: An analysis of covariance of the effects of site and the Na$^+$ concentration of the seed leachate on the salt tolerance of germinating *D. sylvestris*, *H. matronalis* and *A. syriaca* seeds. Salt tolerance is calculated by dividing the average root length (seedling length in *A. syriaca*) in the treatment solution by the average root length in the control solution. The number of degrees of freedom for the error is indicated in parentheses below the mean square. (Na$^+$ = Na$^+$ concentration of the seed leachate).

		D. sylvestris		*H. matronalis*		*A. syriaca*	
Source	df	Mean Square	*F*	Mean Square	*F*	Mean Square	*F*
Na$^+$	1	0.176	5.20*	0.002	0.97	0.004	0.68
Site	1	0.165	4.87*	0.000	0.03	0.005	0.75
Na$^+$ × Site	1	0.250	7.38*	0.000	0.10	0.004	0.61
Error		0.034 (17)		0.017 (10)		0.006 (26)	

$P < .05$.

FIGURE 2.1: Root length after 10 days of germinating *D. sylvestris* seeds collected from oldfields and roadsides and placed in (a) high salinity (70 Mm NaCl), (b) high Mn^{++} (3 Mm $MnSO_4$), and control (distilled water) solutions. Linear connections represent the averages from each maternal family. Dashed lines and ○ represent oldfield populations, solid lines and ● represent roadside populations.

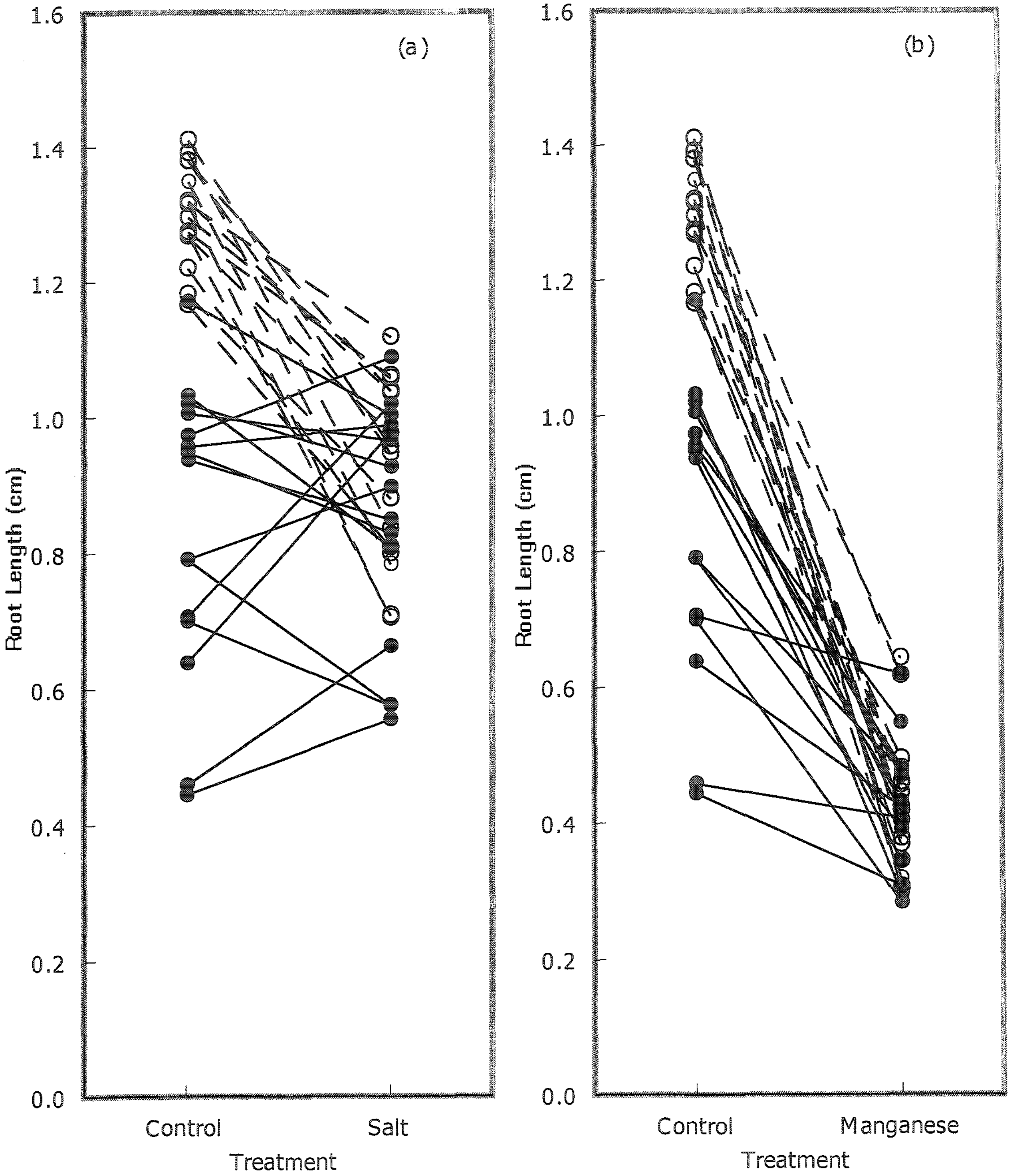

FIG 2.1 BEATON, L. L. and DUDLEY, S. A. 2004

FIGURE 2.2: Root length after 10 days of germinating *H. matronalis* seeds collected from oldfields and roadsides and placed in (a) high salinity (70 Mm NaCl), (b) high Mn^{++} (3 Mm $MnSO_4$), and control (distilled water) solutions. Linear connections represent the averages for each maternal family. Symbols in the control treatment with no lines attached to them had zero seeds germinating in either the salt or Mn^{++} treatment. Dashed lines and ○ represent oldfield populations, solid lines and ● represent roadside populations.

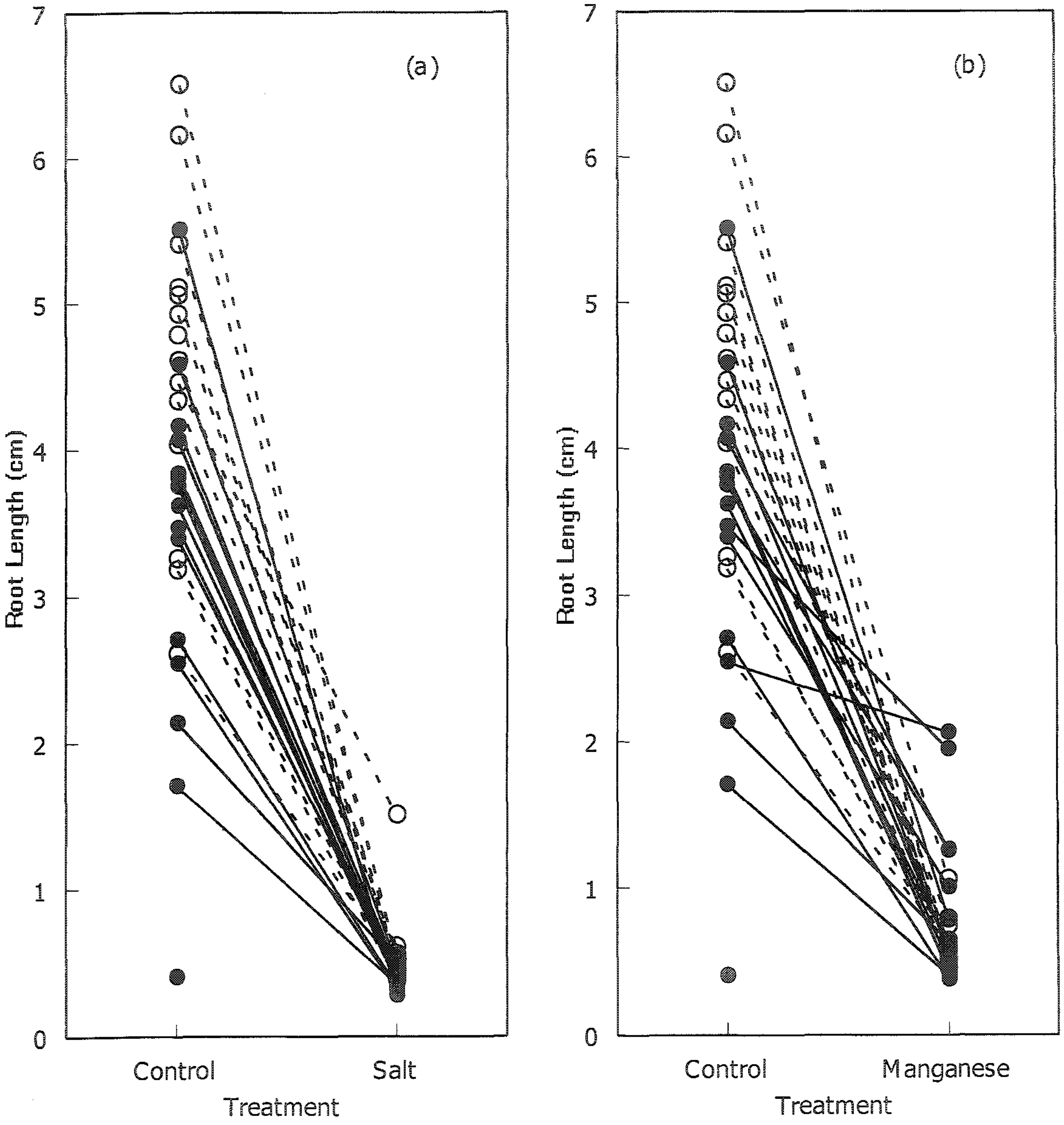

FIG 2.2 BEATON, L. L. and DUDLEY, S. A. 2004

FIGURE 2.3: Root length after 10 days of germinating *A. syriaca* seeds collected from oldfields and roadsides and placed in (a) high salinity (70 Mm NaCl), (b) high Mn^{++} (3 Mm $MnSO_4$), and control (distilled water) solutions. Linear connections represent the averages for each maternal family. Dashed lines and ○ represent oldfield populations, solid lines and ● represent roadside populations.

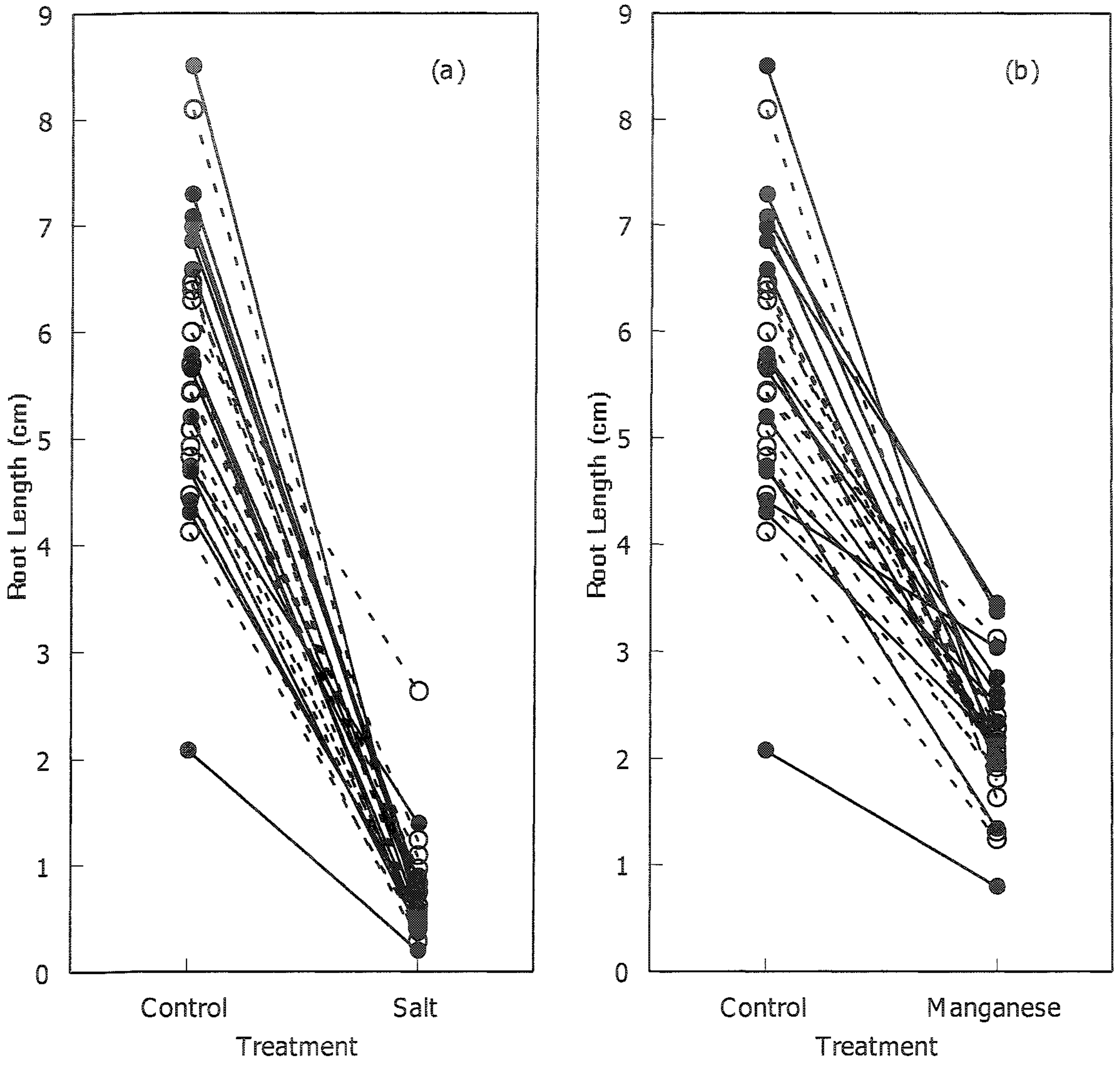

FIG 2.3 BEATON, L. L. and DUDLEY, S. A. 2004

FIGURE 2.4: Scatter plot of average maternal family seed mass versus (a) the average root length after 10 days in distilled water, (b) the average salt tolerance, and (c) the average Mn^{++} tolerance of germinating *D. sylvestris* seeds collected from oldfields and roadsides. Salt tolerance and Mn^{++} tolerance are calculated by dividing the average root length in the treatment solutions by the average root length in the control treatments. Each symbol represents the mean of one maternal family. Dashed lines and ○ represent oldfield populations, solid lines and ● represent roadside populations.

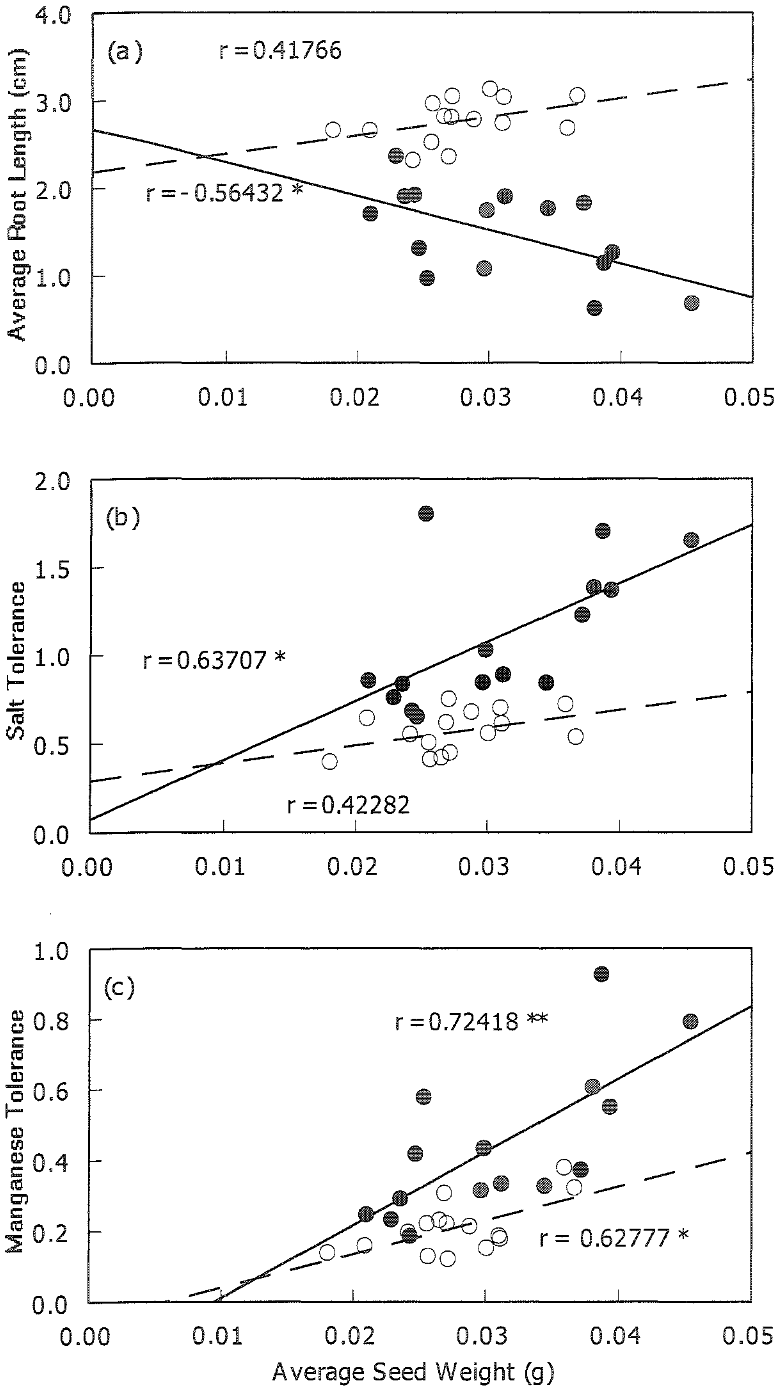

FIG 2.4 BEATON, L. L. and DUDLEY, S. A. 2004

FIGURE 2.5: Scatter plot of the average maternal family salt tolerance versus average Na^+ concentration of the leachate of *D. sylvestris* seeds collected from roadsides and oldfields. Salt tolerance is calculated by dividing the average root length in the salt solution by the average root length in the control solution. Each symbol represents the mean of one maternal family. Dashed lines and ○ represent oldfield populations, solid lines and ● represent roadside populations.

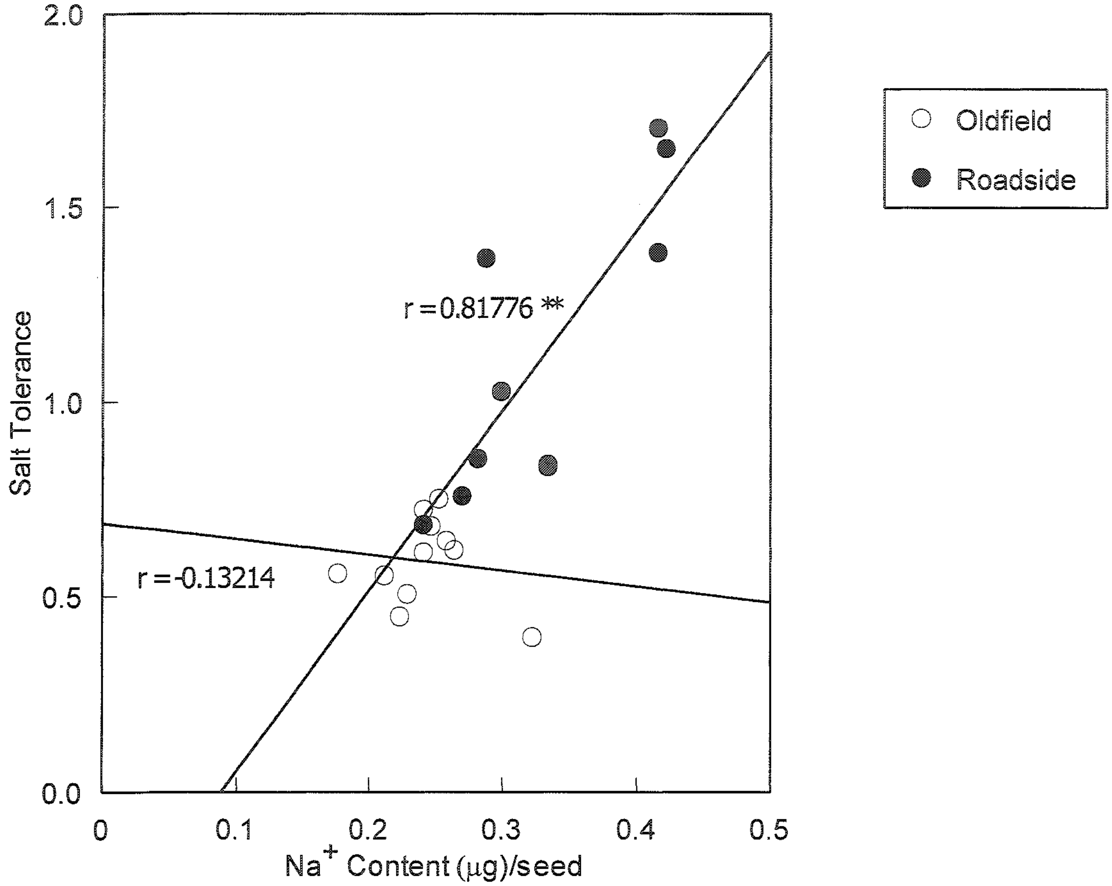

FIG 2.5 BEATON, L. L. and DUDLEY, S. A. 2004

SECTION III

IMPACT OF SOLUTE LEACHING ON THE SALT TOLERANCE DURING GERMINATION OF A COMMON ROADSIDE PLANT *DIPSACUS SYLVESTRIS* HUDS.

3

The impact of solute leaching on the salt tolerance during germination of a common roadside plant Dipsacus sylvestris *Huds.*

(Beaton, L. L. and Dudley, S. A. 2004. A manuscript prepared for publication in Functional Ecology)

3.1 CLARIFICATION OF CONTRIBUTIONS for Beaton and Dudley (2004)

In the following study, I was responsible for proposing the idea, designing and conducting the experiments, analyzing the data and writing the manuscript. Throughout the process, I consulted S.A. Dudley on methodology and statistical analysis and received considerable editorial commentary, which significantly improved the manuscript.

3.2

The impact of solute leaching on the salt tolerance during germination of a common roadside plant *Dipsacus sylvestris (HUDS.)*

by

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In review in Functional Ecology.

BEATON, L. L. and DUDLEY, S. A. 2004. The impact of solute leaching on the salt tolerance during germination of a common roadside plant *Dipsacus sylvestris*

3.3 ABSTRACT

In *Dipsacus sylvestris* (Huds.), seeds collected from roadside plants have higher salt tolerance during germination than seeds from oldfield plants. Several families even demonstrated enhanced growth under saline conditions. The salt tolerance is correlated with seed sodium ion (Na^+) content (Beaton & Dudley 2004). We hypothesized that Na^+ deposition in the seeds may maintain low osmotic potential during germination, so, germinating seeds avoid the osmotic stress imposed by salinity in the roadside. To determine the role of the high Na^+ levels, we leached the soluble components from seeds from both oldfields and roadsides with running water. We compared the salt tolerance of leached seeds to that of unleached seeds. Performance was measured as root length after ten days. As in the previous study, seeds from roadside families showed higher salt tolerance than oldfield seeds and in some cases enhanced growth in high salinity. For oldfield families, leaching the seeds prior to salt exposure had no impact on salt tolerance. For roadside families, leaching the seeds prior to salt exposure caused some families to lose salt tolerance while salt tolerance of other families was unaffected. The results indicated that roadside populations of *D. sylvestris* may possess two distinct salt tolerance strategies. Several roadside families lost their salt enhanced growth following leaching. However, they did not have higher Na^+ levels than oldfield families, suggesting that the seed composition allows them to tolerate the osmotic stress of high salinity but not necessarily through a maternal environmental effect.

ion toxicity, local adaptation, maternal environmental effect, osmotic stress, seed composition

3.4 INTRODUCTION

In the spring, many roadside soils in temperate regions have high salt concentrations resulting from the use of de-icing salts (Thompson *et al.,* 1986). During germination and seedling establishment, plants are much more sensitive to high salinity than during adult stages (Gorham, 1992). In addition to causing ion toxicity, oxidative stress (Gueta-Dahan *et al.,* 1997) and/or nutrient deficiency (Liu & Zhu, 1997) high soil salinity increases the osmotic potential of the soil, making it difficult for plants to take up water, which leads to osmotic stress (Gorham, 1992). Consequently, high soil salinity can be expected to be a strong selection pressure for roadside plant populations.

In a previous study, we compared growth during germination under saline conditions for roadside and oldfield maternal families of *Dipsacus sylvestris* (Huds.). In this study, all maternal families from oldfields were found to be intolerant of salt. In contrast, some maternal families from the roadsides were tolerant of high salinity during germination, while others actually exhibited enhanced growth in salt (Beaton & Dudley, 2004). A very intriguing result of this study was that the more tolerant and salt-enhanced families also showed high levels of Na^+ in the seeds, a character which is presumably affected by the salinity of the maternal environment.

The environment experienced by the maternal plant may influence the phenotype of the seeds and seedlings. Such maternal environmental effects aremost commonly attributed to maternal plant determination of the amount and quality of resources provided to the embryo during germination (though see (Orozco-Segovia *et al.,* 1993). It has frequently been shown that a plant that experiences stressful environmental conditions during seed development will produce smaller seeds of inferior quality compared to a plant inhabiting a more benign environment (Donohue & Schmitt, 1998). In this way, the benign or deleterious effects of the environment experienced by the maternal plant are passed on to the offspring (Roach & Wulff, 1987).

Here we hypothesize that the seed composition, which is partially influenced by the environment experienced by the maternal plant, may have prepared the seedlings for the stressful roadside environment. That is, the salt enhanced growth of *D. sylvestris* seeds collected from roadsides may be due to the accumulation of Na^+ in the seeds resulting from maternal growth in a high Na^+ environment rather than to a genetic trait. When seeds germinate they release a variety of solutes including sugars, organic acids, ions, amino acids and proteins (Bewley & Black, 1994, Simon & Mills, 1983). We observed previously that *D. sylvestris* seeds collected from roadside populations leached out significantly higher levels of Na^+ when placed in distilled water than seeds collected from oldfield populations (Beaton & Dudley 2004). In seeds collected from roadsides, but not oldfields, the amount of Na^+ leached out of the seeds was highly correlated with the ability of the seeds to tolerate high salinity (Beaton & Dudley 2004). Studies of the seeds of some halophytes indicate that the accumulation of Na^+ may aid the seed in tolerating saline environments during germination by creating an osmotic gradient between the seed and its environment (Eddleman & Romo, 1987) allowing the seed to imbibe water more easily; a process called ion enhancement.

Here, we test two predictions from the hypothesis of seed composition conferring salt tolerance. First, if the accumulation of Na^+ in seeds of *D. sylvestris*, considered a glycophyte, leads to salt tolerance and salt enhanced growth in maternal families collected from roadsides, then we predict that the removal of some of the solutes through leaching should reduce adaptation to salt (Fig. 1). Second, we predict that families exhibiting this response should have higher Na^+ levels than other families. Oldfield families, which do not contain elevated levels of Na^+, are included as a control.

In this study, seeds from field-collected maternal families of *D. sylvestris* were either leached under running water for 3 days or placed directly on wet filter paper to imbibe. Seeds were placed in either salt solution or distilled water solutions. Root length was measured after 10 days. We ask the following questions: (1) What is the impact of leaching ions on the root length of germinating seedlings from roadside families?; (2) Does the leaching affect the response to high salinity during germination?; (3) How are these responses related to the salt content of the seeds?

3.5 METHODS

3.5.1 *Study Species*

Dipsacus sylvestris (common teasel), a native of Europe, is found primarily in oldfields and roadsides. A monocarpic perennial, it grows vegetatively for at least one summer as a rosette. In the spring, if the plant has reached a critical size, it will bolt to its reproductive form of an upright flowering stem. Flowering occurs from July to September and seeds are shed throughout the fall. A single plant can produce upwards of 3000 seeds (Werner, 1975).

3.5.2 *Seed Collection*

Six *D. sylvestris* populations (Beaton & Dudley 2004) were chosen (three bordering a major highway (highway 403 between Hamilton and Burlington, Ontario) and three located in oldfields) for study. Highway 403 is a six lane highway carrying 45000 to 75000 automobiles/day (Transportation, 1994). Highway populations were located in full sunlight, 2 - 10 m from the pavement's edge. At all the roadside sites, the surrounding vegetation was primarily various species of grass and/or crown vetch (which is planted following highway construction to stabilize the soil) with a few common oldfield plants (e.g. *Solidago* spp., *Daucus carota*). Oldfield populations were located on Royal Botanical Gardens conservation lands in full sunlight. The surrounding vegetation was dominated by grass with a wide variety of forbs. Seeds were collected from 15 - 20 randomly selected plants from each population. Following collection, seeds were processed and stored in paper envelopes at room temperature.

Five maternal families that differed from those used in previous salt tolerance experiments (Beaton & Dudley 2004), from each population were randomly chosen for experimentation.

3.5.3 *Experimental Design*

Seeds from each of five maternal families from each of the six populations (i.e. fifteen maternal families from the roadside and fifteen maternal families from the oldfield) were placed into one of four treatments: non-leached/distilled water, non-leached/salt, leached/distilled water and leached/salt. Each treatment/seed combination was replicated twice for a total of twenty seeds from each family per treatment.

3.5.3.1 *Salt Treatment*

Seeds were germinated in petri-dishes lined with filter paper and containing either 5 ml of 70 mM NaCl (~12.5 % the salinity of ocean water) solution or 5 ml distilled water. On the third, fifth and seventh day, 2 ml of distilled water was added to each tray to replenish moisture lost to evaporation.

3.5.3.2 *Leaching Treatment*

In the leached treatment, seeds were placed in small bags of tulle under running tap water for the first three days of the experiment. *Dipsacus sylvestris* seeds placed in distilled water begin to germinate after three to four days (unpublished data). The leached seeds did not have any radical growth prior to placement in the petri dishes. Seeds that received the non-leached treatment were placed directly into their treatments in petri dishes lined with filter paper.

3.5.3.3 *Performance*

After ten days, the root length of the emerging seedlings was measured. The root length of the seedling is highly positively correlated with the mass and hypocotyl length of germinating *D. sylvestris* seeds (unpublished data).

3.5.4 *Roadside Soil Na^+ Measurement*

3.5.4.1 *Soil Collection*

At each of the three roadside seed collection sites, five collection locations that were evenly spaced throughout the population and at equal distances from the highway were selected. In early April, 2002, soil samples were collected from each site. An aluminum soil corer was used to collect the top 5 cm of the soil. Any vegetative material collected with the soil was removed from the samples. The samples were stored in plastic resealable bags.

3.5.4.2 *Soil Analysis*

The plastic bags containing the soil samples were opened to allow the soil to dry, after which time, the bags were resealed. The soil samples were ground up using a mortar and pestle and passed through a sieve to remove small stones. 5 g of soil from each sampling location was digested in 100 ml 1 M ammonia acetate. Soil samples were stirred for one hour on a magnetic stir plate and then placed in the refrigerator. After one week, sedimentation had removed enough particulate matter from the solution to allow for Na^+ analysis. Samples were diluted with distilled water by 90-98.25%. The Na^+ content of three replicate samples from each soil solution sample was measured using a Na^+ flame spectrophotometer (Evans Electroselenium Limited).

3.5.5 *Seed Leachate Na^+ Measurement*

The Na^+ content of the seeds was estimated for each maternal family on a separate sample of seeds. Two replicates of ten seeds of each family were placed in 1 ml of distilled water and soaked for 72 hours. The seeds were then removed and the Na^+ concentration of the leachate was measured using a Na^+ flame spectrophotometer. Seeds from several maternal families were not available for the Na^+ analysis.

3.5.6 *Statistical Analysis*

Statistical analysis was conducted using SAS version 7.0 for Windows. Nested mixed model analyses of variance (PROC MIXED) were conducted to examine the effect of the treatments on the root length of germinating seedlings. Because our hypotheses addressed roadsides specifically, with oldfields included as a control, we analyzed the roadsides and oldfields separately. Again, we conducted a nested mixed model ANOVA (PROC MIXED). The experimental design is composed of a hierarchical structure of maternal families within populations and populations within sites, necessitating the use of nested mixed model ANOVA's. Population, maternal family and their interactions with the treatments are considered random variables, while site and the treatment are considered fixed effects.

Our hypotheses regarding the source of the salt enhanced growth in the roadside populations suggested the division of the families into three categories (Fig. 1) based on their responses to the salt and leaching treatments; SD_N/SD_L (Salt Damaged Non-leached/Salt Damaged Leached) includes families that grew significantly shorter roots in the salt solution regardless of the leaching treatment; SE_N/SD_L (Salt Enhanced Non-leached/Salt Damaged Leached) includes families that had enhanced growth in salt when not leached but grew shorter roots in salt when leached prior to salt exposure as well as families that suffered a large decline in root length when leached, but were essentially not impacted by the salt treatment when not leached; and SE_N/SE_L (Salt Enhanced Non-leached/Salt Enhanced Leached) includes families whose root growth was increased by salt exposure regardless of the leaching treatment. Salt tolerance was estimated by dividing the average root length of a maternal family in the salt/non-leached treatment by the average root length in the control/non-leached treatment. The correlation between salt tolerance and the amount of sodium in the seeds was determined for each category of seeds (PROC CORR).

3.6 RESULTS

3.6.1 *Complete Analysis*

The salt treatment greatly reduced the average root length of the germinating seedlings, while the leaching treatment only slightly reduced the average root length (Figure 3.2, Table 3.1). The sites responded differently to the salt treatment; on average oldfield seeds suffered a large decline in root length in the salt treatment while roadside seed root length remained relatively unchanged in the salt treatment (Figure 3.2a, Table 3.1). The average effect of the leaching treatment depended on the salt treatment (Figure 3.2b, Table 3.1). Leaching the seeds had a strong detrimental effect on root length when followed by salt exposure but not by distilled water (Figure 3.2b). Despite the clear differences in the responses of seeds collected from the oldfields and the roadsides to the leaching and salt treatments, there was no significant interaction between the site, leaching treatment and salt treatment (Table 3.1). Instead, most of the variation in root length among the germinating seeds was found among families rather than between sites (Figure 3.4a and b, Table 3.2). Though maternal families did not vary in their response to salt or to leaching, there was significant variation among maternal families in how the leaching treatment affected their response to the salt treatment (Table 3.2). As predicted, roadside families show a qualitatively different response to the treatments than did oldfield families (Figure 3.3a and b). The effect of the salt treatment overall differed between sites, with salt having a negative effect on oldfield but not roadside growth. To further explore these interactions, we have analyzed the effects of treatments separately for each site.

3.6.2 *Analysis of Oldfield Families*

The root length of oldfield seeds was greatly lowered by the salt treatment and slightly lowered by the running water treatment (Figure 3.3, Table 3.3). There was no significant interaction between the two treatments indicating that the leaching process did not impact the ability of the seeds to tolerate high salinity (Table 3.3). Though there was no overall significant interaction at the level of the site or population, there was significant variation among maternal families in how the leaching impacted their salt tolerance (Figure 3.4, Table 3.4). However, all of the families showed the same qualitative SD_N/SD_L response (Figure 3.4).

3.6.3 *Analysis of Roadside Families*

Neither treatment had an overall impact on seeds collected from roadsides (Table 3.3, Figure 3.3). However, there was a significant interaction between the salt and leaching treatments, indicating that leaching the ions from the seeds had a significant impact on their ability to tolerate high salinity (Table 3.3). There was significant variation in how the leaching treatment affected the ability of the maternal families to tolerate salt (Table 3.4). Five of the 15 roadside families were always damaged by the salt, regardless of the leaching treatment (SD_N/SD_L) and four were always enhanced

(SE_N/SE_L) (Figure 3.4). However, six families showed the predicted response (SE_N/SD_L) exhibiting salt-enhanced growth only when not leached (Figure 3.4).

3.6.4 *Roadside Soil Na^+ Measurement*

The soil collected from the location of roadside population 3 had substantially higher Na^+ levels (6.465 ± 0.293) than the soil collected from the locations of roadside populations 1 (0.207 ± 0.027) and 2 (0.387 ± 0.083).

3.6.5 *Seed Na^+ Content*

There was a significant correlation between the Na^+ content and the salt tolerance of the seeds from the SE_N/SD_L category ($r = 0.97551$; $p = 0.0046$) but not in the seeds from the SD_N/SD_L category ($r = 0.14860$; $p = 0.5438$) or the SE_N/SE_L category ($r = -0.48173$; $p = 0.6800$) (Figure 3.5).

3.7 DISCUSSION

Oldfield families were all harmed by salt, while roadside families displayed both increased salt tolerance and salt-enhanced growth. The response of roadside families to high salinity remained consistent with the trends observed in our previous study (Beaton & Dudley 2004) despite the use of different maternal families, indicating that families with salt tolerance and enhancement traits are widespread in roadsides. We had hypothesized that if seedling salt tolerance and enhancement were conveyed by salt from the maternal environment, then we would find first, that leaching would remove salt enhancement; and second, that the loss of salt enhancement would be associated with high levels of Na^+ in the seeds. However, our results supported the first prediction but not the second. We found that leaching eliminated the salt-enhanced growth of many roadside families, though the salt-enhanced growth of other families was not affected by leaching. However, the families that lost their salt-enhanced growth did not have higher Na^+ content in their seeds compared to other families. Although there was a significant correlation between the average seed Na^+ content and the average salt tolerance of roadside families when not leached, which is consistent with notion that high Na^+ levels confer salt tolerance, the small sample size significantly reduces any confidence in the accuracy of this conclusion. Consequently, our results generally do not support the hypothesis that the maternal environment was conferring salt tolerance through the deposition of Na^+ in the seeds. Instead, our results expose underlying variation in salt tolerance mechanisms within the roadside populations.

The differences seen among populations presented an intriguing pattern. Roadside population 1 displays two patterns of response to the leaching treatments (Fig. 4): two families were harmed by the addition of salt regardless of the leaching treatment; and three families that were slightly enhanced by salt when not leached, but were damaged by salt after leaching. Likewise, roadside population 2 displays a mixture of responses with two families displaying SE_N/SD_L patterns and two families showing SD_N/SD_L. The fifth family was the only one of all those studied where leaching increased root growth in salt; it had no root reduction when not leached and showed salt enhanced growth when leached. The loss of salt tolerance/enhancement after leaching suggests that these families owed their salinity tolerance to their seed composition, which is provided by the maternal plant. That they became salt sensitive following leaching suggests that a soluble component(s) of the seed conferred tolerance to the osmotic stress imposed by high salinity rather than Na^+ toxicity. The deposition of different osmotica in the seed by the maternal plant may have lowered the osmotic potential, facilitating imbibition during germination. Although the use of Na^+ as an osmotica has been documented in at least one halophyte (*Sarcobatus vermiculatus*) (Dodd & Donovan, 1999), these families may have utilized a different ion or organic compound as neither population showed elevated Na^+ levels (Figure 3.5). In addition, their sensitivity to salinity following leaching may indicate that they could not tolerate high Na^+ levels in the seed. Some species produce organic substances (i.e. malate, oxalate and proline (Ashraf, 1994)) that allow them to maintain their osmotic potential without taking up high levels of Na^+.

The origin of any potential organic substances produced in roadside populations 1 and 2 to aid in salt tolerance is unknown. The maternal plant may synthesize these compounds and deposit them in the seed during development. Alternatively, the germinating embryo may produce the compounds in response to exposure to high Na^+ levels during germination. Ramagopal (1990) observed in barley that when seeds imbibe salt water they produce different proteins than when they imbibe distilled water. An additional consequence of leaching the seeds prior to salt exposure was that the seeds imbibed water rather than salt water, eliminating the stage where preparation for high salinity may have occurred.

However, it is not likely that the reduced growth of SE_N/SD_L families from roadside populations 1 and 2 exposed to salt following leaching can be attributed entirely to osmotic stress. A comparison of *D. sylvestris* seeds germinating in 70 mM salt solutions and vermiculite of the same osmotic potential showed that reduced water potential stimulates increased allocation of resources to root growth from shoot growth (Chapter 6) while salt exposure led to reduced growth in both the roots and the shoots. Na^+ may compete with K^+ (required for the activation of more than 50 enzymes (Bhandal & Malik, 1988)) at membrane binding sites. Moderately salt tolerant genotypes may be able to better sequester Na^+ in vacuoles and prevent the ion from interfering with K^+ functions. Once the ions and other solutes have been leached out of the seed, they may be just as vulnerable to the osmotic stress of high salinity as SD_N/SD_L families.

Roadside population 3 appears to present another kind of salt tolerance system. Growth of three roadside 3 families was enhanced by high salinity during germination regardless of the leaching treatment, while one family showed a loss of enhanced growth and the other no enhancement regardless of the leaching treatment. One possibility is that these SE_N/SE_L families have evolved the ability to substitute Na^+ for K^+ in metabolic activities. This second salt tolerance strategy has been found in several halophytes. For example, guard cells open and close the stomata by transferring K^+ across the plasma membrane (reviewed in (Robinson *et al.,* 1997)). Under high salinity, Na^+ may replace K^+. In glycophytes, like *Commelina communis*, the replacement of K^+ with Na^+ results in stomata than are able to open but not close in response to changes in light or temperature (Jarvis & Mansfield, 1980). In contrast, the halophyte, *Cakile maritime* uses K^+ in guard cell function when grown at low salinity but uses Na^+ when grown at high salinity (Eshel *et al.,* 1974). The SE_N/SE_L seeds may have developed the ability to substitute Na^+ in metabolic activities normally requiring K^+, so that they benefit not only from the osmotic adjustment provided by Na^+ accumulation but are also protected from K^+ deficiency. Roadside population 3 had significantly higher Na^+ levels than the other two roadside populations (Figure 3.5). However, the high Na^+ levels were not found in all five families. Of the three families that did show elevated Na^+ levels, only one also demonstrated SE_N/SE_L growth, suggesting that salt enhancement and Na^+ deposition in seeds are two separate traits, though they may confer higher fitness when found together. It is likely that both these traits are under selection in this population.

The intensity of a selection pressure is crucial in determining the degree of response. For example, the degree of metal tolerance found in species inhabiting contaminated soil is closely related to the concentration of the metal in the soil

(Antonovics *et al.,* 1971). Correspondingly, the capacity of *D. sylvestris* populations to tolerate high salinity appears to be associated with the level of salt exposure experienced by the parent population. Roadside population 3, which displays the highest degree of salt enhancement, was collected from the parent population that experienced the highest salinity levels in the field. Given that gene flow between roadside populations is likely high due to the seed movement generated by passing cars (Scott & Davison, 1985), SE_N/SE_L traits could spread into populations experiencing low salinity unless there is a tradeoff between success in high and low salinity environments.

Salt tolerance may have a cost to plants not growing in saline environments. A study of *Sonneratia alba* and *S. lanceolata* (Ball & Pidsley, 1995) indicated that salt tolerance is acquired at the cost of reduced growth. The more salt tolerant species, *S. alba* was 50 % smaller under ideal (i.e. low salinity) growth conditions than the less salt tolerant species *S. lanceolata.* The shorter root length of seeds collected from roadsides compared to seeds from oldfields when germinated in distilled water may reflect this cost of tolerance. Alternatively, the low root lengths may be related to the toxicity of high Na^+ levels. Some roadside families had considerably higher root lengths in the control solutions after the leaching process (Figure 3.4) suggesting that the contents of the seed may actually be detrimental to root growth. A plant that has high levels of Na^+ in its tissues may passively deposit the Na^+ in the seed as it provides resources to the endosperm and embryo despite the negative consequences for growth. Oldfield seeds grown in the presence of several roadside seeds had significantly shorter roots than when grown with other oldfield seeds (Chapter 7) indicating that the seed leachate of roadside seeds may actually be harmful to the germinating seed under control conditions.

In conclusion, we cannot support or reject our hypothesis that the maternal deposition of salt in the seeds is an adaptation that confers tolerance to high salinity during germination. However, we have demonstrated that seed composition in important in conferring salt tolerance in some families of roadside plants via a soluble compound that is lost by leaching.

ACKNOWLEDGEMENTS

We would like to thank Dr. Elizabeth Weretilnyk for inspiring conversations that precipitated this research, the Royal Botanical Gardens in Hamilton, Ontario for allowing seed collection on their lands, Sid Lang at the Ontario Ministry of Transport for help in obtaining permission to work on highway verges and Mary DeGuzman for assistance in seed collection.

TABLE 3.1: A mixed model analysis of variance of the effects of the salt treatment, leaching treatment and site, on the root length after ten days of germinating *D. sylvestris* seeds. Seeds that did not germinate are not considered in the analysis. Effects with estimates of zero have been removed from the analysis.

Source	Numerator df	Denominator df	*F*
Leaching	1	4	10.44 *
Salt	1	4	57.08 **
Site	1	4	42.59 **
Leaching×Salt	1	76	9.53 **
Site × Leaching	1	4	0.01
Site × Salt	1	4	30.69 **
Site × Leaching*Salt	1	76	2.64

* $P < 0.05$; ** $P < 0.01$.

TABLE 3.2: A mixed-model analysis of variance for random effects of the effects of the population (nested within site), maternal family (nested within population and site), and their interactions with the leaching and salt treatments on the root length after ten days of germinating *D. sylvestris* seeds. Seeds that did not germinate are not considered in the analysis. Effects with estimates of zero have been removed from the analysis.

Source	Estimate	Std. Err.	*Z*
Maternal Family	0.01272	0.007055	1.80 *
Population×Leaching	0.00557	0.006696	0.83
Population×Salt	0.01153	0.009776	1.18
Maternal Family×Leaching×Salt	0.03105	0.007247	4.28 ***
Residual	0.27030	0.008052	33.57 ***

* $P < 0.05$; *** $P < 0.001$

TABLE 3.3: A mixed model analysis of variance of the effects of the salt treatment and leaching treatment on the root length after ten days of germinating *D. sylvestris* seeds. Roadsides and oldfields are considered seperately for clarity. Seeds that did not germinate are not considered in the analysis. Effects with estimates of zero have been removed from the analysis.

	Oldfield			Roadside		
Source	Num df	Den df	*F*	Num df	Den df	*F*
Leaching	1	2	15.29 †	1	2	3.50
Salt	1	14	306.09 ***	1	2	1.41
Leaching×Salt	1	26	1.36	1	38	9.76 **

*† $P < 0.1$; ** $P < 0.01$; *** $P < 0.001$.*

TABLE 3.4: A mixed-model analysis of variance for random effects of the effects of the population (nested within site), maternal family (nested within population and site), and their interactions with the leaching and salt treatments on the root length after ten days of germinating *D. sylvestris* seeds. Roadsides and oldfields are considered separately for clarity. Seeds that did not germinate are not considered in the analysis. Effects with estimates of zero have been removed from the analysis. (P = population, M = maternal family, L = leaching treatment, S = salt treatment)

	Oldfield			Roadside		
Source	Estimate	Std. Err.	*Z*	Estimate	Std. Err.	*Z*
P	0.01106	0.01595	0.69	---	---	---
M	0.01190	0.01033	1.15	0.01161	0.01016	1.14
P×L	0.000095	0.003727	0.03	0.009148	0.01218	0.75
P×S	0.004887	0.009759	0.50	0.01791	0.01814	0.99
M×L×S	0.02357	0.009577	2.43 **	0.03498	0.01175	2.98 **
Residual	0.2277	0.009577	23.77 ***	0.3132	0.01321	23.71 ***

** $P < 0.05$; *** $P < 0.001$

FIGURE 3.1: The arrows indicate the impact the leaching and salt treatments had on the average salt tolerance of a maternal family with each arrow corresponding to one maternal family. The head and tail of the arrows are Cartesian coordinates with the root length in the non leached treatment corresponding to the x value and the root length in the leached treatment corresponding to the y value. The tail of the arrow indicates the root length in the control (distilled water) treatment and the head of the arrow indicates the root length in the salt treatment. Thus, the arrow is a vector that demonstrates the impact of the salt treatment in the two leaching treatments. The three different categories of the family responses to the salt and leaching treatments are based on the direction of the response. Arrows pointing down and to the left are are families that have greater root growth in distilled water than in the salt solution regardless of the leaching treatment and are included in the SD_N/SD_L category (Salt Damaged in the Non-leached treatment/Salt Damaged in the Leached treatment). Arrows pointing down and to the right are families with enhanced growth in salt, but which lose their ability to grow longer roots in salt if leached prior to salt exposure and are included in the SE_N/SD_L category (Salt Enhanced in the Non-leached treatment/Salt Damaged in the Leached treatment). Finally, arrows pointing up and to the left are families that grow longer roots in salt solution regardless of the leaching treatment and are included in the SE_N/SE_L category (Salt Enhanced in Non-leached treatment/Salt Enhanced in Leached treatment).

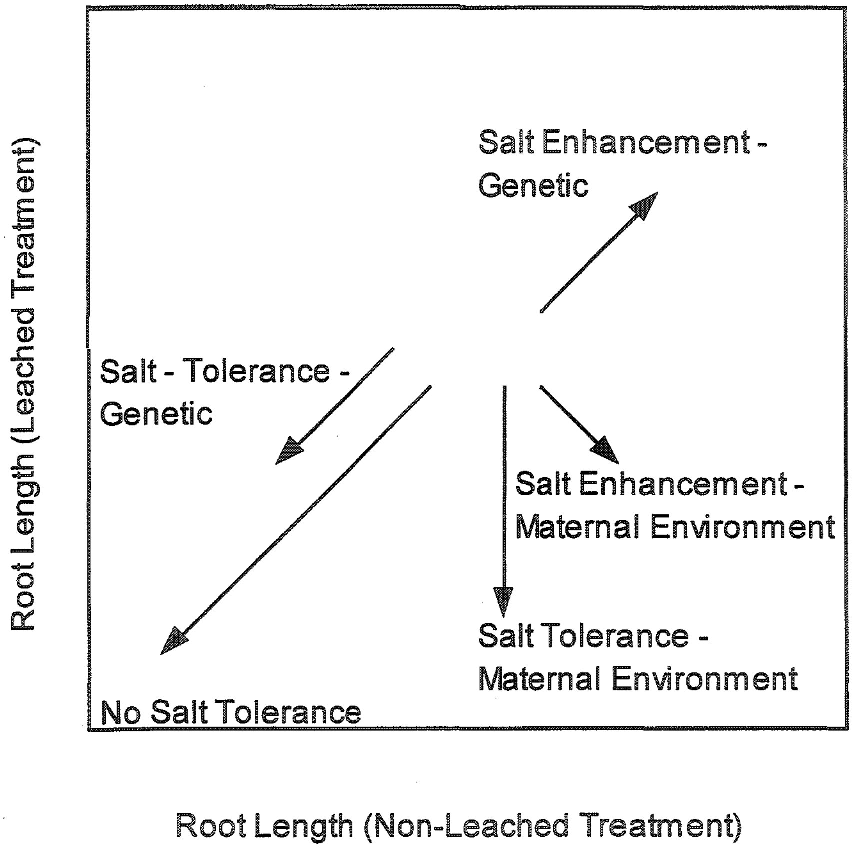

Salt Enhancement - Genetic
Salt - Tolerance - Genetic
Salt Enhancement - Maternal Environment
Salt Tolerance - Maternal Environment
No Salt Tolerance
Root Length (Leached Treatment)
Root Length (Non-Leached Treatment)

FIGURE 3.2: Average root length after ten days of *D. sylvestris* seeds collected from oldfields and roadsides germinating in salt (70 mM NaCl) or control (distilled water) treatments. Seeds collected from oldfields (○) suffered a much larger impact from the salt treatment than did seeds collected from roadsides (●) (a). In comparison to the non-leached treatment (Δ), the leaching treatment (▲) had only a minimal impact on root length unless accompanied by salt exposure (b).

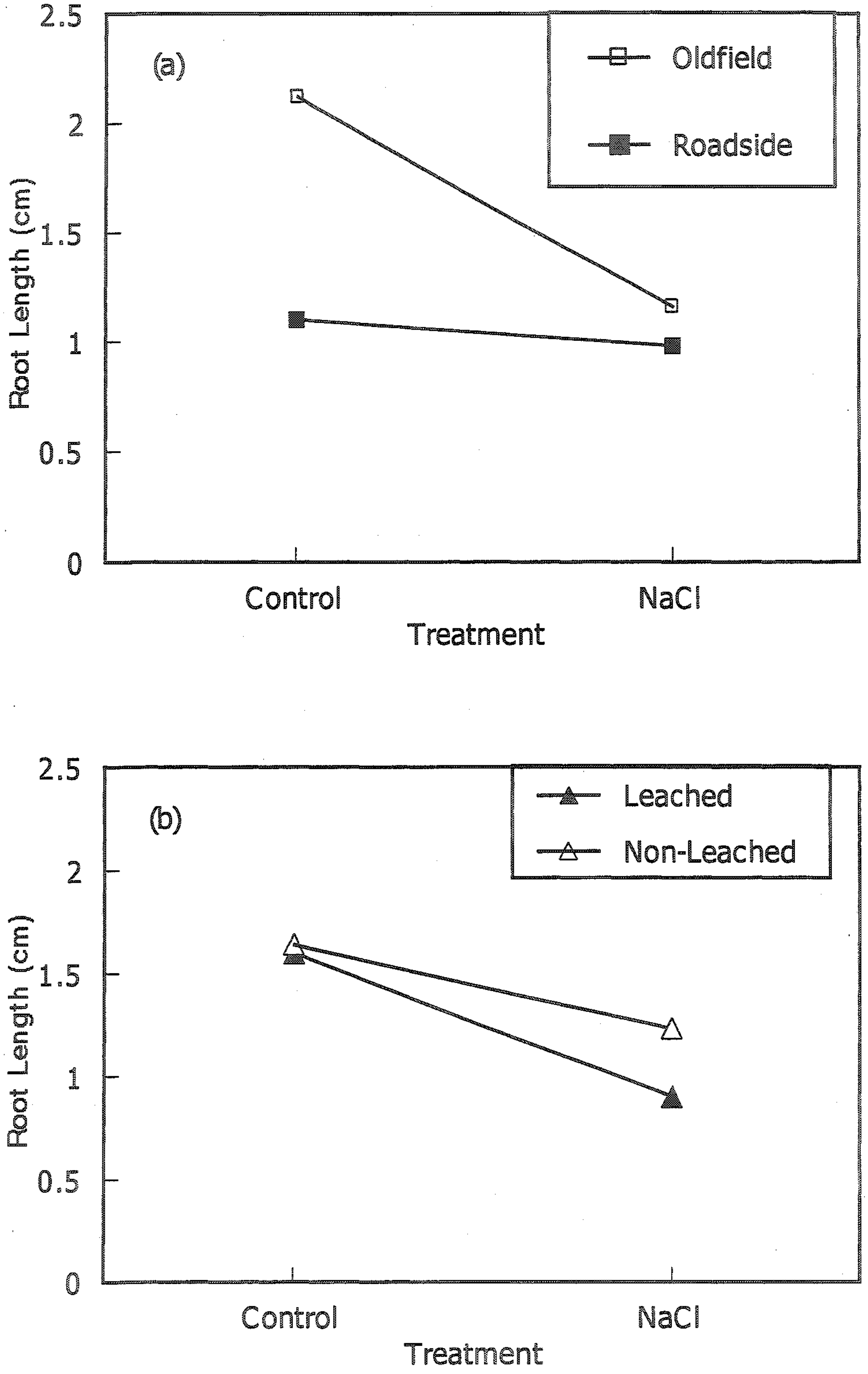

(a)
Oldfield
Roadside
Root Length (cm)
2.5
2
1.5
1
0.5
0
Control
NaCl
Treatment
(b)
Leached
Non-Leached
Root Length (cm)
2.5
2
1.5
1
0.5
0
Control
NaCl
Treatment

FIGURE 3.3: Average root length after ten days of *D. sylvestris* seeds collected from oldfields (a) and roadsides (b) germinating in salt (70 mM NaCl) or control (distilled water) treatments and leached (○) or non-leached treatments (●). Mixed model ANOVA's showed a significant interaction between the salt and leaching treatments for roadside seeds but not for oldfield seeds (Table 3).

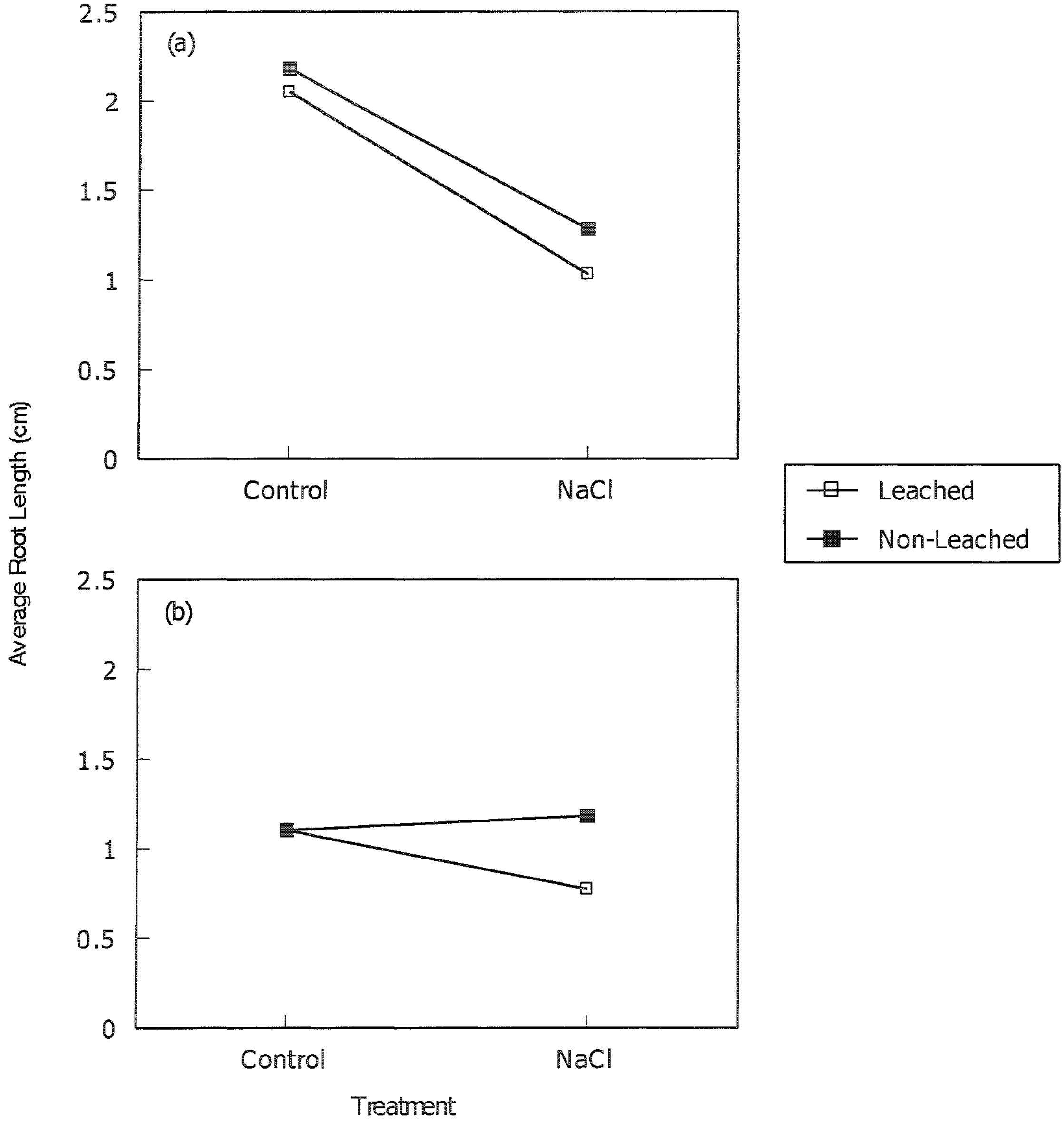
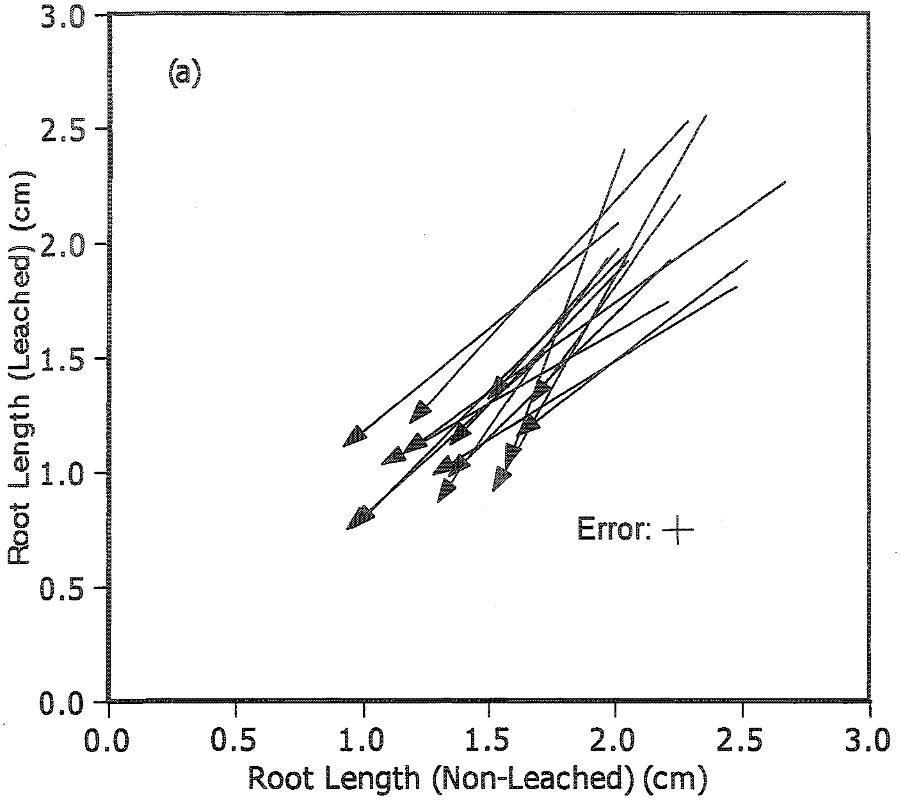

(a)
(b)
2.5
2
1.5
1
0.5
0
Control
NaCl
Average Root Length (cm)
Treatment
Leached
Non-Leached

FIGURE 3.4: Arrows indicate the impact the leaching treatment had on salt tolerance. Each arrow represents one maternal family. The arrow begins at the root length in the control (distilled water) treatment and ends at the root length in the salt treatment (See Fig. 1 for a detailed explanation). Oldfield families (a) show no salt tolerance with all families in the SD_N/SD_L category. Roadside families (b) display a mixture of responses to the salt and leaching treatments with families in the SD_N/SD_L, SE_N/SD_L and SE_N/SE_L categories. See Fig. 1 for a detailed description of categories.


(a)
Root Length (Leached) (cm)
Root Length (Non-Leached) (cm)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Error:

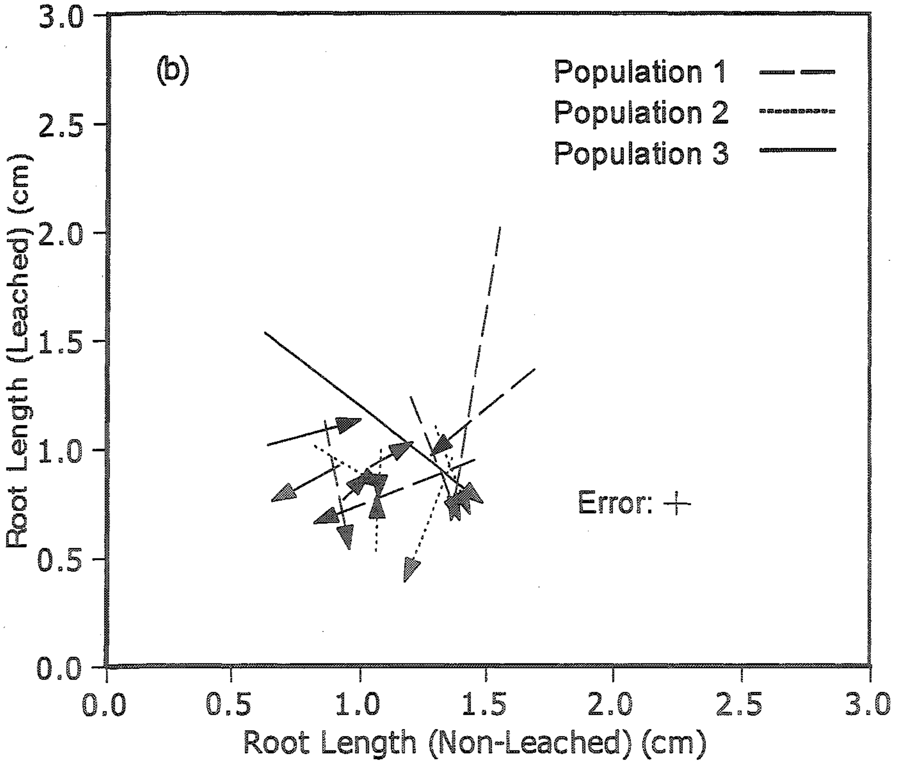
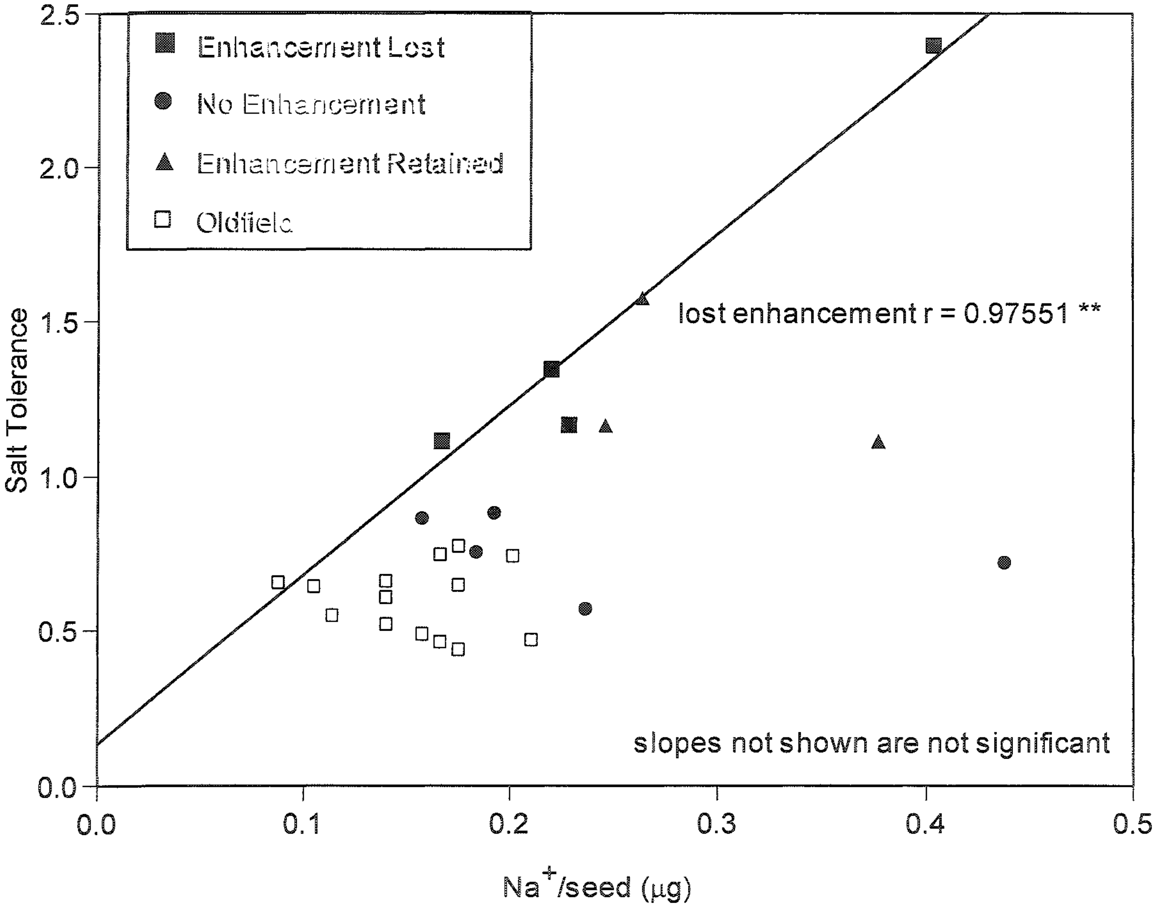

(b)
Population 1
Population 2
Population 3
Root Length (Leached) (cm)
Root Length (Non-Leached) (cm)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Error:

FIGURE 3.5: The relationship between the salt tolerance and the average Na^+ content of maternal families of *D. sylvestris* seeds collected from three oldfield and three roadside populations that were divided into three categories based on their responses to salt and leaching treatments during germination (refer to Fig. 1 for description of categories). Salt tolerance is measured as the root length in the salt treatment divided by the root length in the control treatment.


■ Enhancement Lost
● No Enhancement
▲ Enhancement Retained
□ Oldfield
lost enhancement r = 0.97551 **
slopes not shown are not significant
Salt Tolerance
0.0
0.5
1.0
1.5
2.0
2.5
0.0
0.1
0.2
0.3
0.4
0.5
Na^+/seed (μg)

SECTION IV

LOCAL ADAPTATION IN ROADSIDE VEGETATION

4.1 ABSTRACT

A reciprocal transplant experiment involving three species of common roadside plants (*Dipsacus sylvestris* (Huds.), *Hesperis matronalis* (L), and *Asclepias syriaca* (L.)) was conducted between oldfield and roadside sites. Seeds of each species were collected from ten maternal plants from each of three oldfield and three roadside populations, then planted randomly into two oldfield and two roadside sites. The plants were monitored and the number and size of the leaves and the height of bolting plants were recorded as measures of performance for a period of four years or until the plants either died or reproduced. Following reproduction, plants were dried, and weighed. No individuals survived to reproduce in the roadside. In the oldfield only *D. sylvestris* and *H. matronalis* individuals reached reproductive maturity. Though significant variation was found between populations of *A. syriaca* and *H. matronalis*, no evidence of local adaptation was observed in any of the three species.

4.2 INTRODUCTION

Roadsides present a novel environment with an unusual combination of selective pressures. Roadside soil is often a subsoil high in nitrogen resulting from nitrate emissions (Spencer & Port, 1988) and contaminated with nickel and chromium from chrome plating (Ndiokwere, 1984), cadmium from motor oil, various plated components in automobiles and from its presence as an impurity in the zinc used in tires (Ndiokwere, 1984, Warren & Birch, 1987), zinc from tire rubber and motor oil (Ndiokwere 1984), copper from brake linings, lead (Ndiokwere 1984, Warren and Birch 1987) from the use of leaded gasoline before 1977, manganese from the combustion of gasoline containing the fuel additive methylcyclopentadienyl manganese tricarbonyl (Lytle *et al.,* 1994) and high concentrations of sodium and calcium ions from the use of de-icing salts (Harrison *et al.,* 1985). The aboveground roadside environment imposes a variety of other stresses. Vegetation inhabiting these areas must cope with high levels of carbon monoxide, ethylene, ozone, sulphates and nitrates in the atmosphere as well as high levels of air turbulence and particulate pollution (Thompson *et al.,* 1984). Passing cars cause high levels of turbulence and particulate pollution (Forman & Alexander, 1998).

Despite the various contaminants, roadside plants are larger and produce more flowers and more seeds than plants growing in undisturbed areas, a pattern that has been attributed to the high levels of nitrogen in the soil (Port & Thompson, 1980, Spencer & Port, 1988). The success of aggressive 'weedy' species in roadsides is often attributed to high levels of nitrogen (Forman & Alexander, 1998).

The combination of stressors from the roadside provides a unique environment for plant populations. If populations possess genetic variation for tolerance to these stressors, they may be adapting to the roadside environment. Community-level adaptation to the roadside has been demonstrated, e.g., the changes in species composition from the migration of coastal species along roadsides (Scott & Davison, 1985). In addition, a previous study demonstrated that during germination roadside populations of *Dipsacus sylvestris* had adapted to the high salinity of the roadside and one roadside population of *Hesperis matronalis* had adapted to the high manganese levels (Beaton & Dudley, 2004). However, adaptation to the roadside environment as a whole has not been examined.

In this study, I collected seeds from roadside and oldfield populations of three species of herbaceous plants (*Dipsacus sylvestris*, *Hesperis matronalis* and *Asclepias syriaca*). I planted seeds of known maternal families in both roadside and oldfield environments and monitored them for four years or until the plants had reproduced. Survivorship and the number and length of the largest leaf were recorded approximately monthly throughout the growing seasons. Following reproduction, the plants were dried and weighed. I ask the following questions, 1) What is the impact of the roadside environment? 2) Is there variation between and within roadside and oldfield populations for biomass accumulation? and 3) Have roadside populations adapted to the roadside environment?

4.3 METHODS

4.3.1 *Study Species*

Dipsacus sylvestris (common teasel), a native of Europe, is found primarily in oldfields and roadsides. It grows vegetatively as a rosette for at least one growing season. In the spring, if the plant has reached a critical size, it will bolt to its reproductive form of an upright flowering stem. Flowering occurs from July to September and seeds are shed throughout the fall. A single plant can produce upwards of 3000 seeds (Werner, 1975).

Hesperis matronalis, a native of Asia, grows mainly in semi-shaded habitats including roadsides. Also a monocarpic perennial, it grows vegetatively as a rosette for at least one season. In the spring, if the plant has reached a critical size, it bolts to form an upright flowering stem. Flowering occurs from May to August and seeds are shed throughout the summer. Each plant can produce several hundred seeds (Neilsen *et al.*, 1995).

Asclepias syriaca, a native of northeastern North America is commonly found in roadsides. It is a perennial that reproduces from seed and by adventitious root buds. Flowering occurs from June to August and seeds, which are produced in pods, are released from September to October. Each plant can produce upwards of 2000 seeds, which are dispersed easily by the wind (Bhowmik & Bandeen, 1976).

4.3.2 *Seed Collection*

For each species, three roadside and three oldfield populations were selected. Roadside populations were located in full sunlight, 2 - 10 m from the pavement's edge (of Highway 403 between the cities of Hamilton and Burlington, Ontario, Canada). Highway 403 is a six lane highway with a speed limit of 90 – 100 km/h. Traffic volume ranges between 44 000 to 72 000 vehicles daily (two way traffic) (Ontario Ministry of Transportation 1994). At all the roadside sites, the surrounding vegetation was primarily various species of grass and/or crown vetch (*Coronilla varia*)(which is planted following highway construction to stabilize the soil) with a few common oldfield plants (e.g. *Solidago* sp., *Daucus carota*). Oldfield populations were located on Royal Botanical Gardens (cities of Burlington and Hamilton, Ontario, Canada) conservation lands in full sunlight. All oldfield sites were found > 100 m from the nearest road (in all cases, a two lane road with a 50 - 70 km/h speed limit) and several km from the nearest major highway. The surrounding vegetation of these sites was dominated by a variety of grass species with a wide variety of forbs. Seeds were collected from 15 - 20 plants from each population. To ensure that an adequate number of seeds were collected from each plant, the largest plants from each population were chosen for seed collection. Following collection, seeds were processed and stored in paper envelopes at room temperature. Prior to the establishment of the experiment, seeds were placed in well plates in random order, moistened and stored at 2 degrees Celsius for three months in order to overcome the dormancy of *A. syriaca*.

4.3.3 *Study Sites*

The experiment was conducted at Rock Chapel, an oldfield located on Royal Botanical Gardens conservation lands and at a roadside location near the Aldershot Go Station on the side of the 403 between Hamilton and Burlington. The oldfield site was unshaded and dominated by a variety of grasses and other herbaceous plants including *Solidago* spp. and *Asclepia syriaca.* The roadside site was located behind a guardrail and within 5 m of the edge of the highway. The site was level (i.e. was not sloping into a ditch), unshaded, and the vegetation was dominated by a variety of grasses with only a few other herbaceous plants including *Linaria vulgaris.*

4.3.3 *Experimental Design*

Five seeds from each of ten maternal families from each population collected from each site from each species were planted randomly into a 9 m X 1 m grid at two locations in both the oldfield site and the roadside site. The 900 seeds in each grid were placed in 90 rows of ten and spaced 10 cm apart. The seeds were planted from mid March to early May in the spring of 2000. Plastic sword picks marked the location of the seeds. Data was collected approximately every month from May until November, 2000, from May to November, 2001, from May to September 2002 and from May to September 2003. Data collected included survivorship, number of leaves, the length of the largest leaf and, once the plants had bolted, height. Plants that had bolted were harvested in the late summer (*H. matronalis*) to early fall (*D. sylvestris*). Plants that had not bolted by the end of the 2003 field season were harvested in September. The plants were dried at room temperature and weighed. Plant biomass was divided into reproductive and vegetative components.

4.3.4 *Statistical Analysis*

Statistical analyses were conducted using SAS version 8.2 for Windows. The three species were analyzed separately.

Survivorship data was analyzed using the Wilcoxon test and Logrank test (PROC LIFETEST). Data collected each year was analyzed separately. Multivariate analyses of variance were conducted to determine the effect of transplant site and collection site on the number and length of leaves and to analyze the level of variation between populations and maternal families. The experimental design is composed of a hierarchical structure of maternal families nested within populations and populations nested within sites necessitating the use of nested models. Population, maternal family and their interactions with the transplant are considered random variables, while the transplant site and collection site are considered fixed effects. Nested mixed model analyses of variance (PROC MIXED with the COVTEST option) with random and fixed variables as described above, were conducted to examine the effect of the collection site and transplant site on the final height and biomass of the individuals that reproduced and to analyze the level of variation between populations and maternal families.

4.4 RESULTS

4.4.1 Dipsacus sylvestris

No *D. sylvestris* individuals survived to reproduce in the roadside. Roadside and oldfield plants did not differ in survivorship in either the roadside or the oldfield in 2000, 2001, 2002 or 2003 (Tables 4.1 and 4.2, Figure 4.1). Nor were there any differences in survivorship between populations in either site (Table 4.2).

Plants growing in the oldfield were significantly larger than plants growing in the roadside throughout the experiment (Figure 4.2, Table 4.3). However, there was no difference between the size of the oldfield genotypes and the roadside genotypes (Figure 4.2, Table 4.3), nor was there any interaction between the transplant site and the collection site (Table 4.3). In July and August of season 1, there were significant differences in the size of the plants at the population level but there was no interaction between the population and the transplant site. This effect disappeared by September and did not reoccur in the following seasons (Table 4.3). Throughout the experiment, there was no significant variation in size between maternal families, nor was there an interaction between the maternal family and the transplant site (Table 4.3).

Among plants that reproduced in 2001, roadside genotypes were significantly taller than oldfield genotypes (Table 4.4, Figure 4.3). However, there was no difference in height among plants that reproduced in 2002 or 2003. Nor was there any variation for height among families or populations in 2001, 2002 or 2003 (Table 4.5). Roadside genotype that reproduced in 2001 also had much higher biomass at reproduction than oldfield genotypes that reproduced in 2001 in total biomass and in both vegetative and reproductive biomass (Table 4.6, Figure 4.4).

4.4.2 Hesperis matronalis

No *H. matronalis* individuals survived to reproduce in the roadside (Table 4.1, Figure 4.5). Oldfield genotypes initially had better survivorship in the spring of season 1 in the roadside than did roadside genotypes (Figure 4.5, Table 4.7). In contrast, there was no difference between oldfield and roadside genotypes in the oldfield during any growing season (Table 4.7). There were significant differences in survivorship between oldfield and roadside populations in the oldfield in season 1 and season 3 (Table 4.7, Figure 4.6). Roadside population 3 had lower survivorship in the oldfield than any of the other populations throughout the course of the experiment (Figure 4.6). Oldfield population 3 had lower survivorship than the other oldfield populations and two of the roadside populations throughout season 1 only.

While plants still survived in the roadside, plants growing in the oldfield were significantly larger than plants growing in the roadside (Figure 4.8, Table 4.8). However, there was no difference between the size of the oldfield genotypes and the roadside genotypes (Figure 4.8, Table 4.8), nor was there any interaction between the transplant site and the collection site (Table 4.8). There were no significant consistent differences in the size of the plants at the population level, thought there was a significant variation in the size of the plants from different populations during May, June and July of season 2 (Table 4.8). However, these differences did not persist. No significant interactions between the population and the transplant site occurred at any time during the study (Table 4.8). Throughout the experiment, there was no significant variation in size between maternal families, nor was there an interaction between the maternal family and the transplant site (Table 4.8).

There were no differences in the height of plants that reproduced in 2001 or 2002 (Table 4.9, Figure 4.9). Nor was there any variation for height among families or populations in 2001 or 2002 (Table 4.10). However, there was significant variation for height among maternal families in 2003 (Table 4.10). There was no significant difference between oldfield and roadside genotypes in the total biomass, vegetative biomass or reproductive biomass of the plants that reproduced in either 2001 or 2002 (Table 4.6, Figure 4.9).

4.4.3 Asclepias syriaca

No *A. syriaca* individuals survived past the first season (Table 4.1). Roadside and oldfield families did not differ in survivorship in either the roadside (Wilcoxon $\chi^2 = 3.0042$; $p = 0.0830$; log-rank $\chi^2 = 1.4646$; $p = 0.2262$; or the oldfield (Wilcoxon $\chi^2 = 1.8775$; $p = 0.1706$; log-rank $\chi^2 = 1.8938$; $p = 0.1688$) (Figure 4.10). However, there were significant differences in the survival of populations in the roadside (Wilcoxon $\chi^2 = 14.5596$; $p = 0.0124$; log-rank $\chi^2 = 12.7629$; $p = 0.0257$) but not the oldfield (Wilcoxon $\chi^2 = 5.6307$; $p = 0.3438$; log-rank $\chi^2 = 4.1841$; $p = 0.3438$) (Figure 4.11).

There was no difference in size between the plants growing in oldfield and roadside transacts (Table 4.11, Figure 4.11). Nor was there any difference between the oldfield and roadside genotypes or the interaction between the site genotype and the transplant site (Table 4.11, Figure 4.12). Additionally, there was no consistent significant variation in size among populations or maternal families, though in September, the census immediately preceding the large mortality event where all remaining plants died, there was a significant difference between the populations (Table 4.11). Though there was no significant variation in the response of the populations to the transplant site at any time during 2000, there was significant variation in the size of the maternal families in the two transplant sites in July (Table 4.11). However, this difference did not persist throughout the summer.

4.5 DISCUSSION

The roadside is unquestionably a more stressful environment for plant communities than the oldfield environment. Not one individual of any of the three species studied survived to reproduce in the roadside while there was substantial reproduction by *D. sylvestris* and *H. matronalis* in the oldfield. Previous studies of salt and manganese tolerance in roadside populations of both *H. matronalis* and *D. sylvestris* (Beaton and Dudley 2004) have provided overwhelming evidence that evolutionary change has occurred in roadside populations. However, in this study, local adaptation (i.e. adaptation in the field) could not be observed in either species. Neither plant size nor survivorship differed among genotypes in either the oldfield or the roadside. In particular, early survivorship differences, which would best reflect adaptations during germination, revealed no genotype by environment interactions. The results of the reciprocal transplant experiment involving only *D. sylvestris* that was established in the fall (Chapter 5) suggest that in the field, seed size, a trait conferred by the maternal plant, overwhelmed the influence of genetic traits. The complexity of the natural environment has likely also influenced the ability to assess local adaptation in these species.

The natural environment is complex with many factors affecting the success of plants simultaneously. Though high salinity has been shown to be an important selection pressure during germination in roadside populations of *D. sylvestris* (Beaton and Dudley 2004), the myriad of selection pressures present in the natural habitat may overwhelm adaptations to salt application in roadside populations. Alternatively, the different stresses may impose incompatible selection pressures, such that salt tolerance cannot be achieved simultaneously with adaptation to another aspect of the roadside environment (e.g. metal tolerance). The lack of overlap in tolerance to salt, drought and hypoxia in roadside populations of *D. sylvestris* (Chapter 6) suggests that populations are unable to respond to different selection pressures at the same time. Some families have a greater ability to tolerate drought, others salt and others hypoxia. The greater variability in responses in roadside families with increased stress tolerance when compared to oldfield families indicates that adaptive change is occurring but, is too slight to result in local adaptation.

In addition, selection pressures may vary in importance from year to year. In some years, drought tolerance may be the most important factor impacting plant success; other years, metal tolerance, herbivory tolerance, etc. This variation in environmental conditions from year to year also impacts seedling recruitment. In some years, survival to reproduction may be zero. Selection is not effective if mortality is 100 % as it can only differentiate between genotypes if there is variation in reproductive output. If the selection pressures are so strong that all genotypes are indiscriminately eliminated, no adaptations will be visible. Random variation in weather conditions had strong impacts on the survival of seedlings in the field. Severe droughts in two consecutive years resulted in exceptionally high mortality eliminating the opportunity to observe any adaptations to more benign roadside conditions.

In addition to variation in time, there is considerable heterogeneity in environmental conditions in space. It has frequently been suggested that for seedlings, micro-environmental conditions may overwhelm features of the macro-environment that impact the success of germinating seeds and juvenile plants. For example, seeds that land in a slight depression may have access to more water and be better buffered from diurnal temperature changes or the unpredictable weather of spring than a seed that has landed on the top of a mound of earth. The lack of adaptation found in the field experiments may reflect this unusual feature of seed and seedling ecology. Many studies have demonstrated that local adaptation can occur on extremely fine scales, from metres to mere centimetres (Stratton, 1994, Waser & Price, 1985). Adaptations at these fine scales would not be evident in this research as seeds were collected from three different locations per species and planted into a fourth area along the highway. Adaptations to local conditions that are not continuous along the entire roadside corridor or oldfield habitat type are not observable in this experimental design.

The selection of the study site may have had a profound influence on the outcome of this study. The site was flat to avoid the moisture gradient that would be present in a roadside ditch and was in very close proximity to three roads, (the 403 highway, an exit ramp and an overpass), which may have resulted in higher soil contamination than was present at the seed collection sites, which were typically bordering only the 403 highway. Examination of Na^+ levels at the roadside reciprocal transplant sites indicated that this site was receiving and retaining higher levels of salt than other roadside locations (Appendix C).

In addition to the abiotic differences in the field site, there was also an absence of the study species. The study could not be established inside an existing population of *D.*

sylvestris, *H. matronalis* or *A. syriaca* because there would be serious problems in ensuring the identity of germinating seeds, given the high seed rain for *D. sylvestris*, which reaches up to 1000 seeds/m^2. However, intraspecific competitors may influence the expression of adaptations. In *D. sylvestris* stands, germinating seeds and seedlings must compete not only with an extremely high density of other juvenile plants but also with adult plants for resources. Under these circumstances, the adaptive significance of salt tolerance may have been evident. This is particularly true for roadside *D. sylvestris* populations because the seeds do release a substance(s) that is inhibitory to the germination and growth of other seedlings (Chapter 7).

Finally, the timing of the establishment of this experiment may be responsible for the lack of observable adaptation. The experiment was established in the late spring after salinity levels had already reached their peak suggesting that the period of selection was simply missed. However, a second reciprocal transplant experiment established in the fall (Chapter 5), in which the seeds spent the winter and spring in the salty conditions of the roadside, also did not show any evidence of local adaptation in *D. sylvestris*. Subsequent observations of roadside populations of *D. sylvestris* have shown that there may be significant levels of germination in the fall and they may overwinter as juvenile plants rather than as seeds. However, the harsh conditions of the winter could severely reduce the probability of survival of seedlings to the spring. A future study could investigate the germination ecology of this species and if adaptations are seen if seeds germinate in the fall.

Though both oldfield and roadside genotypes of *H. matronalis* and *D. sylvestris* survived to reproduce in the oldfield, no adaptation to the oldfield environment was observed among oldfield genotypes. However, this does not indicate that adaptation has not occurred. In the absence of stress, many genotypes will perform equally well despite adaptation to harsher environments. The lack of adaptation of oldfield genotypes to the oldfield environment merely indicates that there has been no cost associated with adaptation to the roadside environment among roadside genotypes.

Unlike *H. matronalis* and *D. sylvestris*, no *A. syriaca* seedlings survived to reproduce in the oldfield environment. Unlike the other species, *A. syriaca* is a perennial rather than a monocarpic perennial and is not reliant on sexual reproduction via seeds, but is capable of reproducing by adventitious root buds. Both these characteristics relax selection pressures for survival during seedling establishment. Because plants reproduce over several seasons rather than in one fatal episode, a population will not be significantly harmed if, in any given year, conditions do not favour recruitment. In some years, like the year that this experiment was established, conditions may be extremely severe and no seedlings may survive. However, the plant may have successfully cloned via adventitious root budding and, even if this has not happened, the plant will be afforded the opportunity to reproduce in subsequent seasons.

The lack of adaptive response observed in this study perhaps provides a more interesting result than a positive response. It illustrates the complexity of not only the evolutionary process but the natural habitat as well. It provides perspective for the many examples of rapid evolution documented in laboratory experiments and demonstrates the importance of environmental complexity in the evolutionary process. Even populations subjected to strong selection pressures may not adapt to their local environment because of the impacts of conflicting pressures and the extreme variability of the natural environment compared to controlled laboratory conditions.

ACKNOWLEDGEMENTS

We would like to thank the Royal Botanical Gardens in Hamilton, Ontario for allowing seed collection on their conservation lands, Sid Lang at the Ontario Ministry of Transportation for help in obtaining permission to work on highway verges, Mary DeGuzman for assistance in seed collection and David Guevera and Maria Sanchez for field assistance.

TABLE 4.1: Life history of *D. sylvestris*, *H. matronalis* and *A. syrica* growing in oldfields and roadsides. Data reflect the percentage of seeds planted that emerged (E), survived until the end of the season (S), or reproduced (R).

		2000		2001		2002		2003	
Species	Site	% E	% S	% S	% R	% S	% R	% S	% R
D. sylvestris	Oldfield	60	33	22	2	19	13	1	0
	Roadside	58	37	2	0	0	0	0	0
H. matronalis	Oldfield	49	22	5	2	1	0	0	0
	Roadside	38	0	0	0	0	0	0	0
A. syriaca	Oldfield	25	0	0	0	0	0	0	0
	Roadside	39	0	0	0	0	0	0	0

TABLE 4.2: A Chi-Square analysis of the survivorship of *D. sylvestris* collected from three oldfield and three roadside populations and planted into either oldfields or roadsides in four years. There is no difference in the survivorship between either populations or sites within either the roadside or the oldfield.

	Site					Population				
		Oldfield		Roadside			Oldfield		Roadside	
	df	Logrank	Wilcoxon	Logrank	Wilcoxon	df	Logrank	Wilcoxon	Logrank	Wilcoxon
2000	1	0.1364	0.0914	0.4855	0.5135	5	5.970	5.5343	4.7888	46588
2001	1	0.8924	1.0104	0.0032	0.1473	5	5.1397	5.1285	0.8186	2.4272
2002	1	0.0139	0.2696	0.2013	0.2013	5	2.6968	1.9880	3.4229	3.4229
2003	1	0.1725	0.6034	---	---	5	2.6558	3.8417	---	---

TABLE 4.3: A multiple analysis of variance of the effects of transplant site and seed collection site (both fixed effects) and the random effects of population (nested within site), maternal family (nested within population and site), and their interactions with the transplant site on the number and length of leaves of *D. sylvestris* collected from ten maternal families from three populations in the roadside and three populations in the oldfield growing in the roadside and the oldfield. Plants that did not germinate or died before a particular census are not included in the analysis. Analyses are based on the Pillai's Trace test. (N = numerator, D = denominator, T = transplant site, S = collection site, P = population, M = maternal family).

	July, 2000		August, 2000		Early Sept., 2000		Late Sept., 2000		November, 2000	
Source	N,D df	*F*	N,D df	*F*	N,D df	*F*	N,D df	*F*	N,D df	*F*
T	2,3	32.70**	2,3	80.36 **	2,3	98.77 **	2,3	46.30 **	2,3	98.26 **
T×S	2,3	0.16	2,3	0.66	2,3	0.33	2,3	1.19	2,3	0.15
S	2,3	1.10	2,3	0.16	2,3	4.61	2,3	0.29	2,3	0.14
P	8,108	2.84**	8,108	2.39*	8,108	1.39	8,108	0.56	8,108	1.51
T×P	8,108	1.31	8,108	1.88†	8,108	1.63	8,108	1.24	8,108	0.82
M	8,108	1.05	108,108	1.04	108,108	1.12	108,108	1.03	108,108	1.06
T×M	108,978	1.18	108,884	0.89	108,770	0.91	108,724	1.23 †	108,592	0.98

† $P < 0.1$; * < 0.05; $P < 0.01$.

TABLE 4.3: Continued

	April, 2001		May, 2001		June, 2001		Early July, 2001		Late July, 2001	
Source	N,D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*
T	2,3	53.15**	2,3	28.76*	2,3	75.94**	2,3	85.43**	2,3	299.87 ***
T×S	2,3	0.77	2,3	0.07	2,3	3.28	2,3	0.47	2,3	0.78
S	2,3	1.70	2,3	2.83	2,3	0.54	2,3	1.13	2,3	2.82
P	8,108	1.02	8,108	1.12	8,108	1.73 †	8,108	0.96	8,108	0.92
T×P	8,92	0.74	8,92	1.02	8,92	0.72	8,90	0.73	8,80	0.65
M	108,92	1.02	108,92	0.78	108,92	0.94	108,90	0.99	108,80	1.03
T×M	92,354	0.97	92,332	1.39*	92,316	1.20	90322	0.96	80,254	0.86

** $P < 0.01$.

TABLE 4.3: Continued

	August, 2001		September, 2001		October, 2001		November, 2001		May, 2002	
Source	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*
T	2,3	23.27*	2,3	56.31**	2,3	16.42*	2,2	409.86**	2,3	26.15*
T×S	2,3	2.84	2,3	1.95	2,3	1.81	2,2	363.18**	2,3	0.29
S	2,3	0.22	2,3	0.41	2,3	4.01	2,3	7.68†	2,3	0.43
P	8,108	0.35	8,108	0.42	8,106	0.25	8,106	0.13	8,108	1.95†
T×P	8,26	0.88	8,18	0.77	8,8	0.42	6,6	0.72	8,108	1.07
M	108,26	0.90	108,18	2.63*	106,8	1.23	106,6	2.42	108,108	0.78
T×M	26,154	1.14	18,162	0.39	8,146	1.04	6,150	0.46	108,1992	1.00

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$.

TABLE 4.3: Continued

	June, 2002		July, 2002		August, 2002		September, 2002		May, 2003	
Source	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df/	*F*	N, D df	*F*
T	2,3	30.39 *	2,3	28.23*	2,3	37.00**	2,3	283.32***	----	----
T×S	2,3	0.24	2,3	0.46	2,3	1.18	2,3	39.22**	----	----
S	2,3	1.02	2,3	0.46	2,3	1.18	2,3	39.22**	2,1	0.02
P	8,108	1.09	8,108	0.73	8,108	1.71	8,108	0.69	4,6	2.25
T×P	8,108	0.97	8,108	0.73	8,108	1.71	8,108	0.69	----	----
M	108,108	0.98	108,108	1.00	108,108	1.00	108,108	1.00	----	----
T×M	108,1992	1.24 *	108,1992	0.96	108,1992	0.96	108,1992	1.11	----	----

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 4.4: A mixed model analysis of variance of the effects of the seed collection site on the height of bolting *D. sylvestris* collected from ten maternal families from three populations in the roadside and three populations in the oldfield growing in the oldfield. All individuals located in the roadside site died prior to bolting and reproduction.

Source	Num df	Den df	*F*
2001 – Site	1	9	5.15 *
2002 – Site	1	52	0.99
2003 – Site	1	5	0.24

* $P < 0.05$

TABLE 4.5: A mixed-model analysis of variance for random effects of the effects of the maternal family (nested within population and site) on the site on the height of bolting *D. sylvestris* collected from ten maternal families from three populations in the roadside and three populations in the oldfield growing in the oldfield. All individuals located in the roadside site died prior to bolting and reproduction. Effects with estimates of zero have been removed from the analysis. Population (nested within site) had an estimate of zero in all three seasons.

Source	Estimate	Error	*Z*
2001 – Maternal Family	---	---	---
2001 – Residual	---	---	---
2002 – Maternal Family	11.0611	123.51	0.09
2002 – Residual	721.95	163.89	4.41 ***
2003 – Maternal Family	511.65	324.23	1.58 †
2003 – Residual	0.9981	0	...

† $P < 0.1$; *** $P < 0.001$

TABLE 4.6: An analysis of variance of the effects of the seed collection site on the final vegetative, reproductive, and final biomass of bolting *D. sylvestris* and *H. matronalis* collected from ten maternal families from three populations in the roadside and three populations in the oldfield growing in the oldfield. All individuals of both species located in the roadside site died prior to bolting and reproduction. Too few *H. matronalis* were remaining in 2003 for analyses to be conducted.

	D. sylvestris			*H. matronalis*		
Source	df	Mean Square	*F*	df	Mean Square	*F*
All years - total biomass	1	644.976	2.96 †	1	37.660	0.48
All years – vegetative biomass	1	88.136	1.29	1	17.503	0.85
All years – reproductive biomass	1	180.736	3.83 †	1	7.904	0.44
2001 – total biomass	1	758.052	6.28 *	1	21.057	0.76
2001 – vegetative biomass	1	173.150	5.97 *	1	0.337	0.20
2001 – reproductive biomass	1	206.614	6.41 *	1	5.934	0.91
2002 – total biomass	1	294.202	1.25	1	8.294	0.97
2002 – vegetative biomass	1	67.349	0.95	1	3.248	1.30
2002 – reproductive biomass	1	80.028	1.61	1	1.162	0.60
2003 – total biomass	1	12.605	0.10	---	---	---
2003 – vegetative biomass	1	52.336	0.59	---	---	---
2003 – reproductive biomass	1	2.53	0.16	---	---	---

† $P < 0.1$; * $P < 0.05$.

TABLE 4.7: A Chi-Square analysis of the survivorship of *H. matronalis* collected from three oldfield and three roadside populations and planted into either oldfields or roadsides in four years. There is no difference in the survivorship between either populations or sites within either the roadside or the oldfield.

	Site					Population				
		Oldfield		Roadside			Oldfield		Roadside	
	df	Logrank	Wilcoxon	Logrank	Wilcoxon	df	Logrank	Wilcoxon	Logrank	Wilcoxon
2000	1	0.2299	0.0476	4.0149 *	1.7966	5	16.1328**	19.4719**	10.0178†	5.9312
2001	1	1.9712	1.9749	---	---	5	9.5156†	8.8066†	---	---
2002	1	2.7814†	3.8389†	---	---	5	14.3286*	13.8055*	---	---
2003	1	3.0101†	3.0101†	---	---	5	3.8117	3.0101	---	---

*† $P < 0.1$; * < 0.05; ** $P < 0.01$*

TABLE 4.8: A multiple analysis of variance of the effects of transplant site and seed collection site (both fixed effects) and the random effects of population (nested within site), maternal family (nested within population and site), and their interactions with the transplant site on the number and length of leaves of *H. matronalis* collected from ten maternal families from three populations in the roadside and three populations in the oldfield growing in the roadside and the oldfield. Plants that did not germinate or died before a particular census are not included in the analysis. Analyses are based on the Pillai's Trace test. (N = numerator, D = denominator, T = transplant site, S = collection site, P = population, M = maternal family).

	July, 2000		August, 2000		Early Sept., 2000		Late Sept., 2000		Nov., 2000		April, 2001	
Source	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*
T	2,3	31.93**	2,3	22.39*	2,2	26.38*	---	---	---	---	---	---
T×S	2,3	0.20	2,3	0.06	2,2	0.58	---	---	---	---	---	---
S	2,3	5.79†	2,3	0.25	2,3	0.28	2,3	0.09	2,3	0.85	2,3	0.17
P	8,102	2.04*	8,102	1.68	8,100	1.25	8,100	2.02 †	8,96	1.04	8,78	1.96 †
T×P	8,102	1.34	8,80	1.51	6,24	1.60	---	---	---	---	---	---
M	102,102	0.95	102,80	0.91	100,24	0.99	---	---	---	---	78,78	0.77
T×M	102,574	1.16	80,370	0.94	24,184	0.72	2,95	0.13	---	---	---	---

*† P < 0.1; * < 0.05; ** P < 0.01.*

TABLE 4.8: Continued

	May, 2001		June, 2001		Early July, 2001		Late July, 2001		August, 2001		September, 2001	
Source	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*
T	---	---	---	---	---	---	---	---	---	---	---	---
T×S	---	---	---	---	---	---	---	---	---	---	---	---
S	2,3	0.03	2,3	0.70	2,3	2.46	2,3	0.96	2,2	0.73	2,2	0.01
P	8,74	3.15**	8,74	2.70*	8,74	2.68*	8,70	1.92†	6,38	1.79	6,46	2.03†
T×P	---	---	---	---	---	---	---	---	---	---	---	---
M	74,70	0.75	74,76	0.84	74,78	0.93	70,66	1.34	38,18	0.96	46,14	1.52
T×M	---	---	---	---	---	---	---	---	---	---	---	---

† $P < 0.1$

TABLE 4.8: Continued

	October, 2001		November, 2001		May, 2002		June, 2002		July, 2002		August, 2002	
Source	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*
T	---	---	---	---	---	---	---	---	---	---	---	---
T×S	---	---	---	---	---	---	---	---	---	---	---	---
S	2,2	0.01	2,2	1.28	2,1	0.71	2,1	1.57	2,1	9.15	2,1	2.92
P	6,44	1.35	6,38	1.28	4,12	0.69	4,8	1.44	4,4	0.90	---	---
T×P	---	---	---	---	---	---	---	---	---	---	---	---
M	44,14	1.96†	38,10	0.61	---	---	---	---	---	---	---	---
T×M	---	---	---	---	---	---	---	---	---	---	---	---

* $P < 0.05$; ** $P < 0.01$.

TABLE 4.9: A mixed model analysis of variance of the effects of the seed collection site on the height of bolting *H. matronalis* collected from ten maternal families from three populations in the roadside and three populations in the oldfield growing in the oldfield. All individuals located in the roadside site died prior to bolting and reproduction. Too few plants were remaining in 2003 for analyses to be conducted.

Source	Num df	Den df	*F*
2001 – Site	1	14	0.11
2002 – Site	1	3	0.81

TABLE 4.10: A mixed-model analysis of variance for random effects of the effects of the maternal family (nested within population and site) on the site on the height of bolting *H. matronalis* collected from ten maternal families from three populations in the roadside and three populations in the oldfield growing in the oldfield. All individuals located in the roadside site died prior to bolting and reproduction. Effects with estimates of zero have been removed from the analysis. Population (nested within site) had an estimate of zero in all three seasons.

Source	Estimate	Error	*Z*
2001 – Population	---	---	---
2001 – Maternal Family	---	---	---
2001 – Residual	---	---	---
2002 – Population	80.2642	166.44	0.48
2002 – Maternal Family	295.61	140.53	2.10 *
2002 – Residual	57.8162	50.2526	1.15

* $P < 0.05$.

TABLE 4.11: A multiple analysis of variance of the effects of transplant site and seed collection site (both fixed effects) and the random effects of population (nested within site), maternal family (nested within population and site), and their interactions with the transplant site on the number and length of leaves of *A. syriaca* collected from ten maternal families from three populations in the roadside and three populations in the oldfield growing in the roadside and the oldfield. Plants that did not germinate or died before a particular census are not included in the analysis. Analyses are based on the Pillai's Trace test. (N = numerator, D = denominator, T = transplant site, S = collection site, P = population, M = maternal family).

	July, 2000		August, 2000		Early September, 2000	
Source	N, D df	*F*	N, D df	*F*	N, D df	*F*
T	2,2	0.05	2,3	0.14	2,1	0.80
T×S	2,2	0.54	2,3	0.23	2,1	0.14
S	2,3	0.43	2,3	1.96	2,3	0.02
P	8,106	1.80 †	8,104	1.78 †	8,66	2.95 **
T×P	6,10	0.92	8,10	2.86 †	---	---
M	106,10	0.38	104,10	1.69	---	---
T×M	10,320	3.44 ***	10,240	0.74	---	---

*† $P < 0.1$; ** $P < 0.01$; *** $P < 0.001$.*

FIGURE 4.1: The survivorship of *D. sylvestris* grown from seeds collected from oldfields and roadsides and planted into different oldfield and roadside locations. Reductions in population size that occur between the fall of one year and the spring of the next are primarily a result of plants reproducing rather than dying.

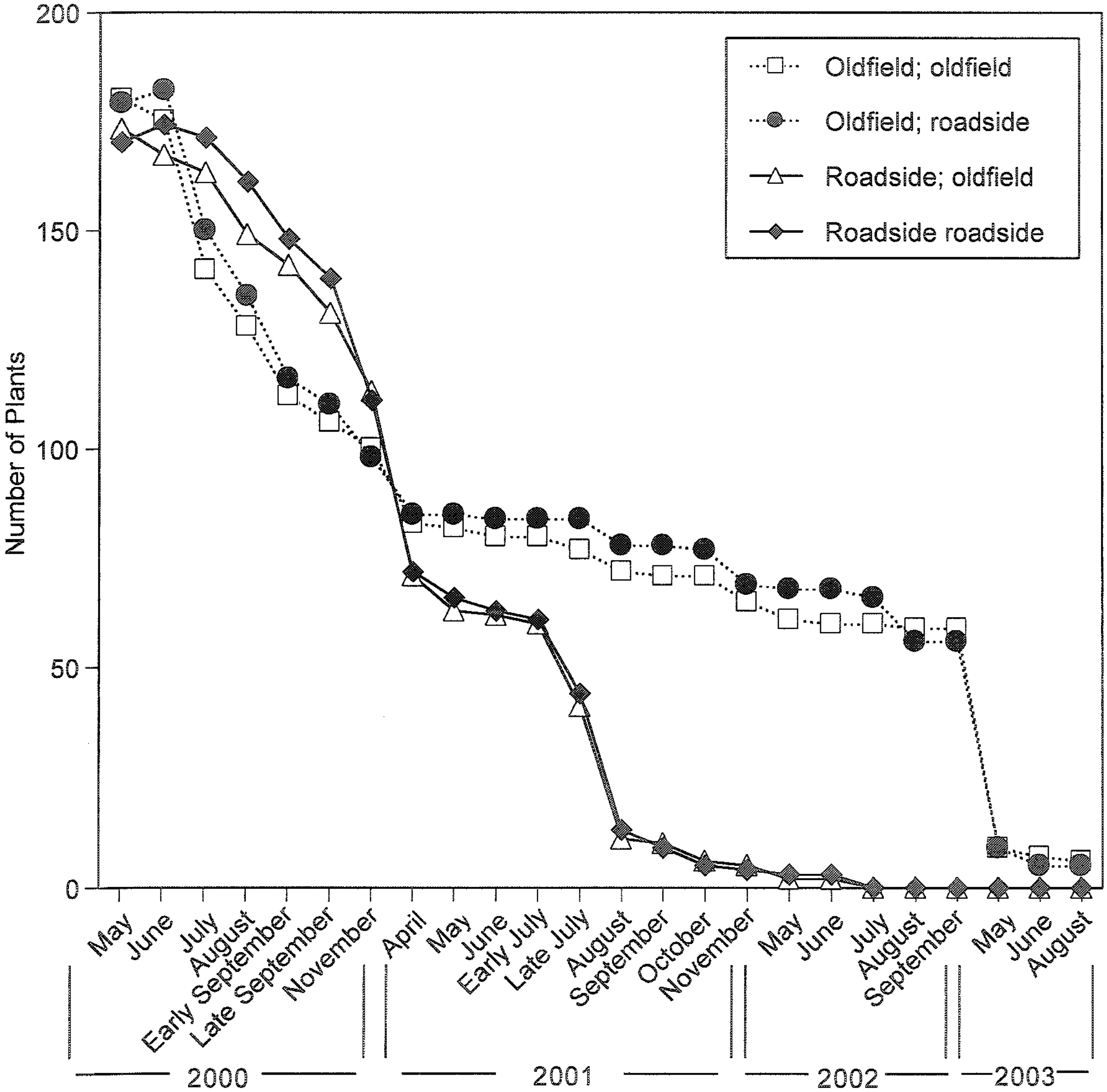

200
150
100
50
0
Number of Plants
Oldfield; oldfield
Oldfield; roadside
Roadside; oldfield
Roadside roadside
May
June
July
August
Early September
Late September
November
April
May
June
Early July
Late July
August
September
October
November
May
June
July
August
September
May
June
August
2000
2001
2002
2003

FIGURE 4.2: Change in the number (a) and length (b) of leaves of *D. sylvestris* seedlings grown from seeds that were collected from oldfields and roadsides and planted into different oldfield and roadside locations. Dead plants are not included.

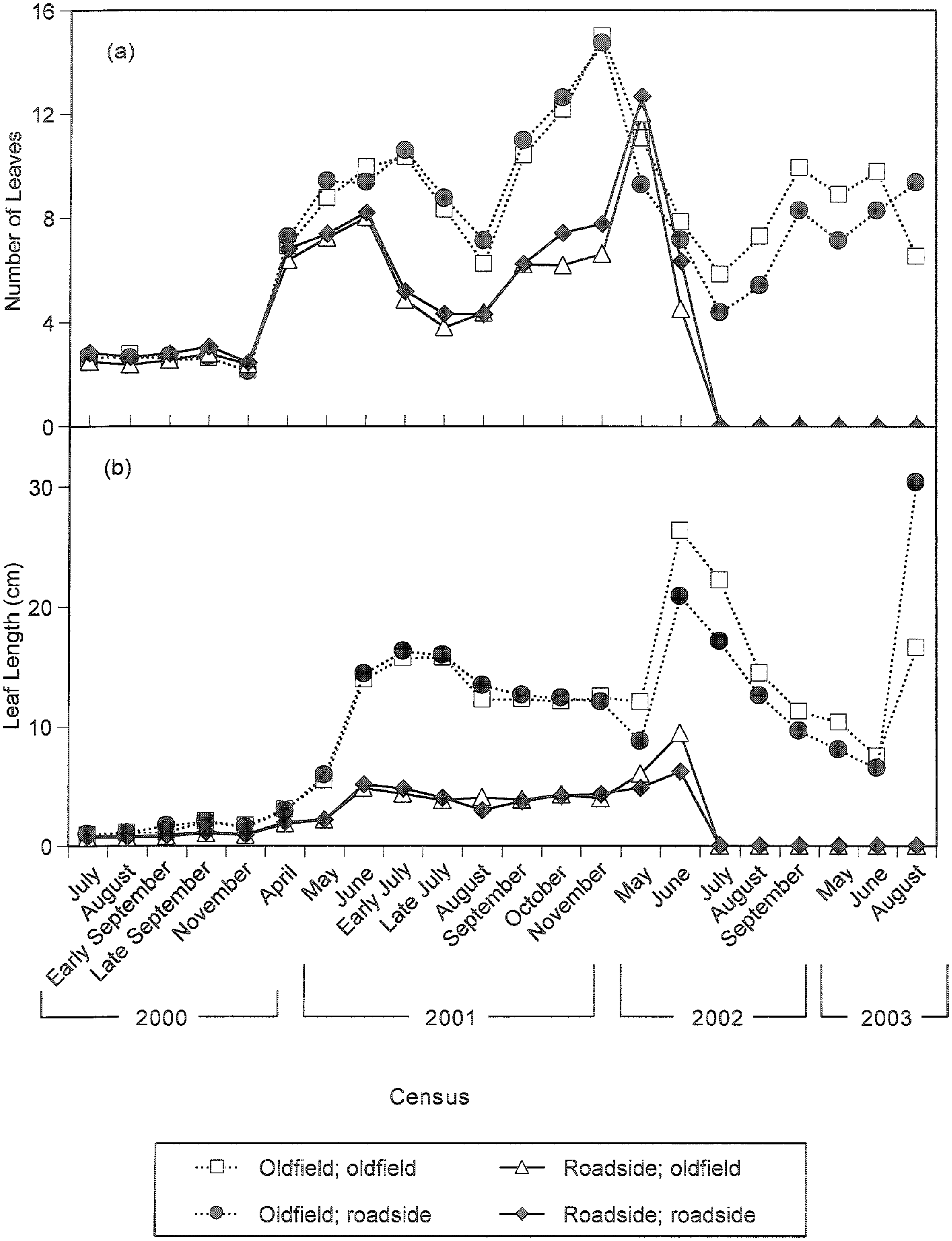

(a)
Number of Leaves
0
4
8
12
16
(b)
Leaf Length (cm)
0
10
20
30
July
August
Early September
Late September
November
April
May
June
Early July
Late July
August
September
October
November
May
June
July
August
September
May
June
August
2000
2001
2002
2003
Census
Oldfield; oldfield
Oldfield; roadside
Roadside; oldfield
Roadside; roadside

FIGURE 4.3: The final height of *D. sylvestris* plants grown from seeds collected from oldfields and roadsides and planted into oldfield and roadside locations. Plants bolted in three successive years. Only plants grown in the oldfield are shown as no individuals survived to reproduce in the roadside. * indicates a difference between oldfield and roadside biomass at $p < 0.05$. NS indicates no significant difference.

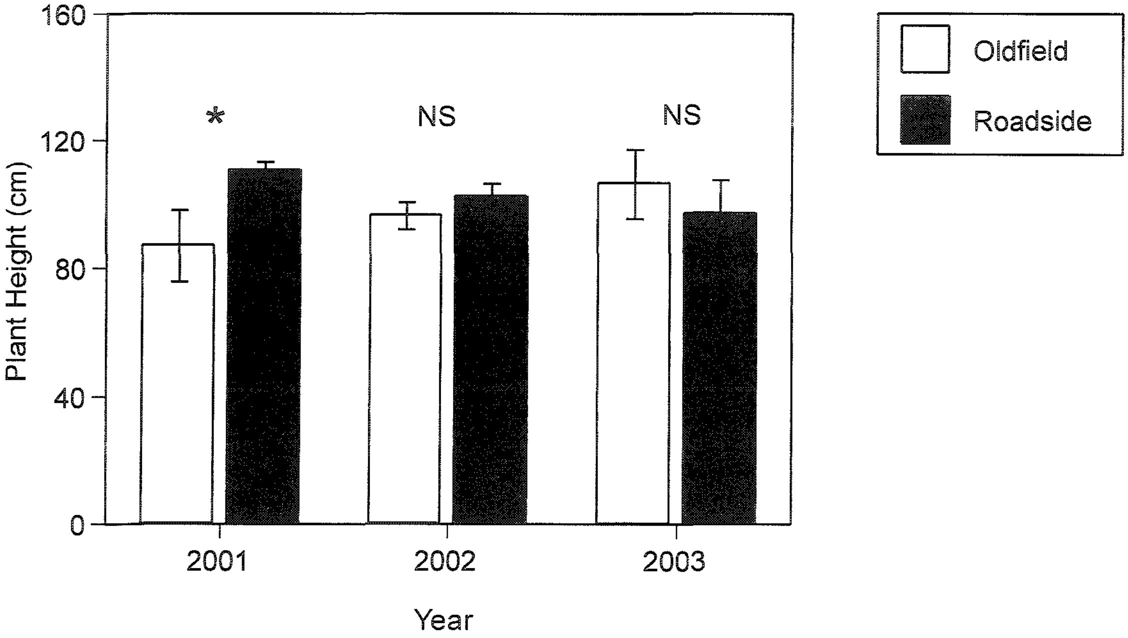
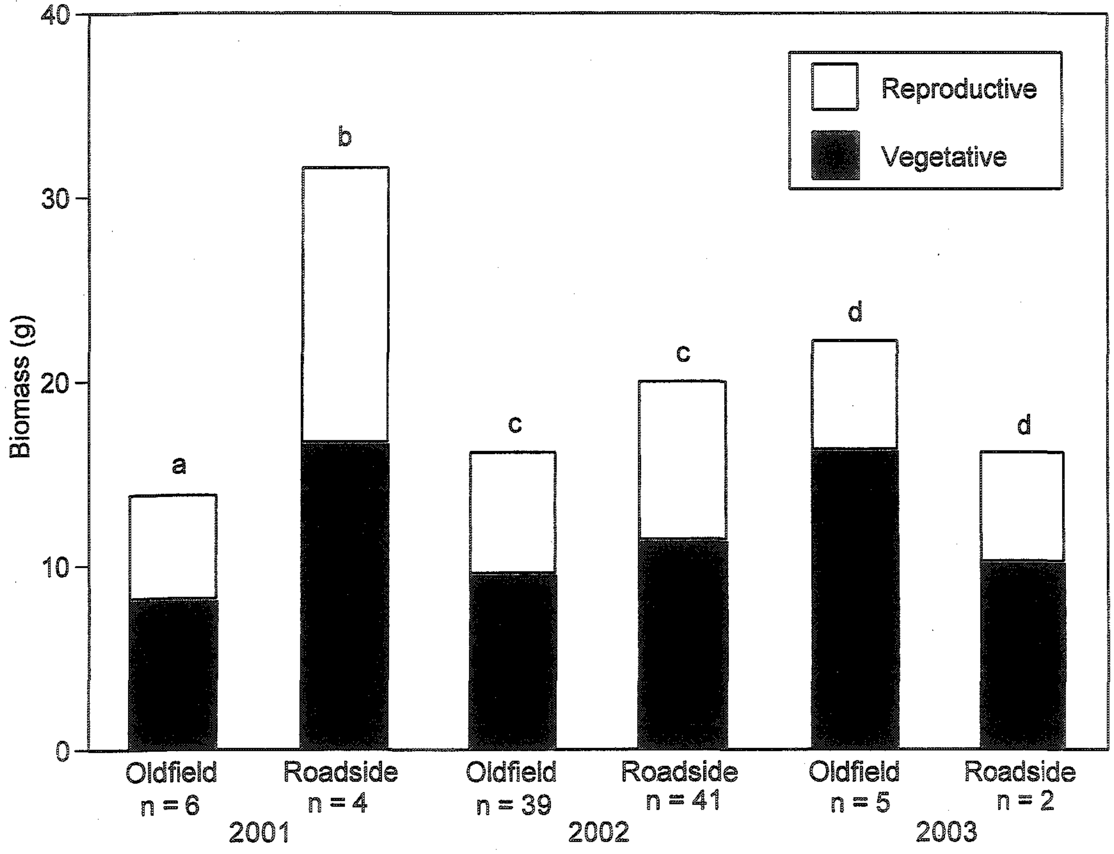
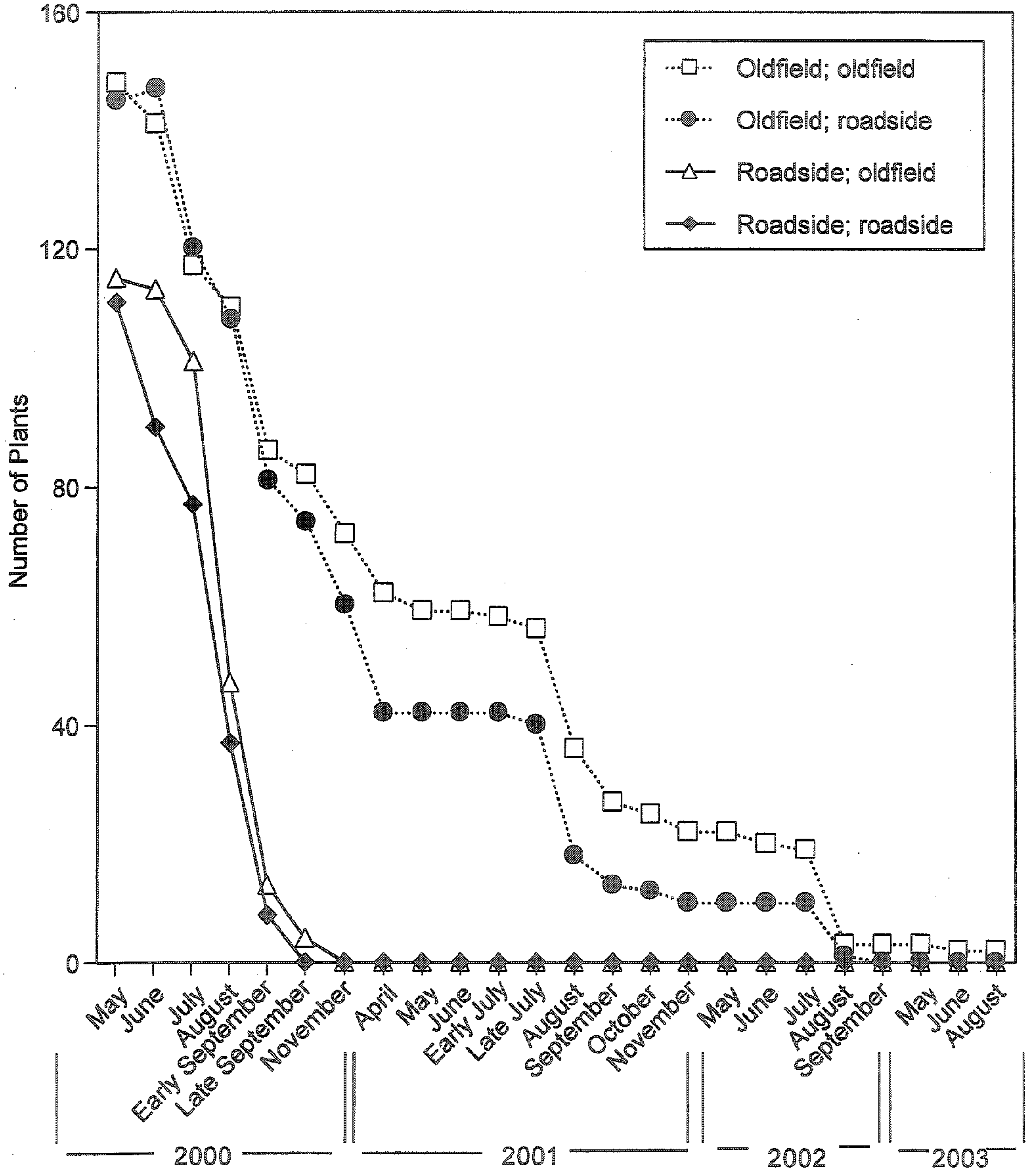

160
120
80
40
0
Plant Height (cm)
*
NS
NS
2001
2002
2003
Year
Oldfield
Roadside

FIGURE 4.4: The final biomass of *D. sylvestris* plants grown from seeds collected from oldfields and roadsides and planted into oldfield and roadside locations. Plant reproduced in three successive years. Only plants grown in the oldfield are shown as no individuals survived to reproduce in the roadside. Different letters indicate a significant difference between oldfield and roadside genotypes within a given year at a level of $p < 0.05$.



Biomass (g)
0
10
20
30
40
Reproductive
Vegetative
a
b
c
c
d
d
Oldfield
n = 6
Roadside
n = 4
2001
Oldfield
n = 39
Roadside
n = 41
2002
Oldfield
n = 5
Roadside
n = 2
2003

FIGURE 4.5: The survivorship of *H. matronalis* grown from seeds collected from oldfields and roadsides and planted into different oldfield and roadside locations. Reductions in population size that occur between the fall of one year and the spring of the next are primarily a result of plants reproducing rather than dying. No individuals survived in the roadside past the summer of 2000.



160
120
80
40
0
Number of Plants
Oldfield; oldfield
Oldfield; roadside
Roadside; oldfield
Roadside; roadside
May
June
July
August
Early September
Late September
November
April
May
June
Early July
Late July
August
September
October
November
May
June
July
August
September
May
June
August
2000
2001
2002
2003

FIGURE 4.6: The survivorship of *D. sylvestris* grown from seeds collected from three oldfield and three roadside populations and planted into different oldfield (a) and roadside locations (b). Reductions in population size that occur between the fall of one year and the spring of the next are primarily a result of plants reproducing rather than dying. No individuals survived in the roadside past the summer of 2000.

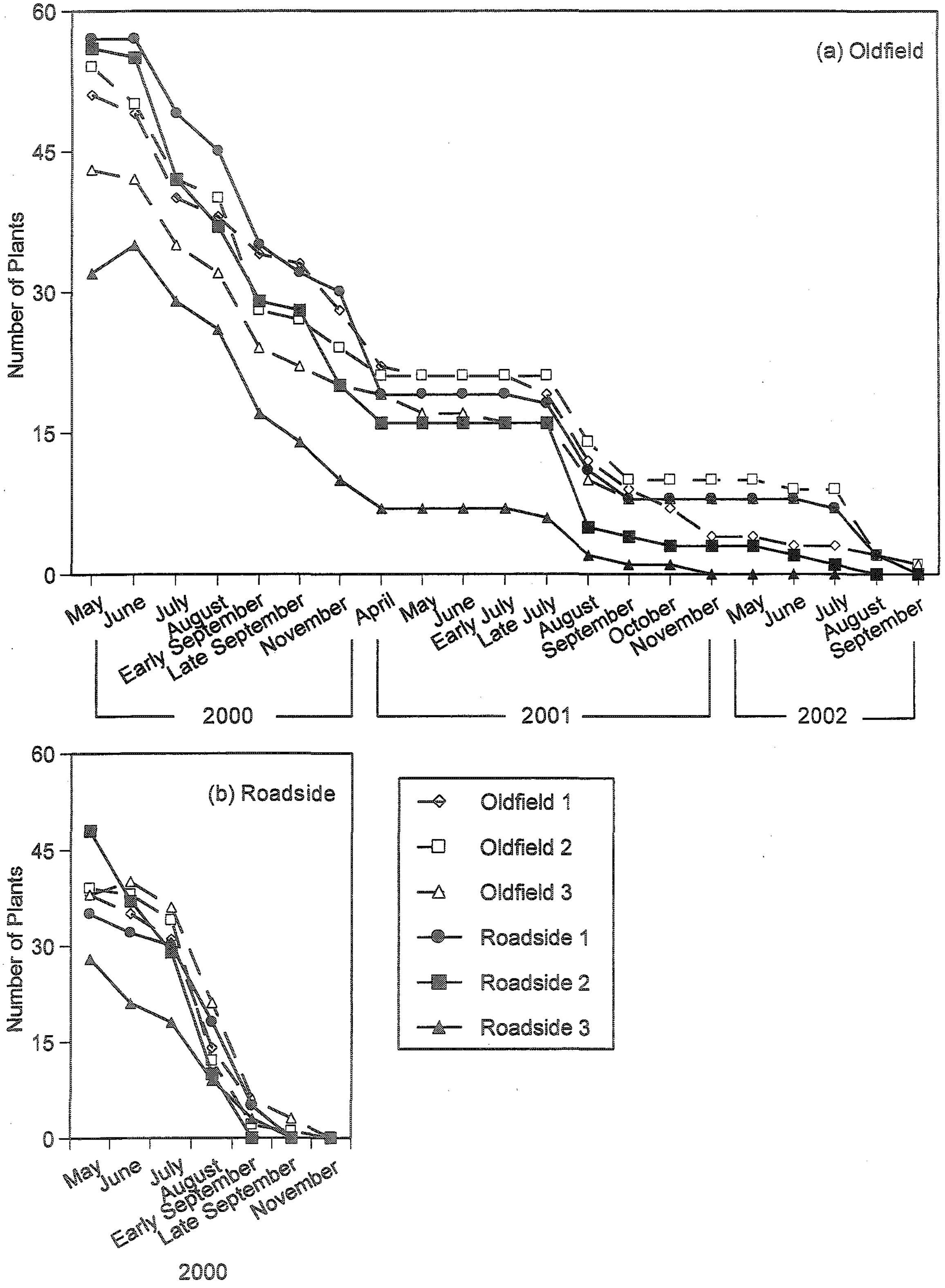

(a) Oldfield
Number of Plants
60
45
30
15
0
May
June
July
August
Early September
Late September
November
April
May
June
Early July
Late July
August
September
October
November
May
June
July
August
September
2000
2001
2002
(b) Roadside
Number of Plants
60
45
30
15
0
May
June
July
August
Early September
Late September
November
2000
Oldfield 1
Oldfield 2
Oldfield 3
Roadside 1
Roadside 2
Roadside 3

FIGURE 4.7: Change in the number (a and c) and length (b and d) of leaves of *H. matronalis* seedlings grown from seeds that were collected from oldfields (a and b) and roadsides (c and d) and planted into different oldfield and roadside locations. Dead plants are not included.

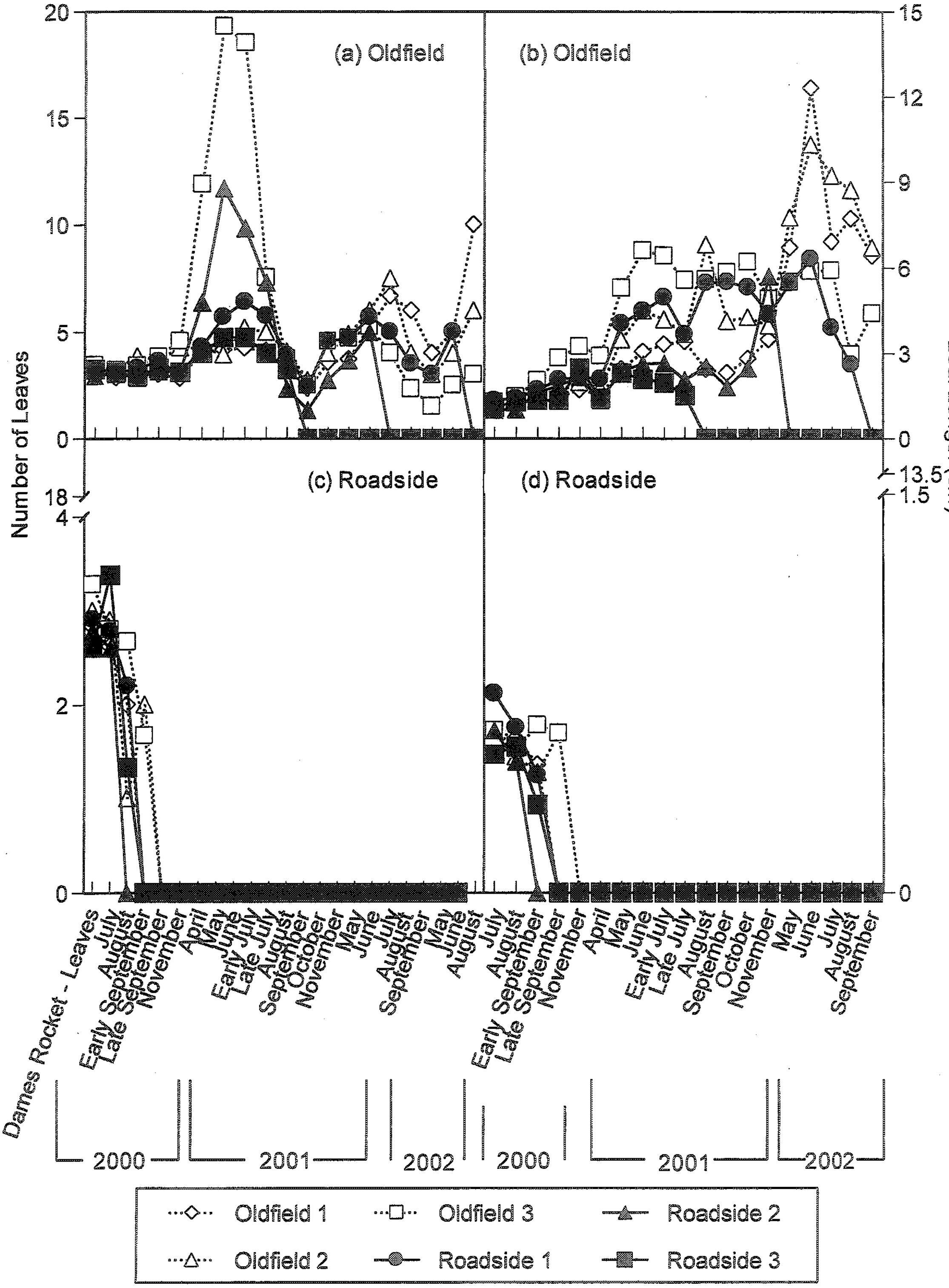

(a) Oldfield
(b) Oldfield
(c) Roadside
(d) Roadside
Number of Leaves
Leaf Length (cm)
20
15
10
5
0
18
4
2
0
15
12
9
6
3
0
13.5
1.5
0
Dames Rocket - Leaves
July
August
Early September
Late September
November
April
May
June
Early July
Late July
August
September
October
November
May
June
July
August
September
May
June
August
2000
2001
2002
2000
2001
2002
Oldfield 1
Oldfield 2
Oldfield 3
Roadside 1
Roadside 2
Roadside 3

FIGURE 4.8: The final height of *H. matronalis* plants grown from seeds collected from oldfields and roadsides and planted into oldfield and roadside locations. Plants bolted in two successive years. Only plants grown in the oldfield are shown as no individuals survived to reproduce in the roadside. NS indicates no significant difference between oldfield and roadside genotypes.

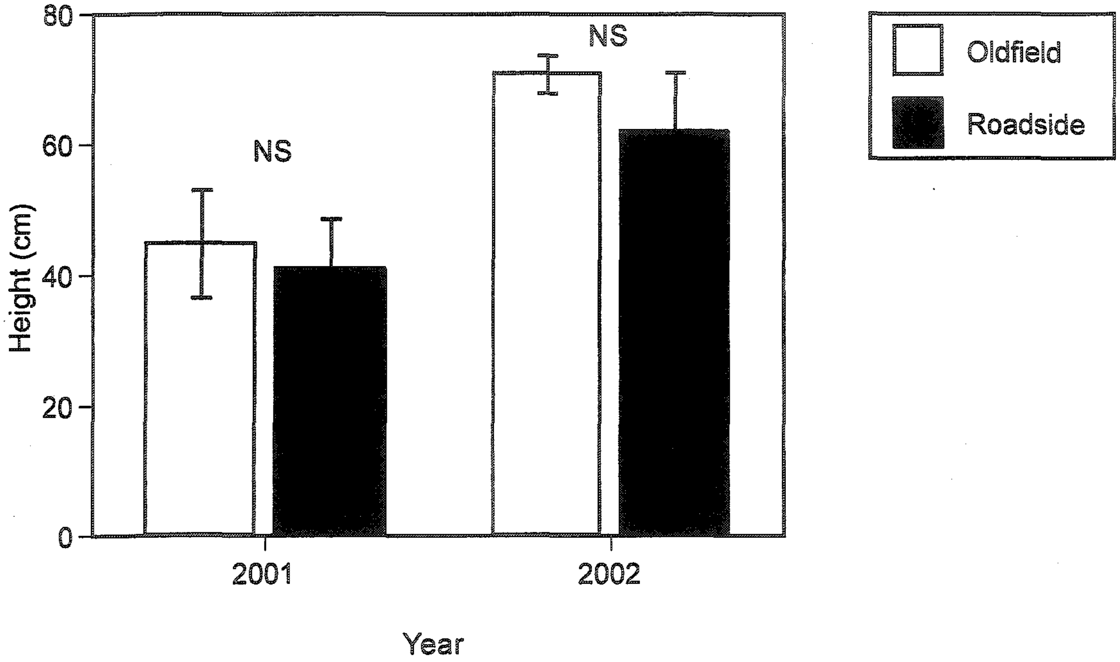

80
60
40
20
0
Height (cm)
NS
NS
2001
2002
Year
Oldfield
Roadside

FIGURE 4.9: The final biomass of *H. matronalis* plants grown from seeds collected from oldfields and roadsides and planted into oldfield and roadside locations. Plant reproduced in two successive years. Only plants grown in the oldfield are shown as no individuals survived to reproduce in the roadside. Different letters indicate a significant difference between oldfield and roadside genotypes within a given year at a level of $p < 0.05$.

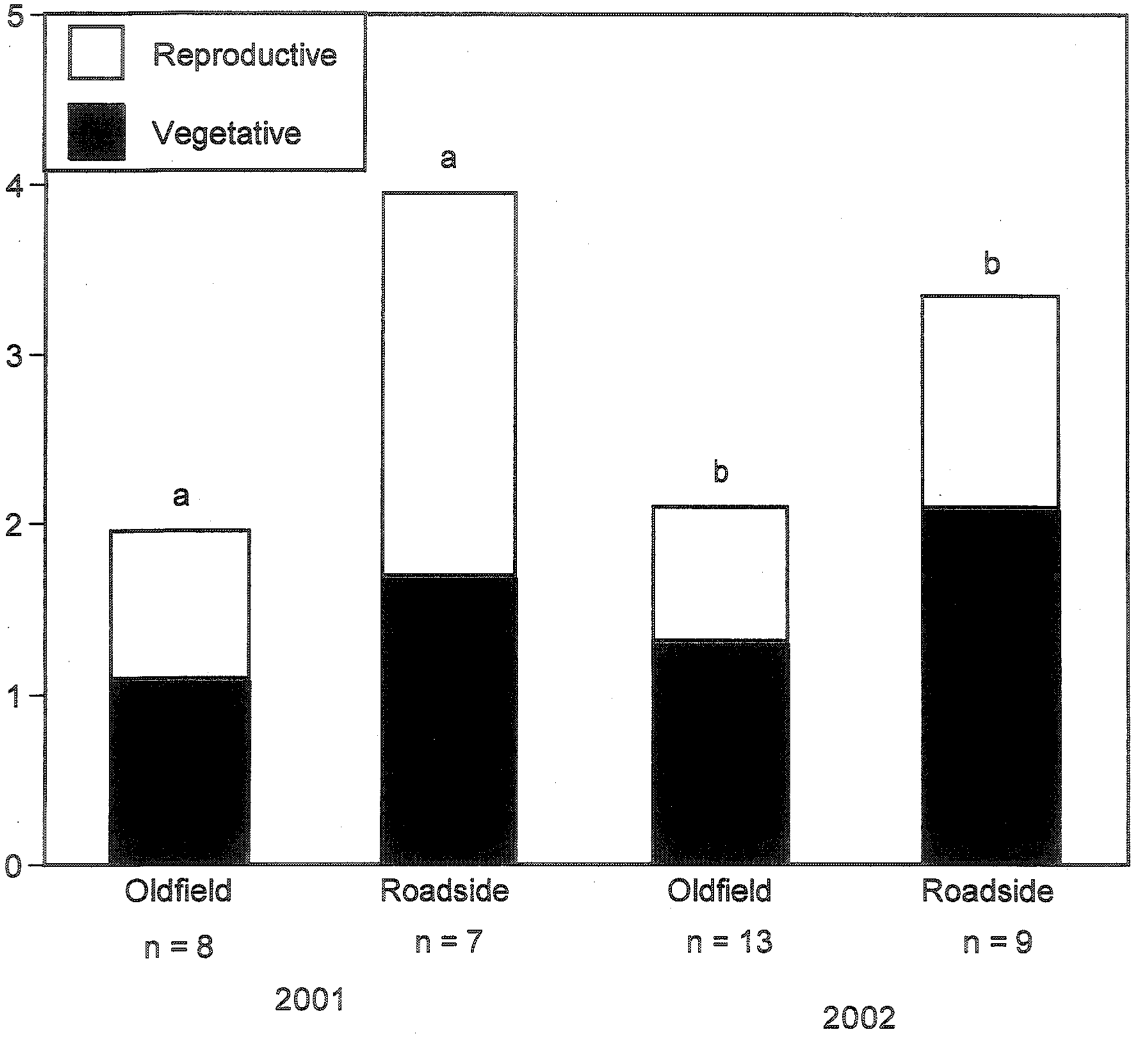

5
4
3
2
1
0
Reproductive
Vegetative
a
a
b
b
Oldfield
n = 8
Roadside
n = 7
Oldfield
n = 13
Roadside
n = 9
2001
2002

FIGURE 4.10: The survivorship of *A. syriaca* grown from seeds collected from oldfields and roadsides and planted into different oldfield and roadside locations. No individuals survived past the summer of 2000.

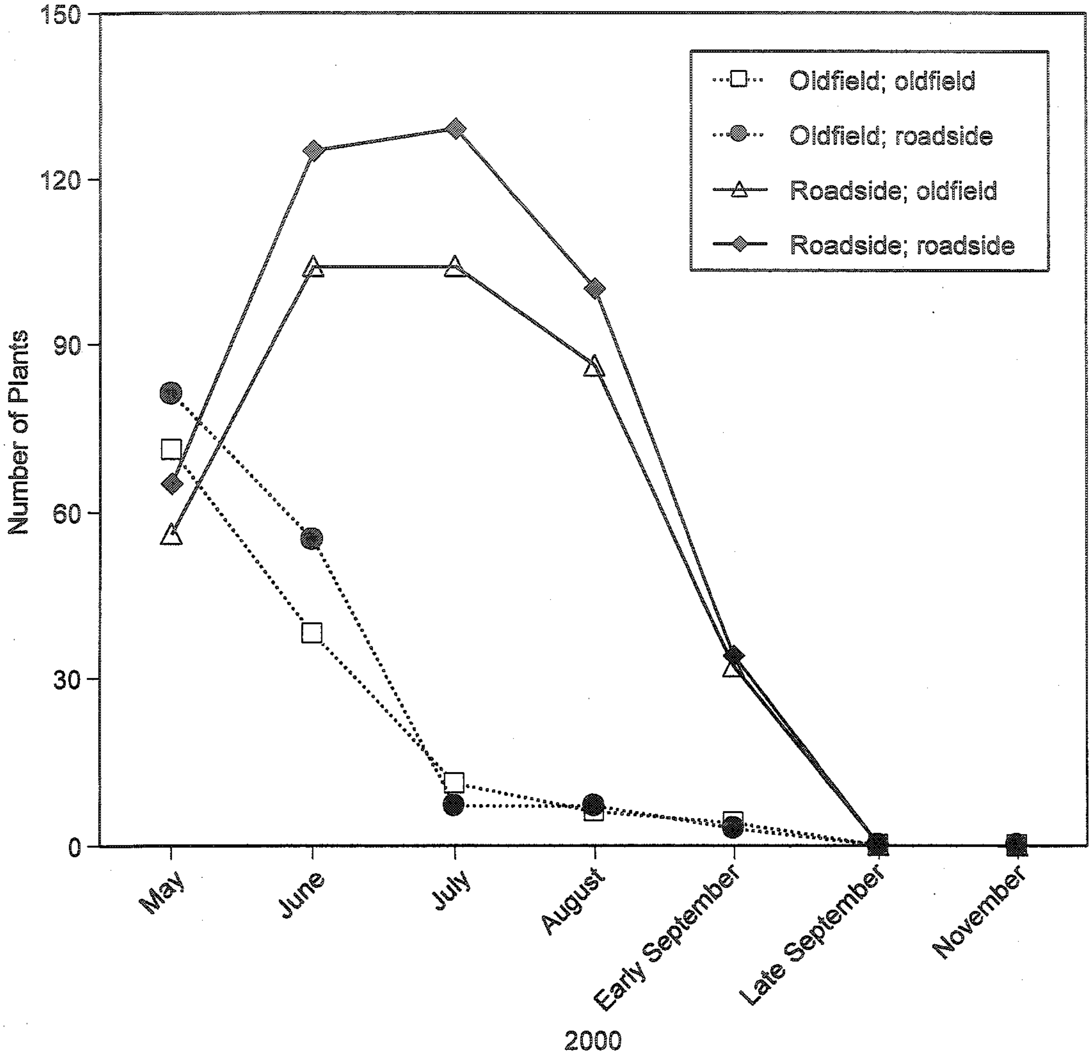

150
120
90
60
30
0
Number of Plants
Oldfield; oldfield
Oldfield; roadside
Roadside; oldfield
Roadside; roadside
May
June
July
August
Early September
Late September
November
2000

FIGURE 4.11: The survivorship of *D. sylvestris* grown from seeds collected from three oldfield and three roadside populations and planted into different oldfield (a) and roadside locations (b). No individuals survived past the summer of 2000.

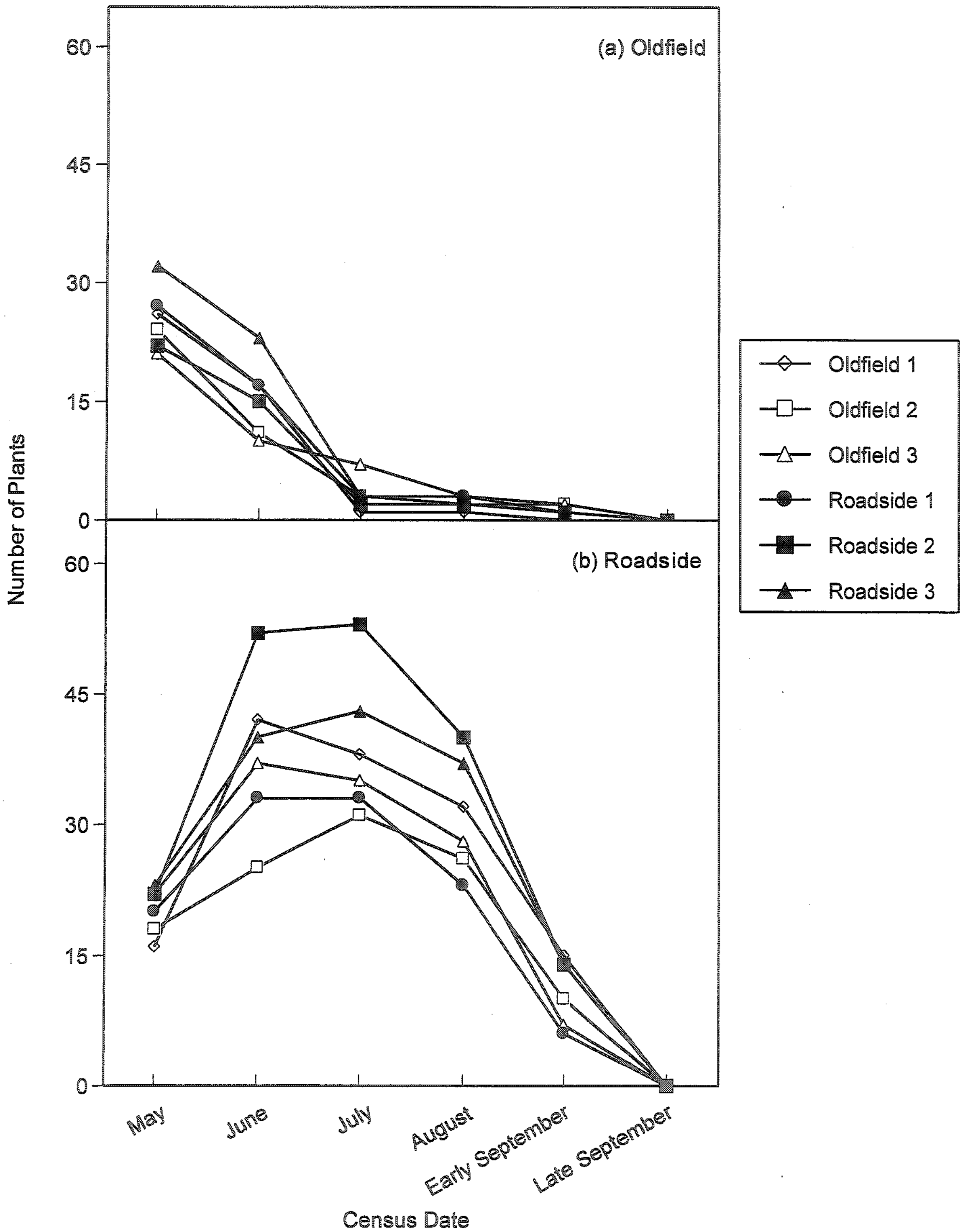
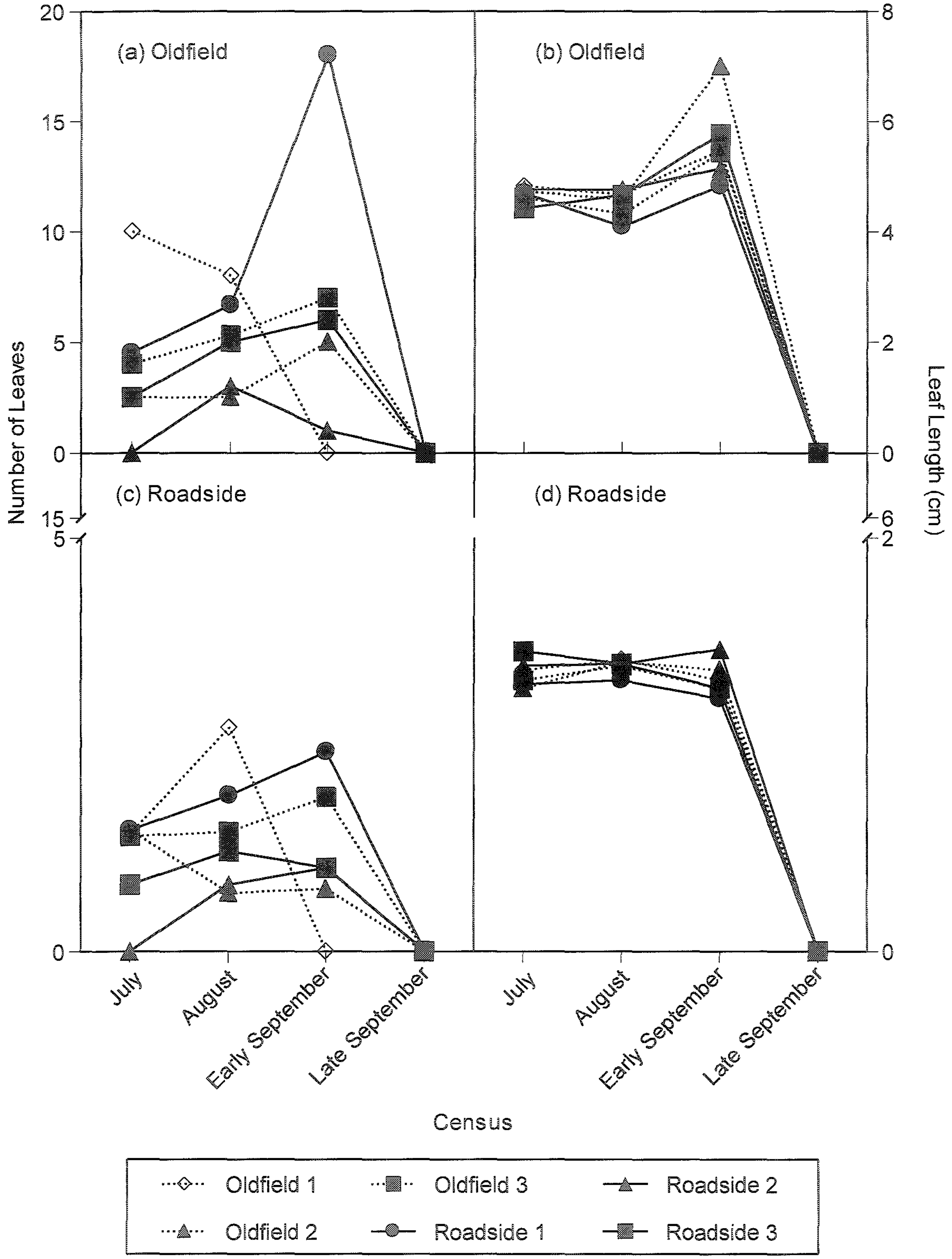

(a) Oldfield
(b) Roadside
Number of Plants
60
45
30
15
0
Census Date
May
June
July
August
Early September
Late September
Oldfield 1
Oldfield 2
Oldfield 3
Roadside 1
Roadside 2
Roadside 3

FIGURE 4.12: Change in the number (a and c) and length (b and d) of leaves of *A. syriaca* seedlings grown from seeds that were collected from oldfields (a and b) and roadsides (c and d) and planted into different oldfield and roadside locations. Dead plants are not included.


(a) Oldfield
(b) Oldfield
(c) Roadside
(d) Roadside
Number of Leaves
Leaf Length (cm)
20
15
10
5
0
15
5
0
8
6
4
2
0
6
2
0
July
August
Early September
Late September
Census
Oldfield 1
Oldfield 2
Oldfield 3
Roadside 1
Roadside 2
Roadside 3

SECTION V

ADAPTATION TO THE HIGH SALINITY OF WINTER AND SPRINGTIME ROADSIDE SOILS IN A COMMON ROADSIDE SPECIES *DIPSACUS SYLVESTRIS* HUDS.

5.1 ABSTRACT

Previous studies have demonstrated that seeds collected from roadside populations of *Dipsacus sylvestris* Huds. Are more tolerant of high salinity during germination than are seeds collected from oldfield populations (Beaton and Dudley 2004). However, a previous examination, which was established in the spring after salinity levels in the roadside had peaked, did not reveal any local adaptations to the roadside. In this study, we investigated the possibility that local adaptation to roadside conditions would be observed if the seeds were planted in the late fall or early winter. So that the seeds experienced the high salt levels of the winter and early spring, ten seeds from each of five maternal plants from three roadside and three oldfield populations were planted into two oldfield and two roadside sites in late November to early December. Throughout the spring and summer, the survivorship and size of the seedlings was monitored. A drought in July resulted in high mortality levels in both the oldfield and the roadside. Seeds collected from roadsides did not show adaptation to the roadside environment in either their survivorship or the size they attained. There was significant variation in performance at the population level. The success of the populations was associated with the average seed mass of the populations and did not vary between oldfield and roadside habitats, suggesting that maternal provisioning of the seed may play an important role in determining the fitness of seeds and seedlings during germination and seedling establishment.

5.2 INTRODUCTION

Local adaptation to adverse environmental conditions has been demonstrated in many different environments and in response to many different selection factors. In any given environment it is difficult to determine to which factors of the habitat a population may by responding, since soil moisture and composition, light intensity and nutrient availability are highly variable (Dudley, 2004). Roadside environments present many novel selection factors for organisms inhabiting them including soil contamination by a variety of metals, high soil salinity, air pollution and turbulence. Soil salinity in roadsides is highest in the spring (Thompson *et al.,* 1986). Many halophytic species inhabiting salt marshes and coastal environments only germinate after heavy rains have reduced the salinity of the soil (Gorham, 1992, Ungar, 1978). Species inhabiting roadsides in temperate areas where de-icing salt is most heavily applied, are less likely to delay germination and seedling establishment because the growing season is restricted in length. We, therefore, predict that individuals inhabiting roadsides that are better able to tolerate high salinity will likely have higher fitness than individuals lacking salt tolerance.

Recent studies of *Dipsacus sylvestris* have demonstrated that many populations inhabiting roadsides are adapted to the high salinity of the soil (Beaton and Dudley 2004). Many seeds collected from roadside populations were unaffected by the addition of salt during germination, as was indicated by root length, and some were even able to grow longer roots in salt than in control solutions. This is in sharp contrast to seeds collected from a less polluted environment, oldfields, that consistently exhibited sharp declines in root length in saline solutions. However, it is unclear if the observed tolerance to salt in laboratory experiments would translate into increased fitness during germination in the roadside environment. In this study, we examine roadside and oldfield populations for local adaptation to roadside environmental conditions during germination by conducting a reciprocal transplant experiment. This type of experiment entails collecting genotypes from different environments and comparing the fitness of each genotype in its own environment and the environment of different genotypes to determine if each population has adapted to its home environment. In a previous reciprocal transplant experiment in which *D. sylvestris* seeds collected from oldfield and roadside locations were planted into the oldfield and the roadside in the spring, no evidence of local adaptation was discovered (Chapter 4). Salt application begins with the first snowfall and continues throughout the winter. Unlike salt sensitive species, salt tolerant species are able to endure exposure to high salinity as dormant seeds and germinate successfully (Ungar, 1978). Exposure to salt throughout the winter months may have a strong impact on the seeds and may be the stage at which the selection event occurs.

In this study we planted 600 oldfield and 600 roadside seeds into two oldfield and two roadside sites. The size of the plants was recorded throughout the spring and summer and was used as a measure of fitness. We ask the following questions: (1) Are *D. sylvestris* families collected from roadside populations better able to grow in roadsides during germination and seedling establishment than seeds collected from oldfield environments indicating local adaptation?; (2) Is there variability for tolerance to winter roadside conditions among populations?

5.3 METHODS

5.3.1 *Study Species*

Dipsacus sylvestris (common teasel), a native of Europe, is found primarily in oldfields and roadsides. It grows vegetatively as a rosette for at least one growing season. In the spring, if the plant has reached a critical size, it will bolt to its reproductive form of an upright flowering stem. Flowering occurs from July to September and seeds are shed throughout the fall. A single plant can produce upwards of 3000 seeds (Werner, 1975).

5.3.2 *Seed Collection*

Six *D. sylvestris* populations were chosen (three bordering a major highway (highway 403 between Hamilton and Burlington, Ontario) and three located in oldfields) for study. Highway 403 is a six lane highway carrying 45000 to 75000 automobiles/day (Transportation, 1994). Highway populations were located in full sunlight, 2 – 10 m from the pavement's edge. At all the roadside sites, the surrounding vegetation was primarily various species of grass and/or crown vetch (which is planted following highway construction to stabilize the soil) with a few common oldfield plants (e.g. *Solidago* sp., *Daucus carota*). Oldfield populations were located on Royal Botanical Gardens conservation lands in full sunlight. The surrounding vegetation was dominated by grass with a wide variety of forbs. Seeds were collected from 15 – 20 randomly selected plants from each population. Following collection, seeds were processed and stored in paper envelopes at room temperature.

5.3.3 *Study Sites*

The experiment was conducted at Rock Chapel, an oldfield located on Royal Botanical Gardens conservation lands and at a roadside location near the Aldershot Go Station on the side of the 403 between Hamilton and Burlington. The oldfield site was unshaded and dominated by a variety of grasses and other herbaceous plants including *Solidago* spp. And *Asclepia syriaca*. *Dypsacus sylvestris* was found in large numbers 10 – 15 m from the site. The roadside site was located behind a guardrail and within 5 m of the edge of the highway. The site was level (i.e. was not sloping into a ditch), unshaded, and the vegetation was dominated by grass with only a few other herbaceous plants including *Asclepias syriaca* and *Linaria vulgaris*. *Dipsacus sylvestris* was found in large numbers at a distance of approximately 20 – 30 m from the edge of the highway.

5.3.4 *Experimental Design*

Five seeds from each of ten maternal families from each population collected from each site were planted randomly into a 3 m X 1 m grid at two locations in both the oldfield site and the roadside site. The 300 seeds in each grid were placed in 30 rows of ten and spaced 10 cm apart. The seeds were planted in late November to mid December to prevent germination of the seeds in the fall. Plastic screws marked the location of the seeds. Data was collected approximately every three weeks from April until September.

In July, a prolonged drought resulted in high plant mortality. Data collected included survivorship, number of leaves and the length of the largest leaf. At the time of the first census in April, many of the seedlings had only their cotyledons.

The average seed weight of each of the ten maternal families from each population was determined by weighing one hundred seeds from each maternal family in ten groups of ten seeds.

5.3.5 *Statistical Analyses*

Statistical analysis was conducted using SAS version 8.2 for Windows. Only one roadside site is considered in the following analyses because although two roadside sites were established, less than 5 % of the seeds at the second site germinated. However, removal of the second roadside site resulted in an unbalanced experimental design. Multivariate analyses of variance of the number and length of the leaves of plants growing in the two oldfield sites revealed that there was no difference between the oldfield sites ($F = 0.25$; $p = 0.7980$). There was also no overall difference between the genotypes at the site level ($F = 1.24$; $p = 0.4054$), the population ($F = 1.01$; $p = 0.4472$) or the maternal family ($F= 1.06$; $p = 0.4721$). Nor were there any significant interactions between the oldfield site and the genotype at the site level ($F = 0.06$; $p = 0.9442$), the population ($F=1.27$; $p=0.3344$) or the maternal family ($F=1.15$; $p = 0.3470$). The two oldfield sites are, hence, pooled together in subsequent analyses.

Survivorship data was analyzed using the Wilcoxon test and the Logrank test (PROC LIFETEST). The Wilcoxon test is more sensitive to differences in early survivorship while the log-rank test is more sensitive to differences in the later stages of the study (Scariot, 2000). Multivariate analyses of variance were conducted to determine the effect of transplant site and collection site on the number and length of leaves and to analyze the level of variation between populations and maternal families. The experimental design is composed of a hierarchical structure of maternal families within populations and populations within sites necessitating the use of nested models. Population, maternal family and their interaction with the transplant site are considered random variables, while the transplant site and collection site are considered fixed effects. A nested analysis of variance (PROC GLM) was conducted to examine variation in the seed mass between the sites and the populations. The p-diff and t-diff options were used to compare seed mass between the six populations.

5.4 RESULTS

Roadside and oldfield seeds did not differ in survivorship in either the roadside (Wilcoxon $X^2 = 0.0001$; $p = 0.9915$; log-rank $X^2 = 0.0001$ $p = 0.9943$) or the oldfield environment roadside (Wilcoxon $X^2 = 1.6012$; $p = 0.2057$; log-rank $X^2 = 0.8743$; $p = 0.3498$) (Figure 5.1). Germination levels and early seedling survivorship levels were higher in the roadside than in the oldfield (Wilcoxon $X^2 = 48.3783$; $p < 0.0001$; log-rank $X^2 = 12.0996$; $p< 0.0005$) (Figure 5.1). However, as a result of a prolonged drought, survivorship levels dropped substantially in both the roadside and the oldfield from July to September. The roadside, however, suffered significantly higher mortality levels and had fewer surviving plants than the oldfield following the drought.

Individuals growing in the roadside, of both oldfield and roadside origin, initially had more leaves than those growing in the oldfield (Figure 5.2, Table 5.1). However, following the drought in June-July, plants inhabiting the roadsides were significantly smaller than those in the oldfield (Figure 5.2, Table 5.1). However, there was no size difference between plants of oldfield origin and plants of roadside origin in either site (Figure 5.2, Table 5.1). Prior to July, (Figure 5.1 and 5.2), there was a significant level of variation between populations (Table 5.1). However, there was no interaction between the population and the transplant site indicating no adaptation at the population level (Table 5.1). There was some initial variation in size between the maternal families (Figure 5.2, Table 5.1). However, there was no interaction between the family and the transplant site indicating no adaptation at the maternal family level. (Table 5.1).

There was significant variation in the seed mass between the different populations (F = 55.33; p = 0.0001) but not between the sites (F = 1.02; p = 0.3690) (Figure 3). Roadside populations two and three had larger seeds than the other populations. However, roadside one had smaller seeds than two of the oldfield populations.

5.5 DISCUSSION

Previous studies have demonstrated that *D. sylvestris* seeds collected from the roadside are more tolerant of high salinity during germination than seeds collected from oldfields (Beaton and Dudley 2004). However, the adaptive significance of this trait was not demonstrated under field conditions. Roadside seeds did not perform better than oldfield seeds in the saline soil of the roadside during germination. There are two possible explanations for this anomaly. First, unlike previous germination experiments, which were conducted in petri dishes, we were not able to use root length as an indicator of performance as it would have resulted in seedling mortality. Instead, we used cotyledon expansion during the early stages of seedling establishment and later the number and length of the leaves as indicators of fitness. Previous studies have shown that although the morphology is affected, the size of the cotyledons is not impacted by high salinity during germination. Our reliance on cotyledon length as a measure of performance made observing differences in performance during the early stages of growth highly unlikely. Adaptations to environmental stresses may only be observable at particular stages of development (Jordan, 1992). Kindell *et al.* (1996) found that as a result of changing environmental conditions, local adaptation in *Aristida stricta* was only observable during the latter stages of development. Any early advantages obtained by roadside plants as a result of an increased ability to tolerate high salinity may not be observable once the seedlings have been established and begun vegetative growth.

Second, the exposure to the highly saline conditions of the roadside soil over the winter may have prepared the oldfield seeds for tolerating the high salinity of the soil in the spring. Ramagopal (1990) observed in barley that when seeds imbibe salt water they produce different proteins than when they imbibe distilled water. Overwintering the seeds in the roadside may physiologically alter the composition of the seeds making them more salt tolerant during the spring. Leaching the soluble contents of *D. sylvestris* seeds collected from the roadside prior to salt exposure significantly reduced the ability of many families to tolerate salt (Beaton and Dudley in review). The leaching not only removed some of the seed contents, but also caused the seeds to imbibe water rather than salt water prior to salt exposure. The loss of salt tolerance suggests that either the seed composition was responsible for the salt tolerance or that imbibition of salt water may prepare the seed for germination in high salinity. Possibly exposing oldfield seeds to sodium prior to germination in salt water prepares the germinating embryo for high salinity.

Prior to the drought, the performance of the six populations in both the oldfield and the roadside can be summarized as follows: roadside 3 > roadside 2 and oldfield 2 > roadside 1 and oldfield 3 > oldfield 1. There is little variation in this pattern between the roadside and oldfield. Interestingly, the average seed mass of the six populations coincides almost identically with the performance measures (roadside 3 > roadside 2 and oldfield 2 > oldfield 3 > roadside 1 > oldfield 1) (Figure 3). Seed size is a critical factor determining competitive outcome and fitness in plants. Turnbull *et al.* (1999) demonstrated that species that produce large seeds are competitively superior to species that produce small seeds when planted at high density. Several other studies have demonstrated that within a species, individuals that germinated from small seeds were significantly smaller plants than individuals that germinated from large seeds (Cideciyan & Malloch, 1982, Stanton, 1984). Any adaptation to the roadside or oldfield could potentially have been surpassed by the impact of seed size on performance.

Nitrogen is the limiting nutrient in many terrestrial ecosystems (Nasholm, 1998). In roadsides, however, the combustion of fossil fuels has substantially increased the level of available nitrogen in the soil (Port & Thompson, 1980, Spencer & Port, 1988). Because of this, plants growing in roadside soils are often larger than plants growing in undisturbed habitats (Spencer & Port, 1988). In addition to growing larger leaves and stems, plants growing under high nutrient conditions have also been shown to produce larger seeds (Aarssen & Burton, 1990, Galloway, 2001, Wulff, 1986). The larger size of the seeds collected from roadsides 2 and 3 may have resulted from high nitrogen levels found in the soil.

Despite the finding that roadside families have elevated tolerance to low water potential (Chapter 6), there is little evidence here to suggest that roadsides may be better able to withstand drought. The main impacts of the drought are seen from July to September, the point at which the effect of the population loses significance (Table 5.2). In addition, all the variation in performance is found at the population level. In contrast, the variation in tolerance of low water potential is found at the site and maternal family levels but not at the population level (Chapter 6).

In order to prevent germination in the fall, this experiment was established in late November to mid December. However, the lack of germination requirements of *D. sylvestris* may indicate that germination actually occurs in the fall immediately following dispersal rather than in the spring. Indeed, subsequent observations of *D. sylvestris* in the field have revealed the presence of newly germinated seedlings in the fall. Plants germinating in the fall are still impacted by high salinity levels in many roadside sites as compared to non-roadside sites. However, the levels are substantially lower than in the spring. The ability to tolerate the exceptionally high salinity levels of the winter soil as a seedling rather than as a seed may be the stage at which the demonstrated salt tolerance of roadside *D. sylvestris* populations could be observed in the field.

ACKNOWLEDGEMENTS

I would like to thank the Royal Botanical Gardens in Hamilton, Ontario for allowing seed collection on their conservation lands, Sid Lang at the Ontario Ministry of Transportation for help in obtaining permission to work on highway verges, Mary DeGuzman for assistance in seed collection and Maria Sanchez for extensive field assistance.

TABLE 5.1: A multiple analysis of variance of the effects of transplant site and seed collection site (both fixed effects) and the random effects of population (nested within site), maternal family (nested within population and site), and their interactions with the transplant site on the number and length of leaves of *D. sylvestris* collected from five maternal families from three populations in the roadside and three populations in the oldfield growing in the roadside and the oldfield. Plants that did not germinate or died before a particular census are not included in the analysis. Analyses are based on the Pillai's Trace test. (N = numerator, D = denominator, T = transplant site, S = collection site, P = population, M = maternal family).

	April		May		June		July		August		September	
Source	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*	N, D df	*F*
T	2,3	44.80 **	2,3	17.61 *	2,3	6.86 †	2,3	1118.79 ***	2,3	7.82 †	2,1	31.98
S	2,3	0.53	2,3	0.82	2,3	6.81 †	2,3	6.56 †	2,3	3.45	2,3	0.74
T×S	2,3	0.65	2,3	1.15	2,3	0.66	2,3	1.14	2,3	0.65	2,1	5.03
P	8,48	3.23 **	8,48	4.45 ***	8,48	2.48 *	8,48	2.11 †	8,44	1.00	8,40	0.92
T×P	8,48	1.12	8,48	1.89	8,48	1.99 †	8,48	1.12	8,24	1.26	4,6	0.79

M	48,48	2.07 **	48,48	1.56	48,48	1.43	48,48	1.16	44,24	1.18	40,6	1.71
T×M	48,970	1.13	48,830	0.75	48,866	0.72	48,676	0.65	24,82	0.82	6,74	0.49

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

FIGURE 5.1: The survivorship of *D. sylvestris* seedlings that were germinated from seeds that were collected from oldfields and roadsides and planted into different oldfield and roadside locations. In July, a severe drought caused extremely high mortality of seedlings in both oldfields and roadsides.

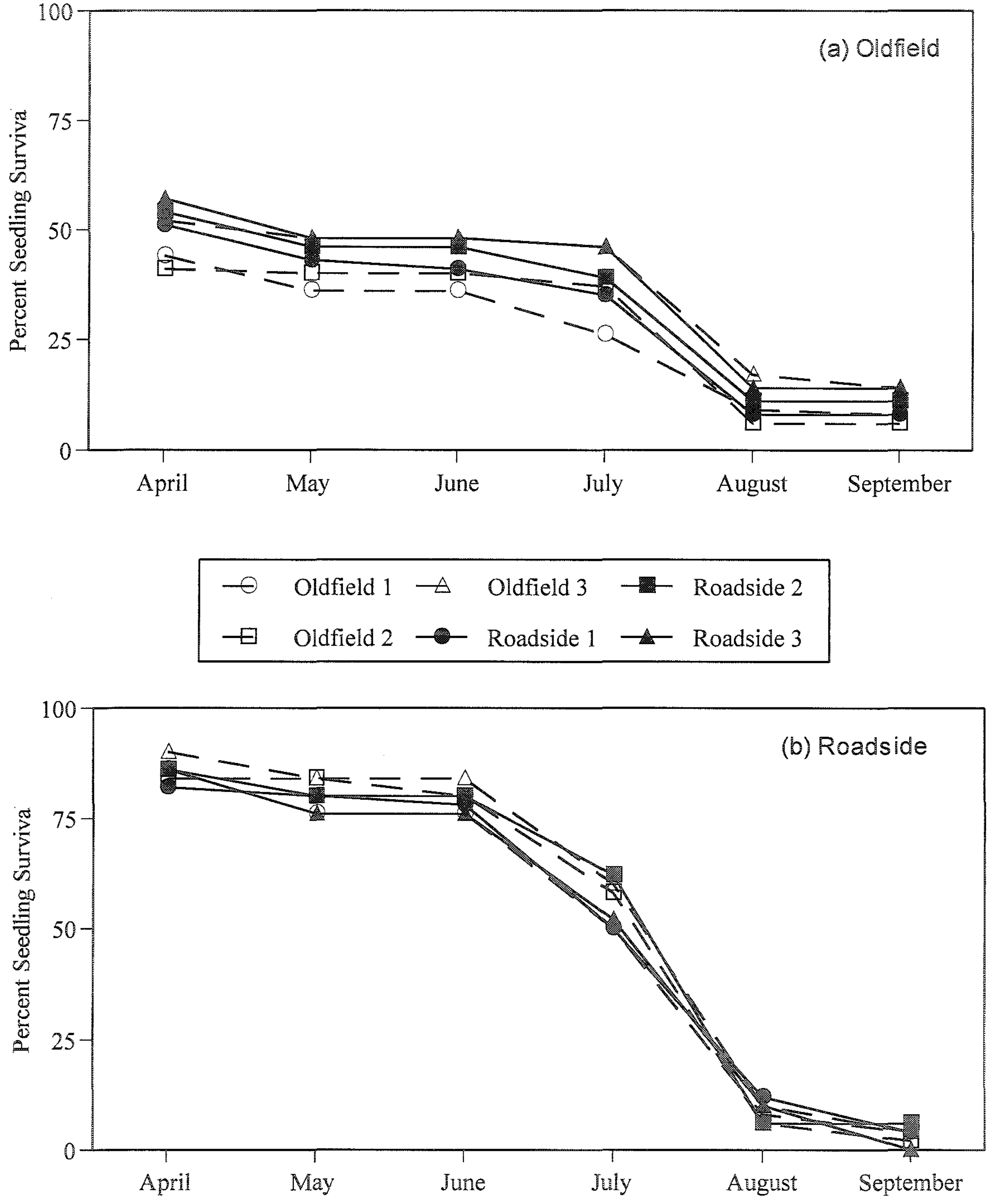

(a) Oldfield
Percent Seedling Surviva
100
75
50
25
0
April
May
June
July
August
September
Oldfield 1
Oldfield 2
Oldfield 3
Roadside 1
Roadside 2
Roadside 3
(b) Roadside
Percent Seedling Surviva
100
75
50
25
0
April
May
June
July
August
September

FIGURE 5.2: Change in the number (a) and length (b) of leaves of seedlings germinated from seeds that were collected from oldfields and roadsides and planted into different oldfield and roadside locations from April to September.

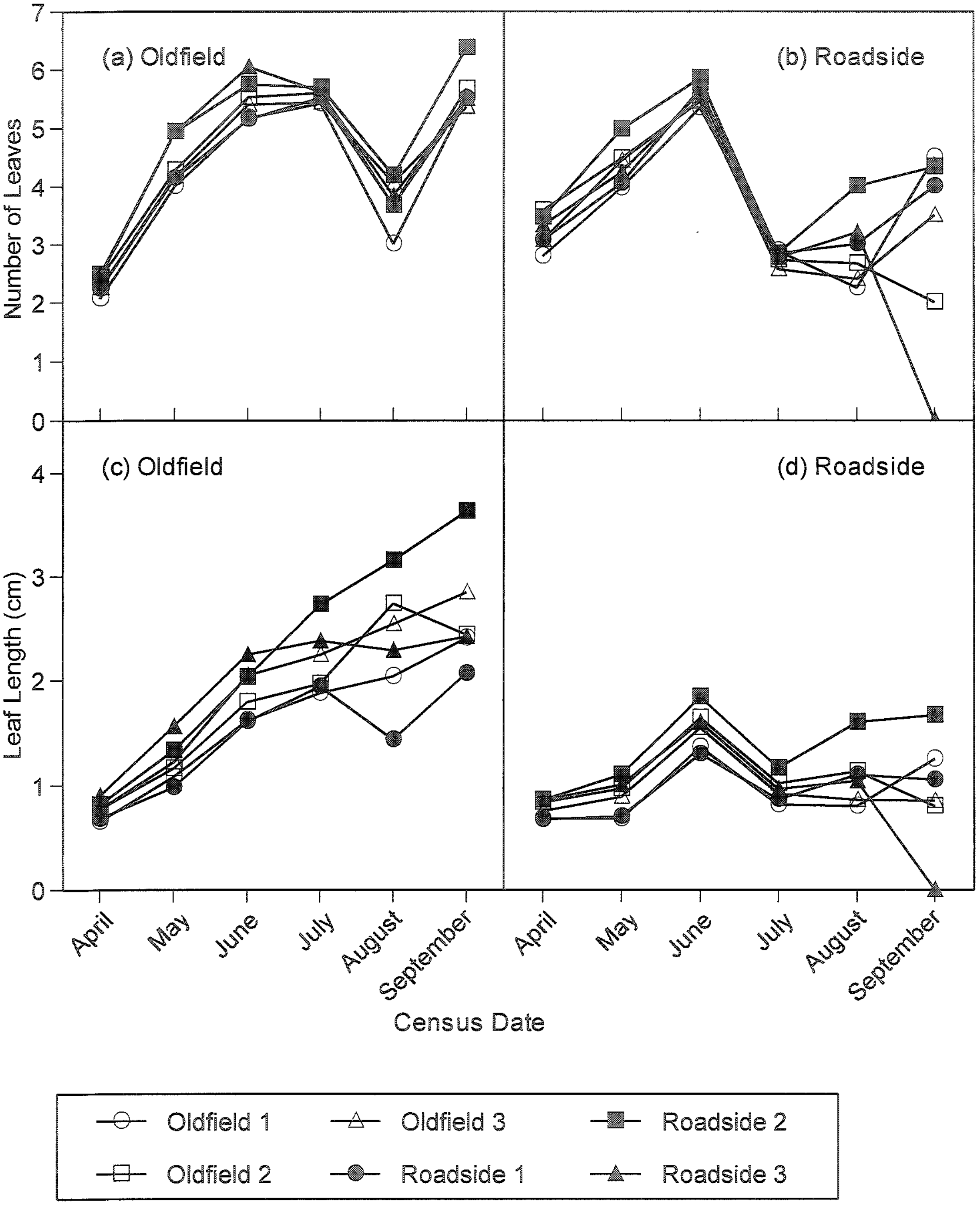
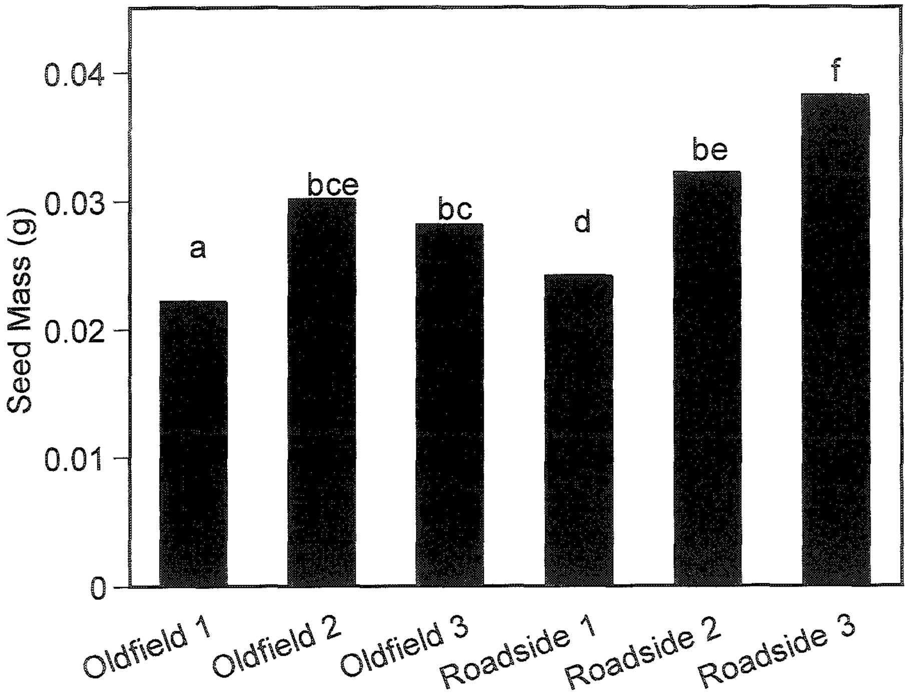

(a) Oldfield
(b) Roadside
(c) Oldfield
(d) Roadside
Number of Leaves
Leaf Length (cm)
0
1
2
3
4
5
6
7
0
1
2
3
4
April
May
June
July
August
September
Census Date
Oldfield 1
Oldfield 2
Oldfield 3
Roadside 1
Roadside 2
Roadside 3

FIGURE 5.3: Average mass of *D. sylvestris* seed collected from three roadside and three oldfield populations. Categories with different letters differ at $p < 0.05$.



Seed Mass (g)
0
0.01
0.02
0.03
0.04
a
bce
bc
d
be
f
Oldfield 1
Oldfield 2
Oldfield 3
Roadside 1
Roadside 2
Roadside 3

SECTION VI

TOLERANCE OF GERMINATING SEEDS TO SALT AND LOW OSMOTIC POTENTIALS OF ROADSIDE AND OLDFIELD POPULATIONS OF *DIPSACUS SYLVESTRIS* (HUDS.)

6.1 ABSTRACT

High salinity is harmful to plants because of the toxicity of sodium (Na^+) and chloride ions and the lowered soil water potential. A previous study demonstrated that seeds collected from roadside populations of *Dipsacus sylvestris* (Huds.) are more tolerant of high salinity during germination than seeds collected from oldfield populations (Beaton & Dudley, 2004). However, it was not clear if the tolerance was of water stress or of ion toxicity. In this study, we have further examined the salt tolerance of roadside populations of this species by exposing germinating seeds to high salinity, low water potentials, created by PEG 8000 solutions or by vermiculite, and distilled water. Root length and cotyledon emergence were used as measures of fitness. PEG solutions are known to cause hypoxia as well as create low osmotic potentials. For this reason, responses to the PEG treatment are a combination of tolerance to low osmotic potentials and hypoxia. Overall, roadside populations differed markedly from oldfield populations with greater tolerance of high salinity, low water potential and hypoxia. In response to water stress, seedlings invested more resources in root growth at the expense of cotyledon emergence. However, cotyledon emergence was high in response to salt. Though there was considerable variation in the root length of the germinating seeds in the three treatments, we found no relationship between increased salt tolerance and increased ability to tolerate low water potentials suggesting that they are two completely separate traits in this species.

6.2 INTRODUCTION

High salinity lowers the osmotic potential of the soil, making it very difficult for plants to take up water. Therefore, salt tolerance and drought tolerance are often considered to be overlapping traits; and plants that are highly drought tolerant are often expected to also have enhanced tolerance to salinity. However, high salinity also exposes plants to elevated levels of sodium (Na^+) and chloride ions, which have harmful effects. Although the exact mechanism of Na^+ toxicity has yet remained undiscovered, it is known that it interferes with plant cell processes involving K^+ by competing for K^+ membrane binding sites (Epstein, 1998). Most plants sequester Na^+ in vacuoles in the leaves to protect their cellular functions, particularly those involving K^+. However, at Na^+ levels that exceed the storage capacity of the vacuoles, salt ions will begin to accumulate in the cytoplasm. Cells will either die from the toxic effects of the ions or begin to pump Na^+ out to the apoplast, where it accumulates on the cell walls. The resultant drastic reduction in water potential in the apoplasm causes cells to rapidly dehydrate and eventually die (Munns, 1993, 2002). Thus, high salinity has drastically more detrimental and varied effects than drought stress alone.

Not all plants are equally susceptible to the toxic effects of high salinity. Both natural and agricultural species possess considerable variation for salt tolerance (Ashraf, 1994, Glenn *et al.,* 1999, Hester *et al.,* 1998). Several examples of the rapid evolution of salt tolerance in newly created saline habitats have indicated that high salinity can impose strong selection pressures for salt tolerance. In temperate regions, the application of de-icing salts, primarily NaCl, has resulted in the formation of highly saline soils along roadsides (Davison, 1971, Thompson *et al.,* 1986, Thompson *et al.,* 1986, Westing, 1969). Recently, it has been shown that roadside populations of *Dipsacus sylvestris* are more tolerant of high soil salinity during germination than populations located in more benign environments (i.e. the oldfield) (Beaton & Dudley, 2004). In this study, we attempt to examine the role drought tolerance plays in conferring the observed tolerance to high salinity.

Investigating the role of low water potential in salt toxicity has proven methodologically challenging. Many different compounds, like malate and a variety of sugars, have been used to impose osmotic stress on plants for comparison to responses to high salinity. However, all these substances can be taken up by the plant, and may then either have toxic effects or be used by the plant for osmotic adjustment. It is impossible to differentiate the effects of the low water potential from the effects of the solute itself. Polyethylene glycol (PEG) has the advantage that it is not taken up by plant cells. Though at one time PEG was thought to be the ideal substance for studies of water stress because it was believed not to have any toxic properties, further research has shown that PEG has deleterious impacts on plants that are unrelated to low water potentials. PEG solutions are extremely viscous and create low oxygen environments for the roots (Mexal *et al.,* 1975). Hypoxic environments are extremely stressful for plants and cause large reductions in root growth (Drew, 1997). An alternative to the use of PEG or other various sugars developed by Sharp *et al.* (1988) takes advantage of the matric potential of vermiculite, rather than the osmotic potential of solutes. Through the careful addition of water, vermiculite can be adjusted to impose the same water potential as a comparable salt solution. Though this appears to be an ideal solution to the methodological problem, vermiculite not only lowers matric potential, but also adds resistance to the growing medium; i.e. it gives the roots something to push through, which can alter their morphology. For these reasons, in this study, we utilized both methods to impose low water potentials.

In this study, we germinated *D. sylvestris* seeds collected from both oldfield and roadside populations in salt solution, two low water potential treatments composed of polyethylene glycol (PEG) and vermiculite, and distilled water as a control. We use root length after ten days and the emergence of cotyledons as measures of performance. We ask the following questions: (1) Is the response of roadside populations to salt stress different than the response to low water potential? (2) Is there variation for growth in low water potentials among the maternal families?

6.3 METHODS

6.3.1 *Study Species*

Dipsacus sylvestris (common teasel), a native of Europe, was first recorded in Canada in 1877. It is found primarily in oldfields and roadsides. *D. sylvestris* exhibits two distinct morphologies. For at least one growing season, plants grow vegetatively as rosettes, and may reach a size of up to 60 cm in diameter. In the spring, if the plant has reached a critical size, it will bolt to its reproductive form of an upright flowering stem (Werner 1975). By July it will have reached its full height of 0.5 - 2.5 m. Flowering occurs from July to September and seeds are shed throughout the fall. A single plant can produce upwards of 3000 seeds (Werner, 1975).

6.3.2 *Seed Collection*

Six *D. sylvestris* populations were chosen (three bordering a major highway (highway 403 between Hamilton and Burlington, Ontario) and three located in oldfields) for study. Highway 403 is a six lane highway carrying 45000 to 75000 automobiles/day (Transportation, 1994). Highway populations were located in full sunlight, 2 - 10 m from the pavement's edge. At all the roadside sites, the surrounding vegetation was primarily various species of grass and/or crown vetch (which is planted following highway construction to stabilize the soil) with a few common oldfield plants (e.g. *Solidago* sp., *Daucus carota*). Oldfield populations were located on Royal Botanical Gardens conservation lands in full sunlight. The surrounding vegetation was dominated by grass with a wide variety of forbs. Seeds were collected from 15 - 20 randomly selected plants from each population. Following collection, seeds were processed and stored in paper envelopes at room temperature.

6.3.3 *Experimental Design*

In order to separate the effects of ion toxicity from osmotic stress in germinating seeds exposed to high salinity, two replicates of ten seeds from each maternal family from each population from each site were placed into one of four treatments: control (distilled water), salt (70 mM NaCl solution with a water potential of −0.5 MPa), PEG (PEG 8000 solution with a water potential of −0.5 MPa) or vermiculite (mixed with distilled water to a water potential of −0.5 MPa). Seeds were germinated in petri dishes lined with filter paper (except those trays receiving the vermiculite treatment) and sealed with paraffin wax to prevent evaporation. The trays were placed in front of a window and received a mixture of natural and artificial light (from a fluorescent source). After ten days, the root length of the emerging seedlings was measured and the emergence of cotyledons was recorded.

6.3.4 *Water Potential Measure*

The water potentials of the three treatments were measured using a Westcor HR-33T Dew Point Microvoltometer. The water potential of the PEG and salt treatments were calibrated by soaking small pieces of filter paper in the solutions. Small pieces of vermiculite were selected randomly from the 300 g sample for measurement. Two successive pieces of vermiculite within the appropriate range of water potential was deemed a satisfactory measure of the water potential of the vermiculite. All containers containing the treatments were sealed with paraffin wax until the time of the experiment (< 24 h).

6.3.5 *Statistical Analysis*

Statistical analysis was conducted using SAS version 8.2 for Windows. A nested mixed model ANOVA (PROC MIXED) with preplanned contrasts (CONTRAST statement) was conducted to examine the effect of the treatments on the root length of the germinating seedlings. The correlation between the root lengths attained by the germinating seeds collected from each site (the roadside and the oldfield) in each of the four treatments was determined (PROC CORR). A Categorical analysis of variance (PROC CATMOD) was conducted to examine the effect of the treatments and the collection site on the emergence of the cotyledons of the germinating seedlings. For clarity, additional analyses were conducted with treatments (i.e. salt and PEG) considered separately. As none of the seeds in the PEG treatment had emerged cotyledons, only the control, salt and vermiculite treatments are considered in this analysis.

6.4 RESULTS

Overall, there was a significant effect of the treatments on the root length of the germinating seeds (Table 6.1). The oldfields and the roadsides were significantly different and there was a significant site by treatment interaction (Table 6.1). However, though there was a significant interaction between the maternal family and the treatment, there was no overall difference between the populations or the maternal families, nor was there a significant interaction between the populations and the treatments.

Seeds collected from oldfields grew significantly longer roots in the control treatment than seeds collected from roadsides (Figure 6.1a, Table 6.3). Exposure to high salinity caused a reduction in root length in all the maternal families from the oldfield but led to longer roots in two of the fifteen roadside families (Figure 6.1a, Table 6.3). Maternal families from the oldfield displayed a mixture of responses in root length to the vermiculite treatment. Approximately half (8 families) grew shorter roots when grown in vermiculite than when grown in the control treatment while the other half (7 families) grew longer roots (Figure 6.1b, Table 6.3). The response of the roadside families to the vermiculite treatment was much more consistent with all but one family growing much longer roots in the vermiculite than in the control treatment (Figure 6.1b, Table 6.3). Overall, the PEG treatment caused a reduction in root length when compared to the control treatment (Figure 6.1c, Table 6.3). All the maternal families had shorter roots in the PEG treatment than in the control treatment (Figure 6.1c, Table 6.3). In contrast, roadside families displayed a mixture of responses to the PEG treatment. Approximately half (8 families) grew shorter roots when grown in PEG than when grown in the control treatment while the other half (7 families) grew longer roots (Figure 6.1c, Table 6.3).

Oldfield families had more seeds with emerged cotyledons than roadside families ($\chi^2 = 4.84$; $p = 0.0017$) (Figure 6.3). There was a significant interaction between the treatment and the site ($\chi^2 = 4.53$; $p = 0.0332$) (Figure 6.3). There were significantly more oldfield seeds with emerged cotyledons in the control treatment than in both the salt treatment ($\chi^2 = 35.82$; $p < 0.0001$) and the vermiculite treatment ($\chi^2 = 346.77$; $p < 0.0001$) (Figure 6.2). In contrast, though there were more roadside seeds with emerged cotyledons in the control treatment than the vermiculite treatment ($\chi^2 = 46.37$; $p < 0.0001$) there were fewer in the control treatment than in the salt treatment ($\chi^2 = 4.78$; $p = 0.0287$) (Figure 6.2).

There was no correlation between the average root length of the maternal families from either site in any of the four treatments (Table 6.4). Examination of the relationship between the impact of the salt, vermiculite and PEG treatments on the root length revealed distinct differences between the oldfield and the roadside maternal families (Figure 6.2). Maternal families that respond in the same manner to two of the three treatments are visually represented in Figure 6.3 by arrows that point either to the lower, left corner or the upper, right corner of the graph. Arrows not aligned along the diagonal demonstrate differences in a maternal families ability to tolerate the treatments. There is striking similarity among the response of oldfield families to the salt and PEG treatments (Figure 6.2a). In contrast, there is no relationship between the two traits among roadside families (Figure 6.2a). The responses of the oldfield and roadside maternal families to the vermiculite and salt treatments are completely different (Figure 6.2b). In addition, there is no relationship in either group of maternal families between the vermiculite and salt tolerance traits (Figure 6.2b). Likewise, there is no similarity between oldfield and roadside maternal families' responses to the vermiculite and PEG treatments nor is there a relationship between the PEG and vermiculite tolerance traits (Figure 6.2c).

6.5 DISCUSSION

Exposure to highly saline soils or solutions causes plants to experience both osmotic stress and ion toxicity. Salt induced stress is often equated to the stress of low water potentials that is induced by drought. However, our results clearly suggest that in *D. sylvestris*, the stresses imposed by high salinity are not equivalent to the stresses imposed by drought. If the same tolerance mechanisms were involved, one would expect families that were capable of tolerating salt stress to also be able to tolerate low water potentials. We found that there was no relationship between the traits. Although, overall roadside families are better at tolerating both low water potentials and high salinity, individual families that possess traits for salt enhanced growth do not possess traits for drought tolerance.

In a previous study of roadside populations of *D. sylvestris* (Chapter 3), we suggested that roadside populations possessed two distinct salt tolerance strategies, with some families relying on their seed composition, which was likely high in various osmotica, to tolerate the low water potential of a saline environment, and others possessing a trait for salt enhanced growth that was not reliant on seed composition. That study suggested that the osmotic stress tolerance traits conferred by the composition of the seeds was separate from salt enhanced growth traits. The results from the current study provide further evidence for this theory. The families that displayed salt enhanced growth were not those that exhibited the strongest ability to tolerate osmotic stress.

The distinction between the salt tolerance and tolerance of water deficits is also supported by trends in the allocation of resources between above and below ground growth. The percentage of oldfield seedlings with emerged cotyledons in the salt treatment is significantly lower than in the control treatment. However, the number of emerged cotyledons of oldfield seedlings in either low water potential treatment is significantly lower than in both salt and the control treatment. In the vermiculite treatment, root length is significantly longer than in either the control or the salt treatment. Although, this is likely related to the resistance of the growing medium (as discussed below), it indicates that seedlings are responding differently to the two treatments. In response to low water potential, a greater proportion of available resources were dedicated to root growth at the expense of shoot growth while, in response to high salinity, resources were not diverted exclusively to below ground growth. The divergence of resources from shoots to roots is a common response to drought stress (Lenzi *et al.,* 1995, Sharp *et al.,* 1988). Investing more resources in root growth increases the likelihood that the plant will gain increased access to water. This is an especially crucial response for seedlings, which inhabit the surface soil. The surface of the soil is the first to dry out when water becomes limiting. The deeper roots are able to penetrate, the greater the likelihood for survival of the seedling. We argue that growing longer roots in response to salinity stress would not provide the same benefits. First, the plant is not likely to encounter a source of non-saline water by investing more in root growth and second, as many plants deposit excess Na^+ in their leaves shoot growth in favour can benefit plants in high salinity. An increase in root surface area may expose the plant to higher Na^+ levels. Additionally, tolerating high Na^+ levels may require a large resource investment that requires actively photosynthesizing tissues.

The germinating seeds responded differently to the vermiculite and PEG treatments. Though both impose low water potentials, the PEG solution has the additional impact of causing hypoxia (Mexal *et al.,* 1975). As oxygen demand increases under low water potentials (Verslues *et al.,* 1998), the reduced oxygen availability would be additionally harmful to the germinating seeds. It is interesting that the roadside families exhibit an increased ability to tolerate the PEG solutions when compared to the oldfield families. In addition to being better able to tolerate low water potentials, the roadside families are also better able to tolerate the low oxygen conditions. Roadside populations are often found in extremely wet areas with poorly draining clay soil. This type of environment is very likely to experience hypoxic conditions. In contrast, in the oldfield, *D. sylvestris* populations are often found in comparatively dry, well drained environments.

A second difference between the vermiculite and PEG treatments is the strength of the media. It has been suggested that the physiological responses of plants growing in liquid media will be different than when they are grown in a solid media. Our results support this theory in that in the vermiculite, the seedlings produce roots that are much longer and thinner than in the solution. However, a significant difference between the ability of roadside and the oldfield families to tolerate low water potentials exists. Several oldfield families have shorter roots in the vermiculite than in the control treatment. In contrast, all but one roadside family grew longer roots in the vermiculite than the control treatments. There is a significant level of variation in the tolerance of the oldfield families to low water potentials. Drought stress occurs as a result of freezing temperatures as well as drought and high salinity. While salinity stress occurs predictably every year in the roadside, freezing and drought occur randomly depending on climatic conditions. Unlike oldfields, which often have a very rich topsoil, the roadside heavy clay soil of the roadside is extremely vulnerable to drying, turning into a cement like substance after water loss. This harsh environment would act as an extremely effective selection pressure. Despite the random nature of the occurrence of droughts, roadside genotypes that are not highly tolerant of low water potentials would be removed during any drought event. The more stable soil of the oldfield supports the retention of variation in the tolerance of low water potentials.

Despite the high availability of nitrogen, the environment experienced by roadside plants is very stressful and inflicts many different selection pressures. The direction of selection is different and potentially conflicting between stressors as exemplified by the lack of overlap in tolerance traits between maternal families. The contrasting responses of genotypes to different selection pressures suggest that the absence of local adaptation found in previous studies (Chapters 4 and 5) may be the result of the complexity of this novel habitat. Additionally, this study has demonstrated that *D. sylvestris* is able to rapidly adapt to many different environmental conditions making in more likely that this species will be able to rapidly spread into new habitats from the roadside and pose a significant threat as an invasive plant.

ACKNOWLEDGEMENTS

We would like to thank the Royal Botanical Gardens in Hamilton, Ontario for allowing seed collection on their lands, Sid Lang at the Ontario Ministry of Transport for help in obtaining permission to work on highway verges, Mary DeGuzman for assistance in seed collection and David Guevera for his expertise in measuring water potentials.

TABLE 6.1: A mixed model analysis of variance of the effects of the treatment (control, salt, PEG and vermiculite), the site (oldfield and roadside) and their interaction on the root length after ten days of germinating *D. sylvestris* seeds. Seeds that did not germinate are not considered in the analysis.

Source	Numerator df	Denominator df	*F*
Treatment	3	12	44.87 ***
Site	1	4	6.92 †
Treatment × Site	3	12	7.22 **

*† $P < 0.1$; ** $P < 0.01$; *** $P < 0.001$.*

TABLE 6.2: A mixed-model analysis of variance for the random effects of the population (nested within site), maternal family (nested within population and site), and their interactions with the treatment (control, salt, PEG and vermiculite) on the root length after ten days of germinating *D. sylvestris* seeds. Seeds that did not germinate are not considered in the analysis. Effects with estimates of zero have been removed from the analysis.

Source	Estimate	*Z*
Population	0.0075	0.43
Treatment×Population	0.0244	0.94
Treatment×Maternal Family	0.1621	5.88 ***
Residual	0.4372	31.51 ***

*** $P < 0.001$.

TABLE 6.3: Preplanned contrasts of the effects of control vs. the treatments and the interactions with site on the root length after ten days of germinating *D. sylvestris* seeds. Seeds that did not germinate are not included in the analysis.

Source	Numerator df	Denominator df	*F*
Control vs. Salt	1	12	34.06 ***
Control vs. PEG	1	12	31.58 ***
Control vs. Vermiculite	1	12	16.20 **
Control vs. Salt × Site	1	12	10.44 **
Control vs. PEG × Site	1	12	16.63 **
Control vs. Vermiculite × Site	1	12	14.94 **

** $P < 0.01$; *** $P < 0.001$.

TABLE 6.4: The correlation of the root length of the germinating seeds from the oldfield and the roadside in each of the four treatments.

Roadside\Oldfield	Control	Salt	PEG	Vermiculite
Control	1	0.495 †	-0.329	0.224
Salt	0.555 *	1	-0.114	-0.123
PEG	0.017	-0.021	1	-0.450 †
Vermiculite	0.322	-0.171	-0.323	1

† $P < 0.1$; * $P < 0.05$.

FIGURE 6.1: Root length after 10 days of germinating *D. sylvestris* seeds collected from oldfields and roadsides and placed in (a) high salinity (70 mM NaCl solution with a water potential of −0.5 MPa), (b) vermiculite (mixed with distilled water to a water potential of −0.5 MPa), (c) PEG (PEG 8000 solution with a water potential of −0.5 MPa), and control (distilled water) solutions. Linear connections represent the averages from each maternal family. Dashed lines and ○ represent oldfield populations, solid lines and ● represent roadside populations.

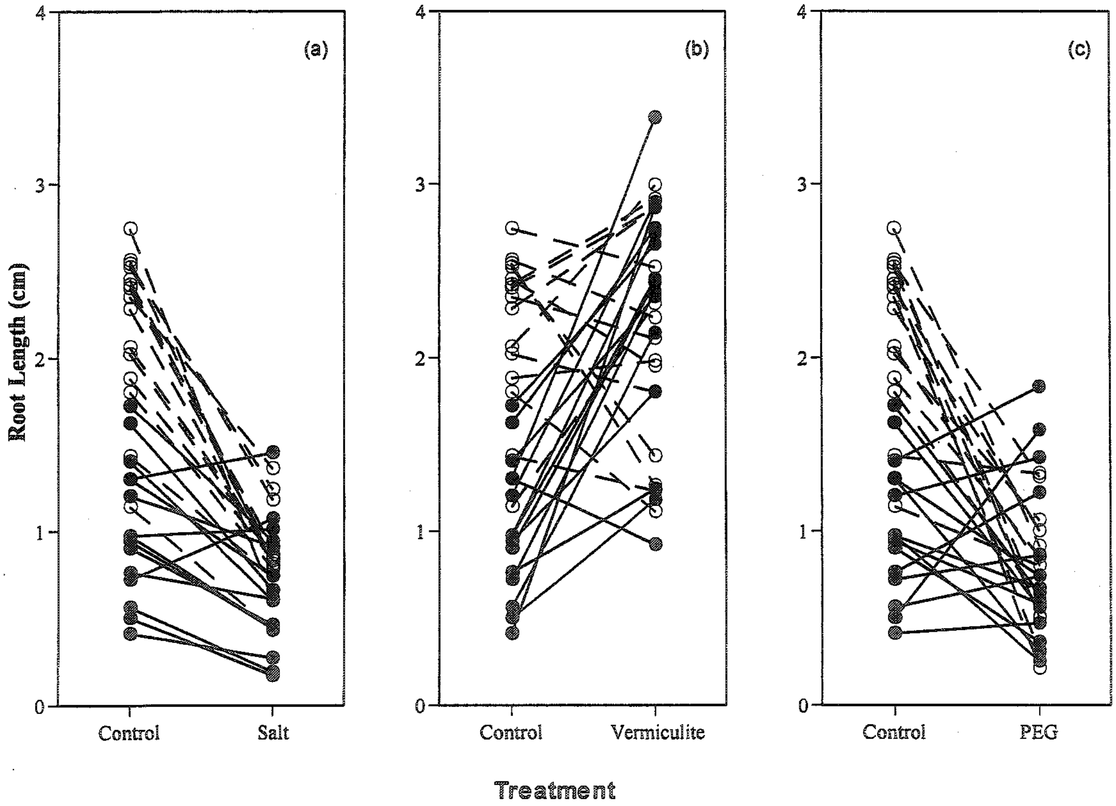

Root Length (cm)
0
1
2
3
4
(a)
Control
Salt
(b)
Control
Vermiculite
(c)
Control
PEG
Treatment

FIGURE 6.2: Percentage of germinating *D.* sylvestris seeds collected from oldfields and roadsides and placed in high salinity (70 mM NaCl solution with a water potential of −0.5 MPa), vermiculite (mixed with distilled water to a water potential of −0.5 MPa), PEG (PEG 8000 solution with a water potential of −0.5 MPa), and control (distilled water) treatments to have their cotyledons emerged from the testa after ten days.

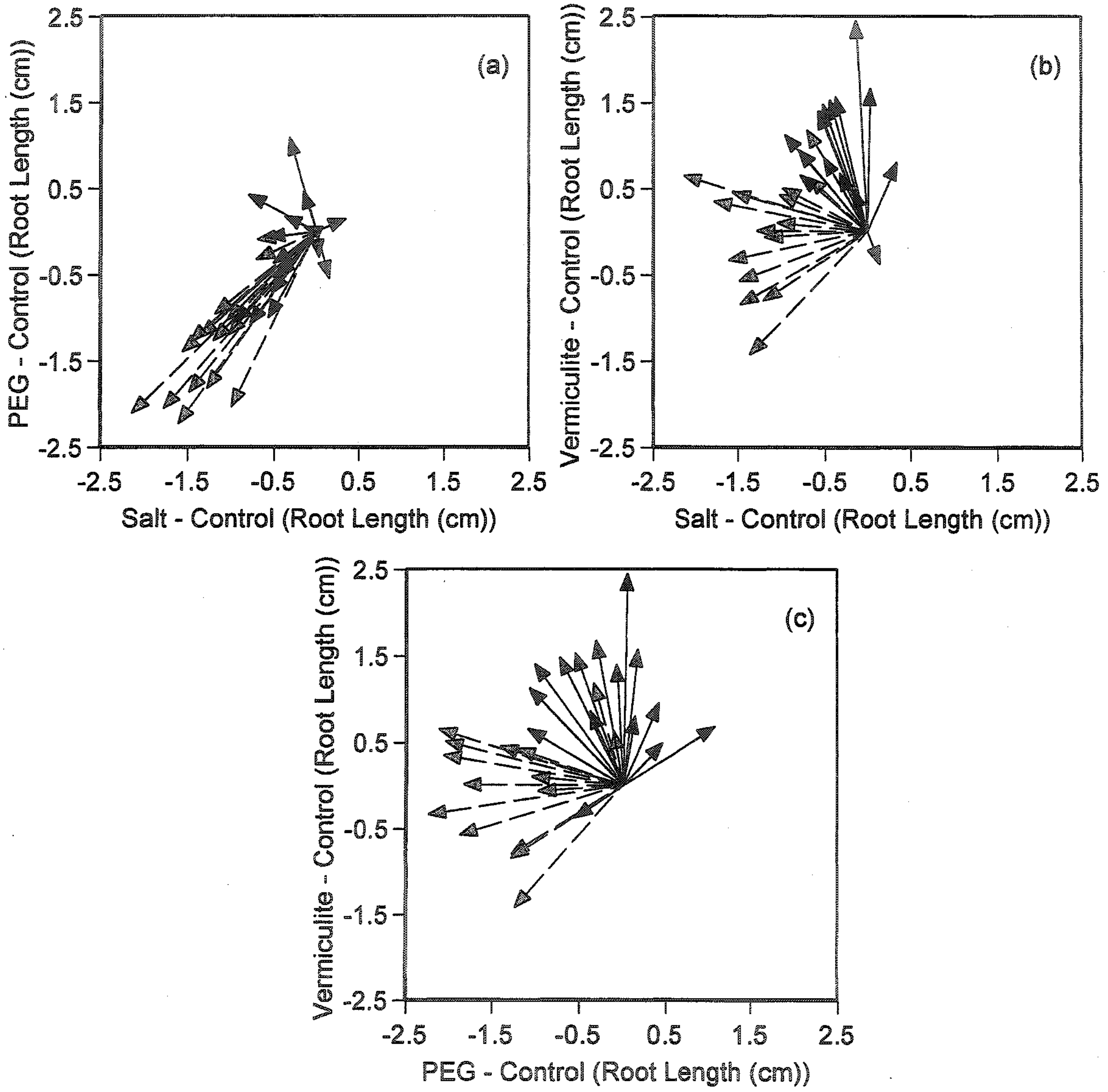
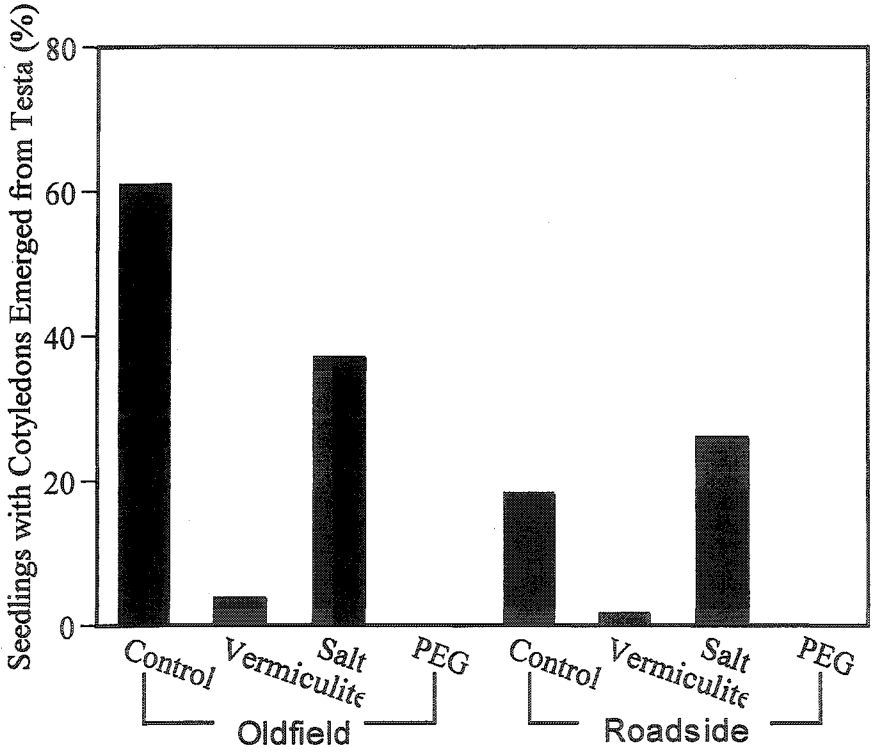

(a)
PEG - Control (Root Length (cm))
Salt - Control (Root Length (cm))
(b)
Vermiculite - Control (Root Length (cm))
Salt - Control (Root Length (cm))
(c)
Vermiculite - Control (Root Length (cm))
PEG - Control (Root Length (cm))
2.5
1.5
0.5
-0.5
-1.5
-2.5

FIGURE 6.3: The root length after 10 days of germinating *D. sylvestris* seeds collected from oldfields and roadsides and placed in a control (distilled water treatment) subtracted from the root length in high salinity (70 mM NaCl solution with a water potential of −0.5 MPa), vermiculite (mixed with distilled water to a water potential of −0.5 MPa), and PEG (PEG 8000 solution with a water potential of −0.5 MPa) treatments. Arrows contrast the impact the treatments had on the root length of the germinating seeds. The arrows begin at the origin of the graph (i.e. 0, 0) and end at the difference in root length between the appropriate treatments (a) PEG vs. high salinity, (b) vermiculite vs. high salinity, and (c) vermiculite vs. PEG) and the control. Each arrow represents one maternal family.


Seedlings with Cotyledons Emerged from Testa (%)
0
20
40
60
80
Control
Vermiculite
Salt
PEG
Control
Vermiculite
Salt
PEG
Oldfield
Roadside

SECTION VII

THE TOXICITY OF THE LEACHATE OF *DIPSACUS SYLVESTRIS* (HUDS.) SEEDS COLLECTED FROM ROADSIDES

7.1 ABSTRACT

Previous studies of *Dipsacus sylvestris* have shown that the seed leachate of seeds collected from roadside populations has significantly higher levels of sodium ions (Na^+) than the seed leachate of seeds collected from oldfield populations. In this study, we compared the toxicity of the leachate of roadside seeds and oldfield seeds. We germinated seeds collected from three oldfield and three roadside populations in the presence of either a high density of oldfield or roadside seeds from the same six populations. The root growth of oldfield seeds was significantly lower in the presence of roadside seeds than in the presence of other oldfield seeds ($t = 2.53$, $p = 0.0200$). In contrast, the surrounding seeds had no impact on the root growth of roadside seeds ($t = -1.03$, $p = 0.3134$). The relationship between the root length of oldfield seeds and the Na^+ concentration of the surrounding solution was significantly different than that of roadside seeds. Root length of oldfield seeds was significantly negatively correlated with the sodium ion concentration of the trays.

7.2 INTRODUCTION

Germination begins with the uptake of water or imbibition. The rate of imbibition is dependent on the water potential gradient between the seed and its surroundings. As a dry seed may contain only 10-15 % water, the water potential gradients are frequently as high as –1000 bars resulting in extremely rapid imbibition (Shaykewich & Williams, 1971). The rapid uptake of water is associated with leakage of a variety of substances including ions, organic acids, proteins and amino acids from the seed (Duke & Kakefadu, 1981, Duke *et al.,* 1983, McKersie & Senaratna, 1983, Simon & Raja Harun, 1972, Spaeth, 1987). The composition of the substances leaked out of the seed is largely determined by the maternal plant. The maternal plant is responsible for provisioning the seed with the resources it will need during germination and seedling establishment. In this way, the maternal plant can pass on traits beyond those determined by its contribution of nuclear genes.

The quality and size of the seed that the maternal plant produces is significantly impacted by the quality of the environment the plant experiences (Galloway, 2001, Roach & Wulff, 1987). A plant in a poor environment will produce smaller seeds of inferior quality than a plant inhabiting a good environment. The impact of the size of the seed on the fitness of the seedling has been extensively investigated. Larger seeds have a greater success during germination and seedling establishment (Stanton, 1984). These early advantages may persist throughout the lifetime of the plant and even into the next generation. Plants germinating from large seeds may eventually produce large seeds as a result of the increased size they attain as a result of their initial advantages. However, little attention has been paid to the impact the maternal environment can have on the composition of the seed and the resultant seedling.

Seeds collected from roadside populations of *Dipsacus sylvestris* have higher levels of sodium than seeds collected from oldfield populations (Beaton and Dudley 2004). The maternal plant is exposed to an environment with extremely high salt levels resulting from the use of de-icing salts (Stanton, 1984). The maternal plant deposits the sodium in the seed during development. Roadside seeds are more tolerant of high salinity during germination than seeds collected from oldfield seeds (Beaton and Dudley 2004). The high sodium levels in the seed are significantly correlated with increased salt tolerance. However, sodium is toxic to plants causing osmotic stress, ion toxicity, nutrient deficiency and oxidative stress (Gorham, 1992, Gueta-Dahan *et al.,* 1997, Munns, 1993, Zhu *et al.,* 1998). When seeds have the sodium ions leached from them prior to soaking in distilled water, they have significantly longer roots indicating that the high sodium levels in the seeds may actually be detrimental to the germinating seeds under non-saline conditions (Beaton & Dudley, 2004).

In order to determine if the high sodium levels in the seeds collected from roadside populations are detrimental to the germinating seedling under non-saline conditions, we compared the growth of seeds collected from oldfields and roadsides when they were grown in the presence of several oldfield or roadside seeds. We ask the following questions: Are germinating oldfield and roadside seeds affected by the presence of other oldfield or roadside seeds? Does the response of roadside and oldfield seeds to the presence of the surrounding seeds differ? Is there any relationship between the difference in root length between oldfield and roadside treatments and the amount of sodium in the seeds?

7.3 METHODS

7.3.1 *Study Species*

Dipsacus sylvestris (common teasel), a native of Europe, was first recorded in Canada in 1877. It is found primarily in oldfields and roadsides. *D. sylvestris* exhibits two distinct morphologies. For at least one growing season, plants grow vegetatively as rosettes, and may reach a size of up to 60 cm in diameter. In the spring, if the plant has reached a critical size, it will bolt to its reproductive form of an upright flowering stem. By July it will have reached its full height of 0.5 – 2.5 m. Flowering occurs from July to September and seeds are shed throughout the fall. A single plant can produce upwards of 3000 seeds (Werner, 1975).

7.3.2 *Seed Collection*

Six *D. sylvestris* populations were chosen (three bordering a major highway (highway 403 between Hamilton and Burlington, Ontario) and three located in oldfields) for study. Highway 403 is a six lane highway carrying 45000 to 75000 automobiles/day (Transportation, 1994). Highway populations were located in full sunlight, 2 – 10 m from the pavement's edge. At all the roadside sites, the surrounding vegetation was primarily various species of grass and/or crown vetch (which is planted following highway construction to stabilize the soil) with a few common oldfield plants (e.g. *Solidago* sp., *Daucus carota*). Oldfield populations were located on Royal Botanical Gardens conservation lands in full sunlight. The surrounding vegetation was dominated by grass with a wide variety of forbs. Seeds were collected from 15 – 20 randomly selected plants from each population. Following collection, seeds were processed and stored in paper envelopes at room temperature.

7.3.3 *Experimental Design*

Three oldfield or roadside seeds from each of the six populations (i.e. three roadside and three oldfield) were germinated in 5 cm diameter petri dishes lined with filter paper and containing 5 ml of distilled water and in the presence of either 25 oldfield seeds or 25 roadside seeds. The seed treatments were composed of a mixture of seeds from three maternal families from each of the three populations per site. Each population-treatment combination was replicated twice for a total of 180 trays. Small dots of nail polish were painted on the three focal seeds in order to distinguish them from the treatment seeds. The nail polish had no impact on the ability of the seeds to germinate or grow roots (unpublished data). 2 ml of distilled water was added to the trays on the third, fifth and seventh days of the experiment to replace that lost to evaporation. After ten days the root length of the seedlings and the presence or absence of emerged cotyledons was recorded.

The liquid remaining in the trays following the experiment was collected. The sodium ion content was measured using a sodium flame photometer (Evans Electroselenium).

7.3.4 *Statistical analysis*

A mixed model analysis of variance (PROC MIXED) with genotype site and treatment site considered fixed effects and genotype population and treatment populations and their interaction with the fixed effects and each other considered random effects was conducted to determine if the treatments had a significant effect on the germinating seeds, if the genotypes differed overall and if they differed in their response to the treatments. As there was no significant variation between the populations within the genotype site or treatment site or their interactions, the population level is not included in subsequent analyses. The p-diff and t-diff options of the LSMEANS statement (PROC GLM) was used to determine if the root length of germinating oldfield and roadside seeds was impacted by whether they were in the presence of other oldfield or roadside seeds. Categorical analyses (PROC CATMOD) were conducted to determine if there were any differences between the number or seeds with emerged cotyledons from either site or in either treatment. A t-test (PROC GLM) was conducted to examine the difference in sodium ion content of the liquid remaining in the trays between the two treatment sites. An ANCOVA was conducted to examine the difference in the relationship of the sodium ion content of the trays and the average root length of three focal seeds between the oldfields and roadsides genotypes.

7.4 RESULTS

The type of seeds that the roadside and oldfield seeds germinate with does not have a significant overall effect on the root length attained by the germinating seeds (Table 7.1). However, there is a significant difference between the genotypes with oldfield seeds growing significantly longer roots than roadside seeds in both treatments (Table 7.1, Figure 7.1). In addition, there was a significant difference in the response of the oldfield and roadside seeds to the two treatments (Table 7.1). When oldfield seeds are grown in the presence of roadside seeds they produce significantly shorter roots than when grown in the presence of other oldfield seeds ($t = 2.53$; $p = 0.0200$) (Figure 7.1). In contrast, there is no significant difference in the length of the roots attained by roadside seeds when they are germinated in the presence of other roadside seeds or oldfield seeds ($t = -1.03$; $p = 0.3134$) (Figure 7.1).

Oldfield seeds were much more likely to have their cotyledons emerged from the testa than were roadside seeds ($\chi^2 = 63.14$; $p < 0.0001$) (Figure 7.2). There was a significant interaction between the treatment site and the site ($\chi^2 = 8.44$; $p = 0.0033$) (Figure 7.2).

Roadside seeds leach significantly higher levels of sodium ions than oldfield seeds (Figure 7.3). Overall there was no significant effect of the sodium ion concentration of the seed leachate (Table 7.2) on the root length attained by the germinating seeds. However, there was a significant difference in the relationship between the sodium ion content of the seed leachate and the root length attained by the oldfield and roadside seeds (Table 7.2, Figure 7.4). The concentration of sodium ions in the trays was significantly negatively correlated with the root length of germinating oldfield seeds ($r = -0.27792$; p 0.0080) (Figure 7.4). In contrast, there was no correlation between the sodium ion concentration of the seed leachate collected in the trays and the root length of the germinating roadside seeds ($r = 0.11843$; $p = 0.2690$) (Figure 7.4).

7.5 DISCUSSION

The seed leachate of seeds collected from roadside populations of *D. sylvestris* significantly hindered the root growth of germinating seeds collected from oldfield populations, but had no impact on the ability of other seeds collected from roadsides to grow roots. In contrast, seeds collected from oldfields had no obvious impact on the ability of roadside seeds to grow roots. The impact of the seed composition of roadside seeds on their own ability to grow roots remains unclear. Previous studies have shown that, first, when germinated in distilled water roadside seeds have significantly shorter roots than oldfield seeds. Second, seedlings grown from roadside seeds that were leached prior to germination to remove some of the contents of the seeds had longer roots suggesting that roadside seeds may be detrimentally impacted by their seed composition when germinated at low salinity (Beaton and Dudley in review). However, reducing the density of the roadside seeds (i.e. by germinating the seeds in the presence of oldfield seeds rather than roadside seeds) did not significantly impact the root length of the germinating seeds. In fact, when germinated with other roadside seeds, the average root length was slightly, though not significantly, higher. The leachate of roadside seeds had a significantly higher concentration of Na^+ than that of oldfield seeds (Figure 7.2). A previous study (Beaton and Dudley 2004) has shown that the growth of seeds collected from roadside populations is enhanced during germination by elevated salinity (70 Mm NaCl). The high Na^+ concentration of the solution resulting from the high density of germinating roadside seeds may have actually stimulated root growth in roadside populations. Although it is clear that oldfield seeds suffer from the toxicity of the seed leachate of roadside seeds and that roadside seeds are tolerant of their own leachate, it remains unclear what the impact, if any, the seed composition of roadside seeds has on themselves.

Roadside populations of *D. sylvestris* may accumulate Na^+ and other solutes in their seeds as a way to protect the germinating embryo from osmotic stress imposed by the highly saline conditions of the roadside. The shorter root lengths of roadside populations of *D. sylvestris* when grown in distilled water suggested that the seed composition may actually have a deleterious effect on the germinating seed when grown in non-saline conditions. This hypothesis is extremely difficult to test. By exposing oldfield seeds to the leachate of germinating roadside seeds, we have demonstrated that roadside seeds do, in fact, contain harmful substances that are not found in oldfield seeds and to which roadside seeds are tolerant. This variation in seed composition is a maternal effect resulting from the environment experienced by the maternal plant, the genotype of the maternal plant, or an interaction between the maternal plant's genotype and its environment. The relationship between the Na^+ content of the seed leachate collected in the trays and the reduction in root length in the oldfield seeds when exposed to the leachate of roadside seeds suggests that the saline environment experienced by the maternal plant played a role in determining the seed composition. However, plants inhabiting roadsides are also exposed to a wide variety of other harmful substances (metals, including lead (Piron-Frenet *et al.,* 1994), chromium, cadmium, nickel, zinc, manganese, copper and iron (Hamilton *et al.,* 1984, Ndiokwere, 1984, Warren & Birch, 1987), high nitrogen levels (Port & Thompson, 1980, Spencer & Port, 1988), and a variety of PAH's (Maltby *et al.,* 1995) any of which may be impacting the composition of the seeds.

Although the dispersal of oldfield seeds into dense populations of roadside seeds is likely to occur only extremely infrequently, the harmful effect of the roadside seeds on the oldfield seeds' root growth is interesting because it indicates that the seeds release a substance that is inhibitory to the root growth of other seeds. Given the size of the seedlings at the time of root length measurement, it is unlikely that they were impacted by quantity or quality of light received by other seedlings. Hence, light competition is not likely to be responsible for the difference in root lengths. The seedlings were germinating in distilled water. Therefore, competition for nutrients is also not likely responsible for the root length differences. Finally, competition for water is unlikely to have caused the variation in root length as the growing conditions experienced by the seeds were never water limiting. It seems far more likely that the substances leaked from the seeds were harmful to the root elongation of the oldfield seeds.

The root length of oldfield seeds may have been affected by the Na^+ content of the roadside seeds. However, it is extremely unlikely that sodium levels of <0.35 Mm Na^+ (Figure 3) would cause any reduction in root length. A previous study of the effect of 3 Mm $NaSO_4$ on the root length of germinating *D. sylvestris* seeds showed no reduction in root length by oldfield seeds (Appendix B).

A second possibility is that the roadside seeds leach out more osmolytes than oldfield seeds resulting in lower osmotic potential of surrounding solution. A previous study has shown that when exposed to low water potentials, germinating *D. sylvestris* seeds invest more resources in root growth than shoot growth, as is seen by a large reduction in the number of seedlings with emerged cotyledons (Chapter 6). In contrast, when exposed to high salinity solutions of the same osmotic potential, many more seeds have cotyledons despite reductions in root growth. In this study, oldfield populations displayed a small reduction in root length but a large reduction in the number of seedlings with emerged cotyledons, suggesting that differences in root length in the oldfield populations may be due in part to changes in the osmotic potential of the surrounding solution.

Finally, variation in root lengths between the oldfield and the roadside seedlings may be due to the release of an allelopathic substance by the roadside seeds. The seed leachate of the seeds of many species has been shown to have allelopathic effects on the germination of other species (Kushima *et al.,* 1998, Suman *et al.,* 2002, van Staden & Grobbelaar, 1995). Although not ecologically relevant because an oldfield seed is never likely to encounter a high density of roadside seeds during germination, other species do germinate in close proximity with roadside populations and could be potentially harmed by any substance(s) leached from the roadside *D. sylvestris* seeds during germination. Roadside populations of *D. sylvestris* are often much more robust and dense than oldfield populations (personal observation). Like the noxious weed *Sesbania punicea*, seeds of which are known to contain the allelopathic substance sesbanimide (van Staden & Grobbelaar, 1995), the higher degree of success of *D. sylvestris* in the roadside may reflect an enhanced competitive ability during germination resulting from the release of toxic substances by roadside seeds. This would present a novel system in that there is an exceptionally high level of variation within a species for the production of allelopathic substances. The maternal plant has considerable influence on the composition of the seed. The relationship between the reduction in the oldfield seeds and the Na^+ content of the leachate may indicate that the salty environments experienced by roadside plants has some influence over the toxicity of the leachate of roadside seeds.

However, though speculation regarding putative toxic substances in roadside seeds is interesting, it remains speculation. A further study investigating the impact of roadside leachate on other roadside species would provide better evidence for this theory. Were *D. sylvestris* to possess a trait for leachate toxicity, it would support the theory that the success of many invasive plants is due to the release of allelopathic substances (Hierro & Callaway, 256). Perhaps the most intriguing result of this paper is the clear differences between seeds collected from the oldfield and the roadside not only in their response to the treatments but in their influence on the germination of other seeds.

ACKNOWLEDGEMENTS

We would like to thank the Royal Botanical Gardens in Hamilton, Ontario for allowing seed collection on their lands, Sid Lang at the Ontario Ministry of Transport for help in obtaining permission to work on highway verges and Mary DeGuzman for assistance in seed collection.

TABLE 7.1: A mixed model analysis of variance of the effects of the genotype site (seeds collected from the roadside or the oldfield), treatment site (germinated in the presence of either 25 oldfield or 25 roadside seeds) and their interaction on the root length after 10 days of germinating *D. sylvestris* seeds. Seeds that did not germinate are not considered in the analysis. Effects with estimates of zero have been removed from the analysis.

Source	df	Mean Square	*F*
Genotype Site	1	18.421	19.72 *
Treatment	1	0.1964	1.10
Treatment×Genotype Site	1	1.154	6.33 *

$P < 0.05.$

TABLE 7.2: A mixed-model analysis of variance for random effects of the effects of the genotype population (nested within the genotype site), treatment population (nested within treatment site), and their interactions with each other and the genotype site, treatment site and their interaction on the root length after ten days of germinating *D. sylvestris* seeds. Seeds that did not germinate are not considered in the analysis. Effects with estimates of zero have been removed from the analysis.

Source	Estimate	Std. Err.	*Z*
Genotype Population	0.02447	0.01994	1.23
Treatment Population×Genotype Site	0.005063	0.006607	0.77
Treatment Population×Genotype Population	0.007640	0.007008	1.09
Residual	0.2135	0.01354	15.76 ***

*** $P < 0.001$.

TABLE 7.3: An analysis of covariance of the effects of the sodium ion content of the trays and the genotype (seeds collected from the roadside or oldfield) and their interaction on the average root length after ten days of germinating *D. sylvestris* seeds.

Source	df	Mean Square	*F*
Na+ Concentration	1	0.122	1.04
Genotype Site	1	8.034	68.21 ***
Na+ Concentration×Genotype Site	1	0.983	8.35 **

** *P < 0.01;* *** *P < 0.001.*

FIGURE 7.1: Root length after ten days of *D. sylvestris* seeds collected from roadside and oldfield populations germinating with a high density of roadside *D. sylvestris* seeds or a high density of oldfield *D. sylvestris* seeds.

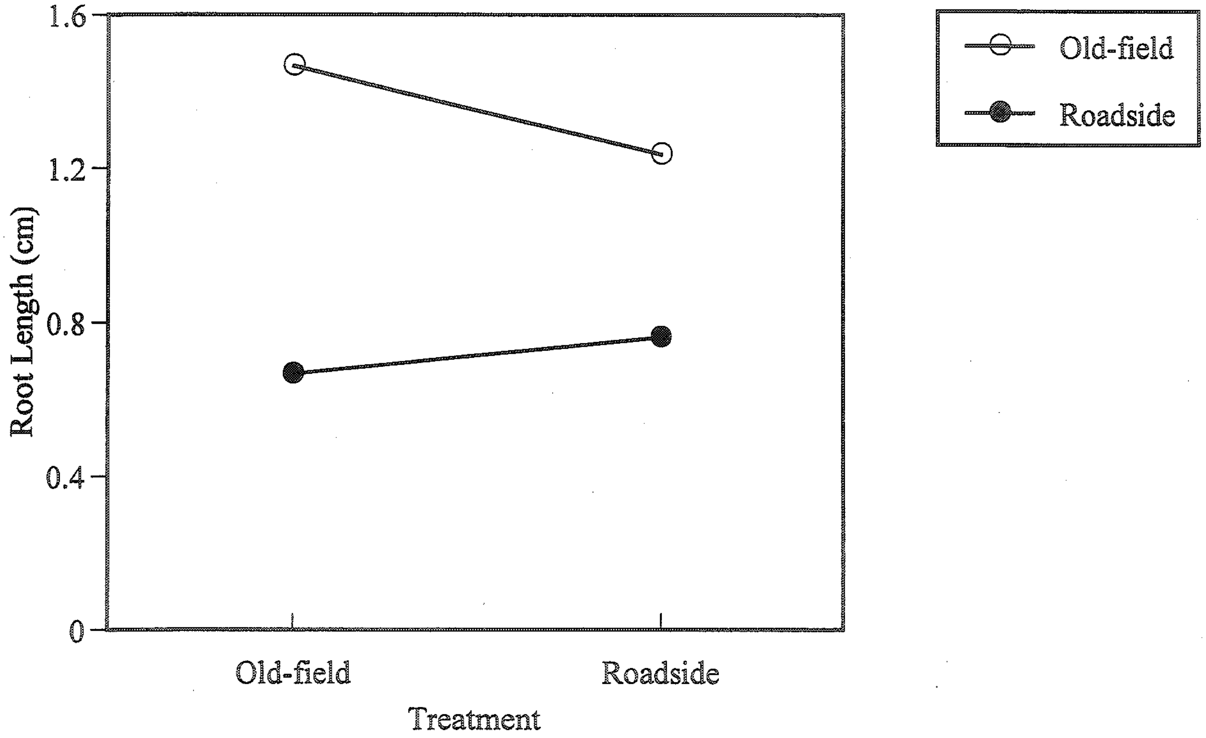
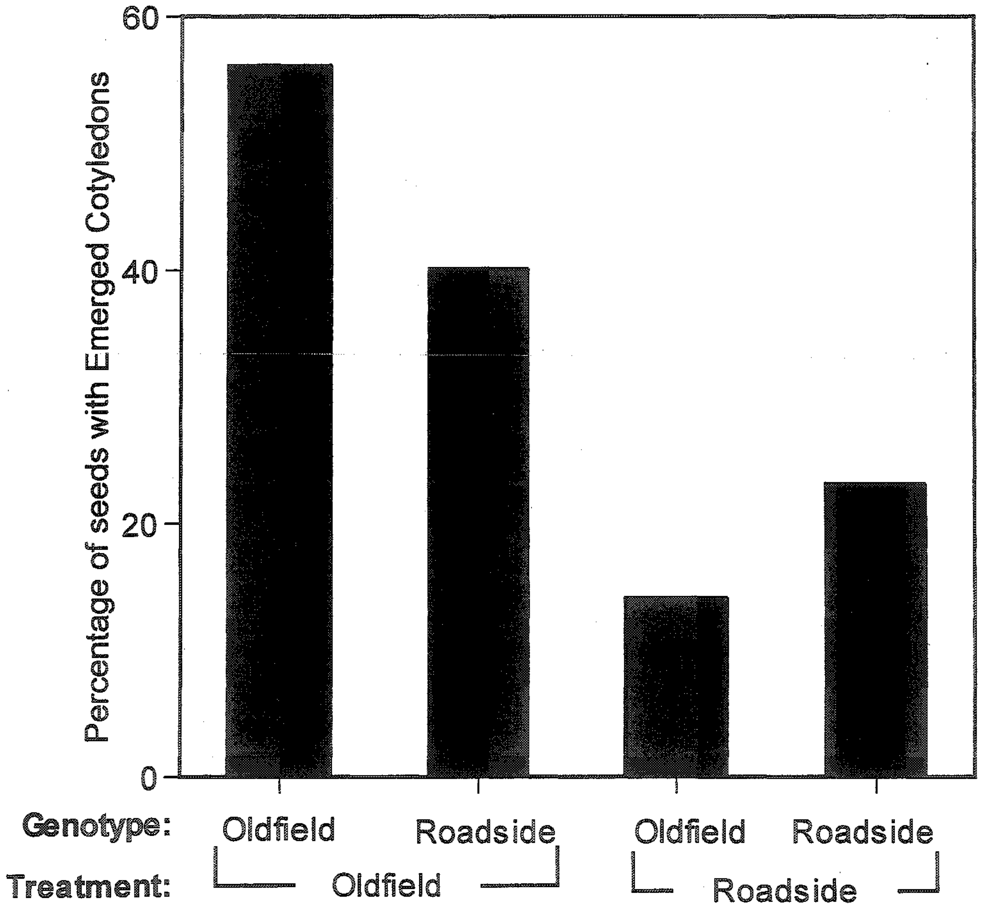

1.6
1.2
0.8
0.4
0
Root Length (cm)
Old-field
Roadside
Treatment
Old-field
Roadside

FIGURE 7.2: The percentage of *D. sylvestris* seeds collected from roadside and oldfield populations germinating with a high density of roadside *D. sylvestris* seeds or a high density of oldfield *D. sylvestris* seeds to have their cotyledons emerged from the testa after ten days.



60
40
20
0
Percentage of seeds with Emerged Cotyledons
Genotype:
Oldfield
Roadside
Oldfield
Roadside
Treatment:
Oldfield
Roadside

FIGURE 7.3: The Na^+ concentration of the leachate remaining in the trays following germination of *D. sylvestris* seeds collected from oldfields and roadsides. Each tray contained either 25 oldfield seeds or 25 roadside seeds plus three focal seeds germinating in 5 ml of distilled water.

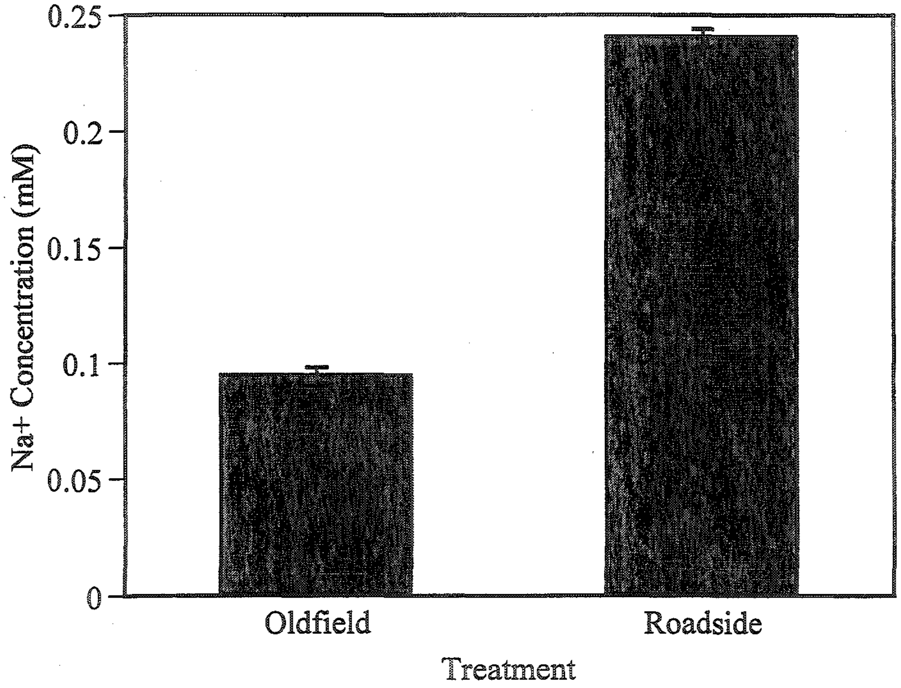
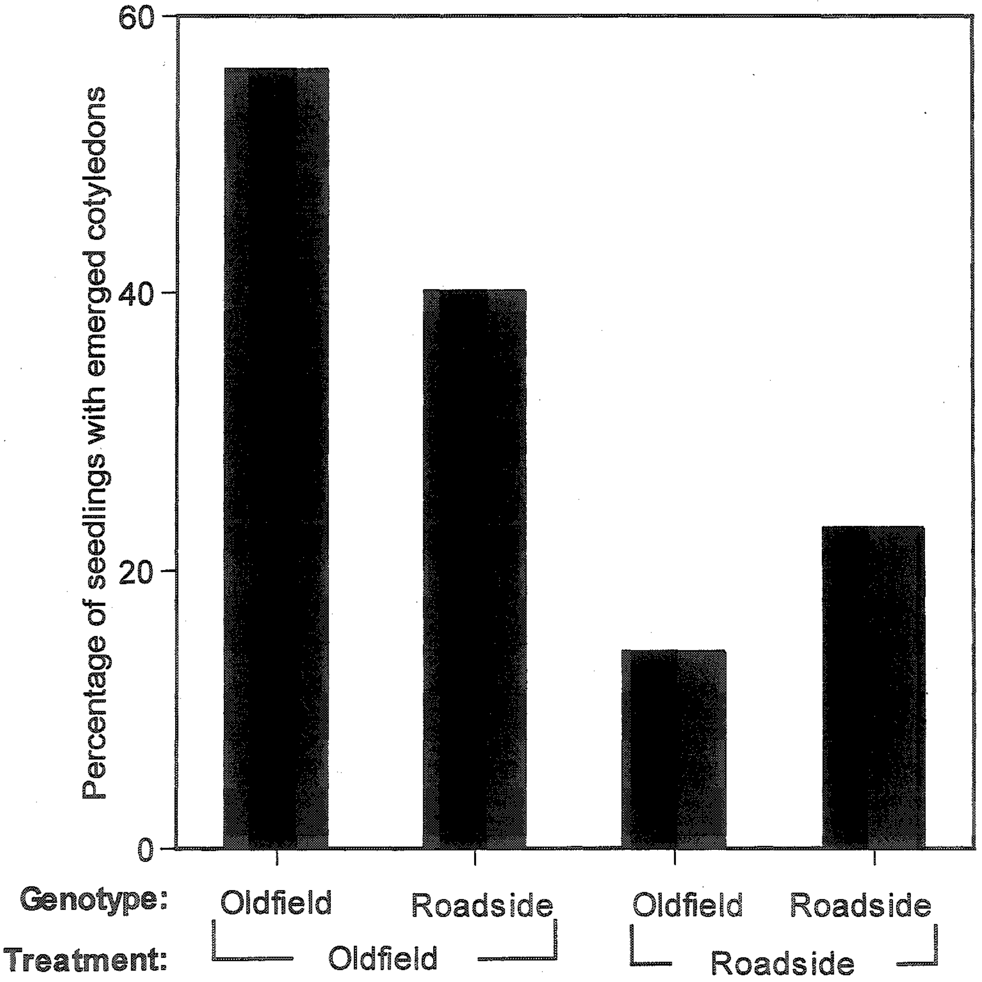

0.25
0.2
0.15
0.1
0.05
0
Na+ Concentration (mM)
Oldfield
Roadside
Treatment

FIGURE 7.4: The relationship between root length of *D. sylvestris* seeds collected from oldfields and roadsides and the Na^+ concentration of the solution in the trays. Variability in the Na^+ concentration resulted from differences in the amount of Na^+ leached from the treatment seeds (either a high density of oldfield or roadside seeds).


60
40
20
0
Percentage of seedlings with emerged cotyledons
Genotype:
Oldfield
Roadside
Oldfield
Roadside
Treatment:
Oldfield
Roadside

SECTION VIII

CONCLUDING DISCUSSION AND GENERAL SUMMARY

In the previous six chapters, I have presented evidence that roadside species are evolving in response to the selection pressures of the roadside environment. Exceptionally high levels of variation were found in both the roadside and the oldfield populations in all three species in laboratory studies of seedling traits (Chapters 2, 3, 6 and 7). Roadside populations of the only native species examined, *A. syriaca*, did not have an enhanced ability to tolerate either high manganese or high salt levels (Chapter 2). Some *H. matronalis* families collected from roadside habitats had an increased ability to tolerate high manganese levels during germination when compared to oldfield populations (Chapter 2). Roadside populations of *D. sylvestris* had increased tolerance to high salinity, drought and hypoxia (Chapters 2 and 6). However, the apparent evolutionary changes witnessed in laboratory studies were not demonstrated in field experiments (Chapters 4 and 5). In part, seed size, which is a trait of the maternal plant, and its interaction with the environment, may overwhelm genetic differences for success during germination (Chapter 5). Further examination of the salt tolerance of roadside populations of *D. sylvestris* demonstrated that there were different mechanisms conferring tolerance in different populations (Chapter 3). Again, maternal influences of seed composition were implicated in one of the salt tolerance mechanisms. The other type of salt tolerance resembled the behaviour of a halophytic plant, with high Na^+ levels being associated with tolerance. Finally, comparisons of the responses of different roadside families to salt and drought stress revealed that tolerance to these stresses are two separate traits (Chapter 6). Examination of the leachate of roadside populations of *D. sylvestris* indicated that the seeds may possess some allelopathic properties (Chapter 7).

Evolutionary change has occurred in roadside populations of both *D. sylvestris* and *H. matronalis* and is visible in the laboratory examinations of tolerance to specific abiotic stresses (Chapter 2). The tolerance to high manganese levels observed in *H. matronalis* was only observed in a subset of maternal families from one of the three roadside populations. This could be indicative of variability in the strength of selection pressure for manganese tolerance among roadside locations. The absence of high manganese levels in roadsides of the Greater Toronto Area (Bhuie & Roy, 2001) suggests that high levels of manganese and, especially, high levels of manganese that are available to plants, are likely extremely patchy along roadsides in southern Ontario. Alternatively, the presence of the tolerance trait in only two of the five maternal families from the roadside population may indicate that this is a new trait that has not yet had the opportunity to spread widely within and between roadside populations.

In contrast, the salt tolerance and salt enhanced growth observed in *D. sylvestris* was found in all three roadside populations examined indicates the ubiquitous selection for enhanced salt tolerance among roadside populations (Chapter 2). Further examination of the tolerance (Chapter 3) has revealed that between and even within populations, different mechanisms for salt tolerance exist, highlighting the exceptional levels of variation found in these populations. Two of the three seed collection sites of *D. sylvestris* had substantially lower Na^+ levels than the other roadside locations (Appendix C). Yet, seeds collected from these sites possessed significant variation for salt tolerance.

Migration rates between populations along the roadside corridor are likely very substantial resulting in the presence of salt tolerance traits even in populations that are not exposed to high salinity levels. The existence of these traits in non-saline habitats strongly suggests that there is little cost to salt tolerance, despite the low rates of root growth observed under control conditions in the germination experiments (Chapters 2,3, 5 and 6).

Despite the substantially reduced growth of roadside families of both *H. matronalis* and *D. sylvestris* in control treatments in Chapters 2, 3, 6 and 7, roadside families performed very well in the field experiments (Chapters 4 and 5). This contradiction in results illustrates the importance of the complexity of the natural environment in evolutionary change and how inferences from experiments made under control conditions may not be valid in the natural environment.

Many different aspects of the evolutionary change in roadside species of plants have been investigated in the preceding chapters. I have investigated adaptations to specific stresses in laboratory experiments, local adaptation in field experiments, the physiology of salt tolerance in *D. sylvestris* and the role of maternal effects in conferring adaptations. My investigations have resulted in the unveiling of a complex system. Roadside vegetation has very high levels of genetic variation. In particular, *D. sylvestris* and *H. matronalis* roadside populations had higher levels of variation than the oldfield populations. Environmental stress is a strong evolutionary force. Not only does it impose strong directional selection pressures, it also reduces population sizes, which reduces the frequency of migrants between populations, increasing differentiation between populations. Under stressful conditions, mutation and recombination rates also rise, increasing the variation present within populations (Hoffman & Hercus, 2000). Finally, many environmental stresses are not constant. When there is a significant interaction between an environmental stress and the genotype of organism that is not continually present, increased levels of genetic variation will be maintained (Hoffman & Hercus, 2000). My examination of roadside habitats has demonstrated that this environment possesses the characteristics necessary to promote the high levels of variation found in roadside populations; there are many stresses acting in concert on a single genotype that vary in importance from year to year and from place to place depending on the weather and the distance that a seed is deposited from the roadside. Environmental conditions change very rapidly from the edge of the highway to the edge of the highway corridor (Forman & Alexander, 1998), with selection pressures varying with equal magnitude. The high levels of variation present in roadside populations mirrors the extreme variation in the environment.

These studies have clearly demonstrated that species are able to adapt very rapidly to anthropogenic activities. Genetic diversity is the focal point of conservation strategies, but the existence of a significant level of variation that exists only because of the presence of habitats created and maintained by anthropogenic activity has not been adequately addressed. The issue of whether this variation is more or less important in the conservation of species has been ignored. The high level of success of roadside genotypes in natural environments (Chapters 4 and 5) indicates that there is no barrier to gene flow between populations in human environments and natural habitats. It is pertinent to discuss if contamination of natural populations by disturbance tolerant genotypes is of concern to the preservation of species. This is particularly relevant for native species that inhabit roadsides. Dispersal along roadsides is very high and genetic variation for tolerance of human habitats is more likely to enter undisturbed populations from roadsides simply because the pervasiveness of roadsides puts virtually all natural population in close proximity to them (Forman & Alexander, 1998). If these disturbance tolerant genotypes are not viewed as desirable, then management practices must be undertaken to ensure that they do not infiltrate native populations. In contrast, the adaptability of exotic species to human activities demonstrated in the Chapter 2 is also important for natural populations. The continued expansion of human habitats results in greater and greater degrees of disturbance in natural habitats. If exotic plants, which make up the majority of the species inhabiting roadsides (Forman & Alexander, 1998, Wester & Juvik, 1983), are afforded the opportunity to adapt to human disturbances, while native species are not simply because the populations are protected in sanctuaries, the increasing levels of human disturbance may make the populations more vulnerable to competitive exclusion by exotic plants. In this case, establishing native populations in human altered ecosystems and allowing them to adapt to the conditions present may afford native species greater protection from the competitive onslaught of exotic species. Restoring roadsides to a prairie habitat would not only create additional habitat for species whose range has been obliterated and link up fragmented populations, but would also reduce the habitat present for exotic species, potentially inhibiting their spread, and may potentially result in the evolution of disturbance tolerant genotypes that would increase the likelihood that species could avoid extinction, though it may mean a loss of genetic diversity in populations that are not disturbance tolerant and hence are competitively inferior in the face of human expansion.

These results support the theory that the invasive nature of some exotic species could be due to their ability to rapidly adapt to new environmental conditions (Lee, 2002). The examination of the role of maternal environmental effects indicates that adaptations that are conferred not through genetic changes but by the influence of the maternal plant could considerably speed up the response to selection. If tolerance to a specific stress is conferred through maternal environmental effects, then survival through one season would create offspring adapted to the environment they will face during germination and seedling establishment.

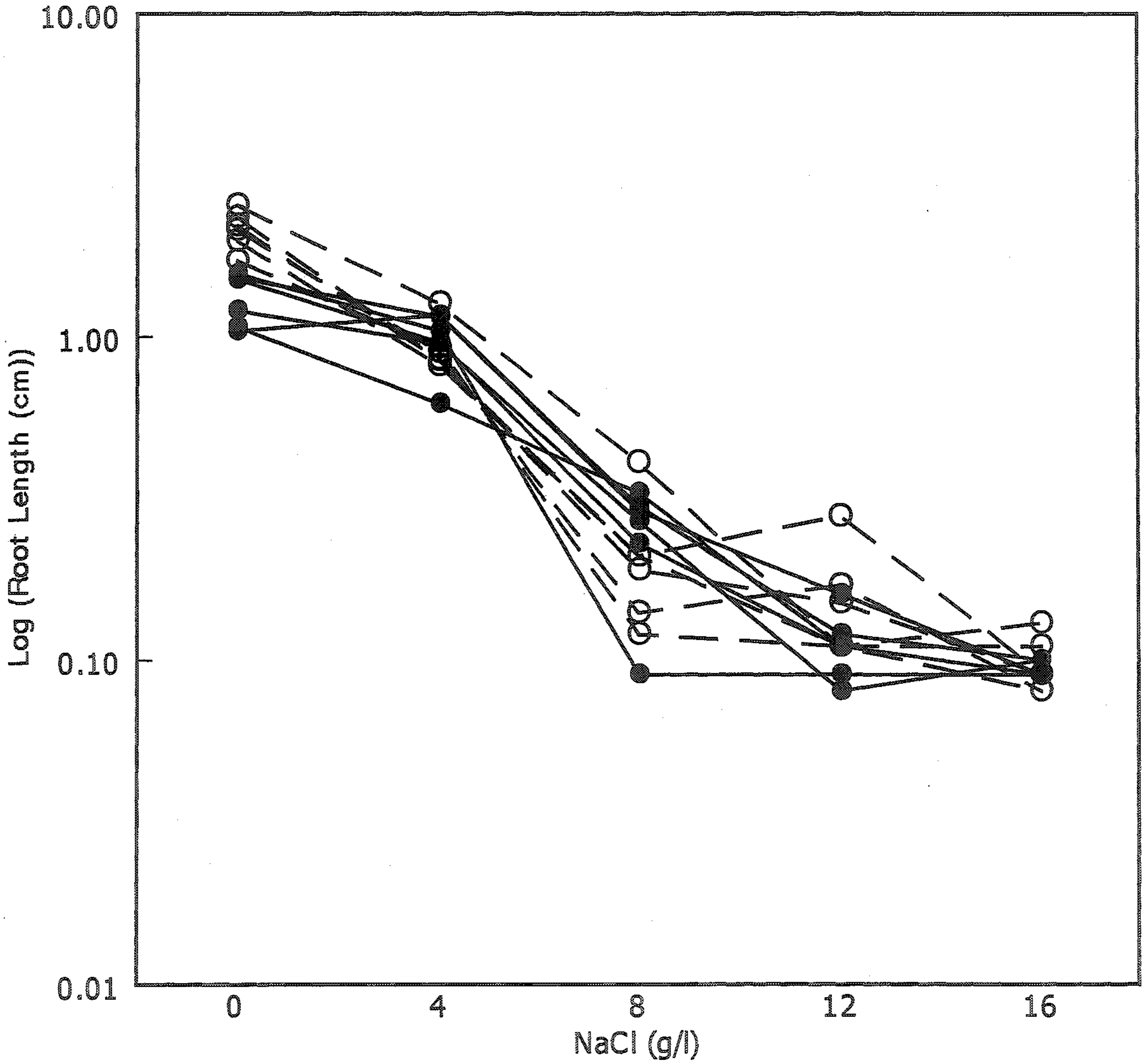
In this examination of evolutionary change in the roadside, I have examined the problem from several angles, through laboratory experiments to specific abiotic stresses, through field studies of adaptation to the environment as a whole, through examination of the physiology of tolerance and through the role of the maternal environment in conferring tolerance. These studies have led me to conclude that the roadside is an important habitat and warrants additional research into the impacts of the increased availability of nitrogen, the control of the spread of exotic species and their dispersal patterns. The selection pressures present in the roadside are creating populations of plants that are extremely tolerant of disturbance and possess considerable genetic variation. Roadside ecology is a growing field and constitutes many fascinating avenues of future research.

APPENDICES

APPENDIX A

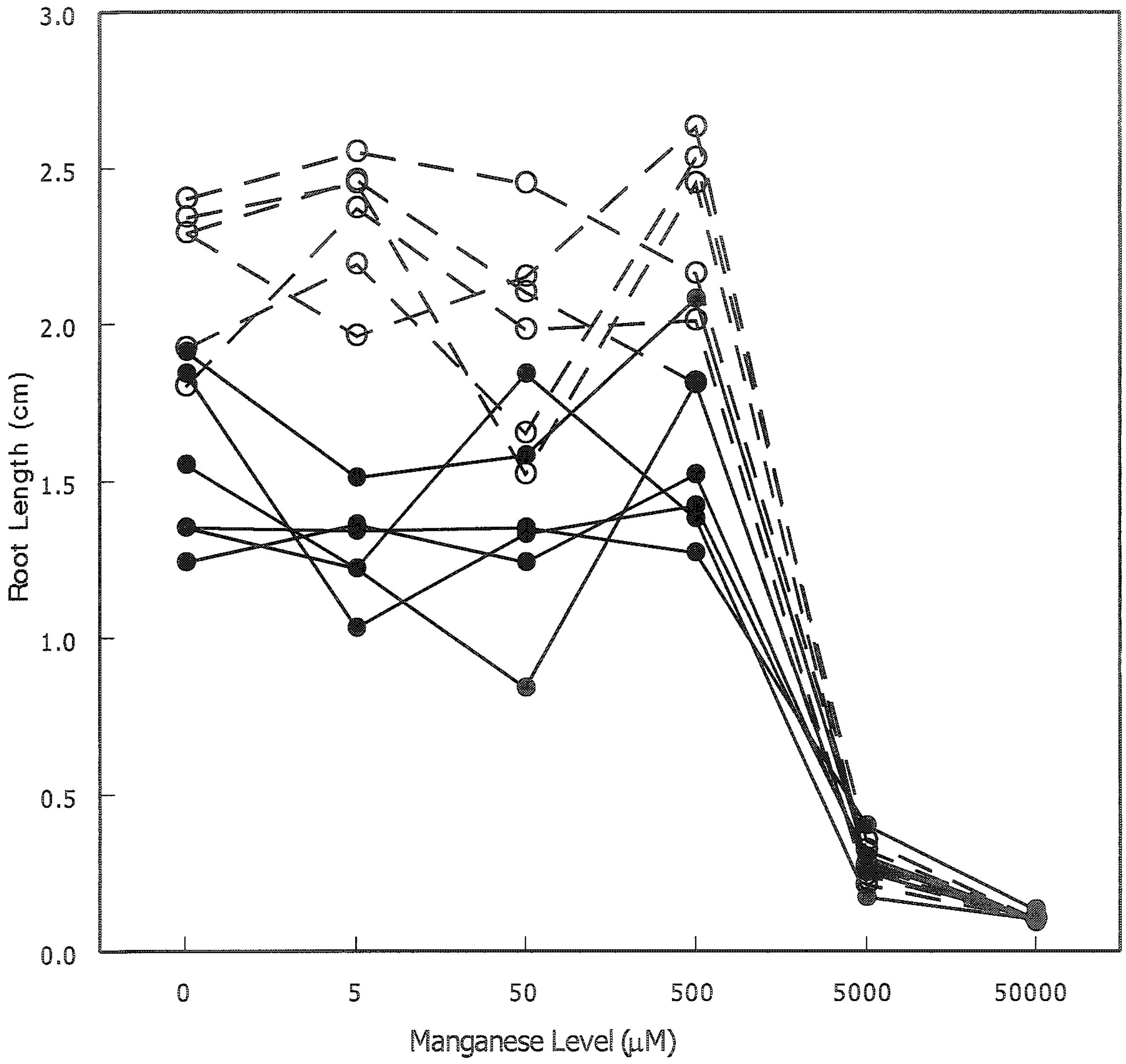
SALT AND MANGANESE TOLERANCE TRIALS – *D.SYLVESTRIS*

A.1 Root length after ten days of *D. sylvestris* seedlings collected from oldfield and roadside populations germinating in 0, 4, 8, 12 and 16 g/l NaCl. Linear connections represent the averages from each maternal family. Dashed lines and ○ represent oldfield populations, solid lines and ● represent roadside populations.


10.00
1.00
0.10
0.01
Log (Root Length (cm))
0
4
8
12
16
NaCl (g/l)

APPENDIX A (continued)

A.2 Root length after ten days of *D. sylvestris* seedlings collected from oldfield and roadside populations germinating in 0, 5, 50, 500, 5000 and 50 000 μM $MnSO_4$. Linear connections represent the averages from each maternal family. Dashed lines and ○ represent oldfield populations, solid lines and ● represent roadside populations.


3.0
2.5
2.0
1.5
1.0
0.5
0.0
Root Length (cm)
0
5
50
500
5000
50000
Manganese Level (µM)

APPENDIX B

EXAMINATION OF THE IMPACT OF SO_4 ON THE GERMINATION OF *D. SYLVESTRIS*, *H. MATRONALIS AND A. SYRIACA*

B.1 METHODS

Seeds from each of two maternal lines of the six populations (three roadside and three oldfield) from the three species (*D. sylvestris*, *H. matronalis* and *A. syriaca*) were placed in petri-dishes lined with filter paper (10 seeds from one maternal line per dish) and containing either 5ml of 3 mM $MnSO_4$, 3 mM $NaSO_4$ or distilled water (control treatment). Each treatment/seed combination was replicated twice. *A. syriaca* seeds displayed a strong dormancy that was overcome by soaking the seeds for one week in the test solutions and then scarifying the seeds by scraping away the seed coat with a razor blade. Seeds were incubated at room temperature under artificial lighting. On the third, fifth and seventh day, 2 ml of distilled water was added to each tray to replenish moisture lost to evaporation. After 10 days, the root length of the emerging seedlings was measured. Unlike *H. matronalis* and *D. sylvestris*, *A. syriaca* seedlings do not display a clear differentiation between the hypocotyl and the root. For this reason, the length of both the hypocotyl and the root was recorded for this species.

Analyses of variance (PROC GLM) were conducted to determine if the treatments had an effect on the root length of the germinating seeds and if the effect differed between seeds collected from roadsides and seeds collected from oldfields. Each species was analyzed separately. The p-diff and t-diff options of the LSMEANS statement (PROC GLM) were used to make pair-wise comparisons between the three treatments

APPENDIX B (continued)

B.2 RESULTS

The sulfate in the manganese treatment did not have a deleterious effect on root length in any of the three species as can be seen by comparing the control treatment to the sulfate treatment (Figure 1). In fact, both *D. sylvestris* and *H. matronalis* had slightly longer roots in the $NaSO_4$ treatment than in the control treatment. The cause of this stimulatory effect is unknown but may be related to the increased acidity resulting from the addition of the salt. In contrast, the $MnSO_4$ treatment had a strong deleterious effect on the root lengths of all three species. Thus, it is possible to conclude that the effects of the sulfate in the manganese treatment were overwhelmed by the deleterious effects of the manganese.

APPENDIX B (continued)

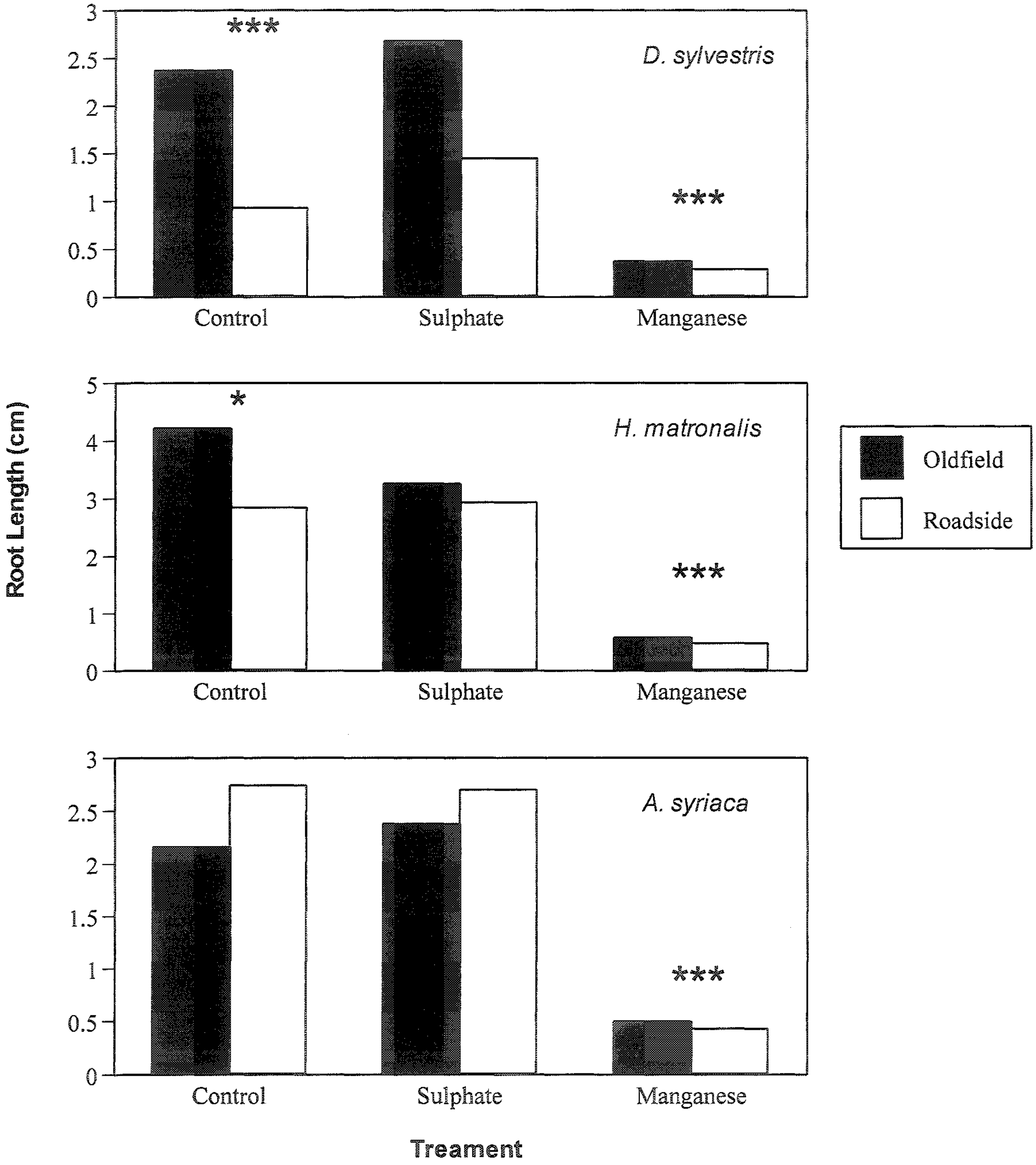
B.3 An analysis of variance of the effect of the treatment, site and the interaction of the treatment and site on the root length after ten days of *D. sylvestris*, *H. matronalis* and *A. syriaca* seeds collected from roadside and oldfield population and germinating in distilled water, 3 mM $MnSO_4$ and 3 mM $NaSO_4$.

		D. sylvestris		*H. matronalis*		*A. syriaca*	
Source	df	Mean Square	*F*	Mean Square	*F*	Mean Square	*F*
S	1	182.220	544.09 ***	5.520	3.87 †	5.520	3.31 †
T	2	169.410	505.84 ***	216.805	161.63 ***	194.011	116.34 ***
S×T	2	39.036	116.56 ***	1.158	0.86	2.905	1.74

*† $P < 0.1$; *** $P < 0.001$*

APPENDIX B (continued)

B.4: Root length after ten days of *D. sylvestris*, *H. matronalis* and *A. syriaca* seedlings collected from oldfield and roadside populations germinating in distilled water, 3 mM $MnSO_4$ and 3 mM $NaSO_4$. Asterisks represent significant differences between the $NaSO_4$ treatment and either the $MnSO_4$ or distilled water treatment. (* $P < 0.05$; *** $P < 0.001$).


D. sylvestris
H. matronalis
A. syriaca
***
***
*
***
***
Root Length (cm)
0
0.5
1
1.5
2
2.5
3
0
1
2
3
4
5
Control
Sulphate
Manganese
Oldfield
Roadside
Treament

APPENDIX C

MEASUREMENT OF SODIUM ION LEVELS IN ROADSIDE SOIL

C.1 COLLECTION SITES

Four sites were chosen for soil sample collection. Three sites corresponded to the *Dipsacus sylvestris* roadside populations collection sites described previously. The fourth site was the location of the reciprocal transplant experiments. Roadside population 1 was located on highway 403 in Hamilton at the top of the escarpment. Soil was collected at a distance of approximately 10 m from the highway's edge at the periphery of the *D. sylvestris* population. The site was elevated above the ditch running next to the highway. The soil was a dense clay. Due to highway construction activity at this site, the collection of samples was restricted to the spring (March to early May). Roadside population 2 was located on highway 403 in Hamilton in Mercer's Glen near Cootes Paradise Marsh. Soil was collected at a distance of approximately 3 m from the edge of the highway at the top of a very steep ditch. The soil was silty with large pieces of gravel. Roadside population 3 was located on highway 403 between Hamilton and Burlington. Soil was collected at a distance of approximately 3 m from the edge of the highway in a shallow ditch. The soil was very dense clay. The site of the reciprocal transplant experiments was located along the 403 between Hamilton and Burlington near the Aldershot Go Station. The area is near an overpass and is bordered by both the 403 and the Waterdown exit ramp. The area is level with the highway (i.e. is not sloped). The soil was very dense, red clay.

APPENDIX C (continued)

C.2 SOIL COLLECTION

Five collection locations were selected at each site. At the three sites corresponding to the *D. sylvestris* collection sites, locations were chosen to be evenly spaced throughout the population at equal distances from the highway. At the site of the reciprocal transplant experiments sampling locations were evenly spaced along the length of the experimental sites at equal distances from the highway. The sampling locations were marked with flags. Every three to four weeks from March to September, 2002, soil samples were collected from each site. An aluminum soil corer was used to collect the top 5 cm of the soil. Any vegetative material collected with the soil was removed from the samples. The samples were stored in plastic resealable bags.

C.3 SOIL ANALYIS

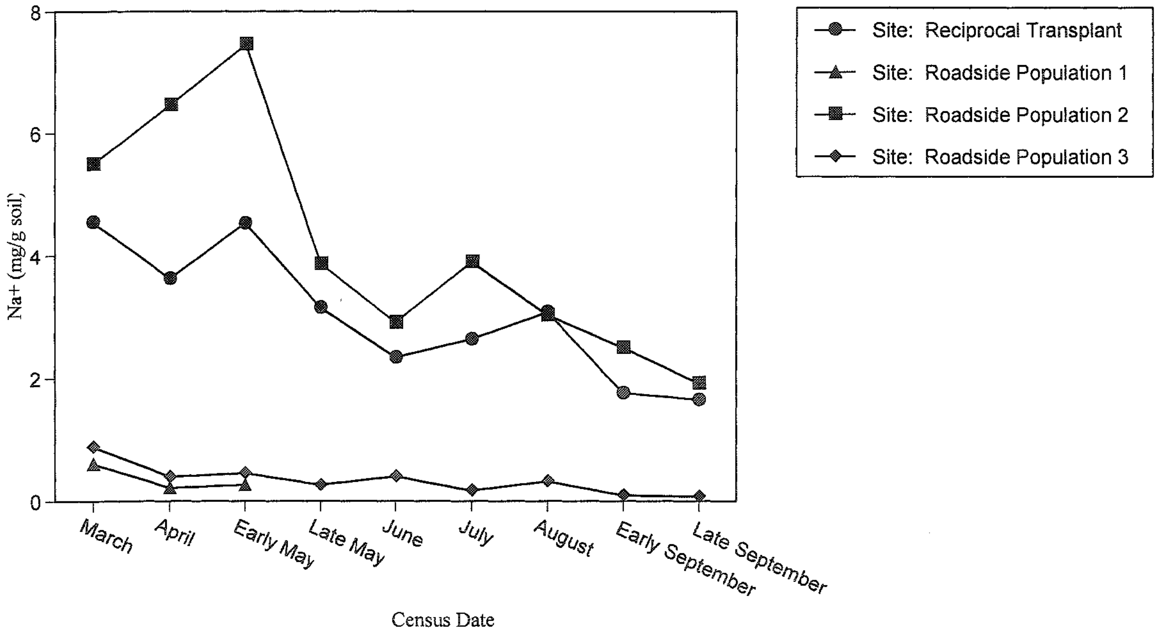
The plastic bags containing the soil samples were opened to allow the soil to dry, after which time the bags were resealed. The soil samples were ground up using a mortar and pistil and passed through a sieve to remove small stones. 5 g of soil from each sampling location was combined with 100 ml 1 M ammonia acetate. Soil samples were spun for one hour on a magnetic spinner and then placed in the refrigerator. After one week, sedimentation had removed enough particulate matter from the solution to allow for sodium ion analysis. Samples were diluted with distilled water by 90-98.25%. The sodium ion content of three replicate samples from each soil sample was measured using a sodium flame spectrophotometer.

C.4 RESULTS

All four sites had high Na^+ levels in the spring (Fig. 1). The Na^+ levels at the roadside population 3 site increased throughout the early spring. Salt that has accumulated on the pavement throughout the winter may have continued to be transported into the roadside soil by runoff. The roadside population 3 site and the site of the reciprocal transplants had substantially higher Na^+ levels throughout the season than roadside population 1 site and the roadside population 2 site. The roadside population 1 site was located much farther from the highway than the two sites with higher Na^+ levels, which may have contributed to the lower levels. The roadside population 2 site, unlike the other three sites, had a gravelly, silty soil which would allow ions to leach much more easily than the dense clay subsoils present at the other three sites. Both the roadside population 1 site and the site of the reciprocal transplant experiment were very close to the elevation of the highway with no intervening ditch. Both sites had very dense soils that would likely have retained ions very effectively. At both sites, Na^+ levels increase from June to September. The increase was likely the result of a drought that lasted throughout the growing season of 2002. By late September, the Na^+ levels at both sites remained substantially higher than at the roadside population 1 site and roadside population 2 site, indicating that Na^+ may accumulate from year to year in roadside soil.

APPENDIX C (continued)

C.5 Na^+ levels at four sites located beside highway 403 between Hamilton and Burlington, Ontario from early spring to fall of 2002. Roadside populations refer to *D. sylvestris* collection sites.


Na+ (mg/g soil)
0
2
4
6
8
March
April
Early May
Late May
June
July
August
Early September
Late September
Census Date
Site: Reciprocal Transplant
Site: Roadside Population 1
Site: Roadside Population 2
Site: Roadside Population 3

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