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Influences of Environmental Variability, Genetics and Plant Size on Variation in Sexual and Clonal Reproduction and Allocation of Resources in Three Wetland Plant Species

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INFLUENCES OF ENVIRONMENTAL VARIABILITY, GENETICS AND PLANT SIZE ON VARIATION IN SEXUAL AND CLONAL REPRODUCTION AND ALLOCATION OF RESOURCES IN THREE WETLAND PLANT SPECIES.

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Dedication

My dissertation is dedicated to the

memory of Dr. Tarun Mal

1962-2005

Teacher,
Mentor,
Friend.

“To live in the hearts we leave behind is not to die.”
--Thomas Campbell

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INFLUENCES OF ENVIRONMENTAL VARIABILITY, GENETICS AND PLANT SIZE ON VARIATION IN SEXUAL AND CLONAL REPRODUCTION AND ALLOCATION OF RESOURCES IN THREE WETLAND PLANT SPECIES.

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ABSTRACT

Optimal Partitioning Theory (OPT) states organisms will give more resources to structures and functions that enhance fitness. OPT can be applied to reproduction in clonal plants, which allocate resources between two modes of reproduction—sexual through fruits and clonal through spacers and ramets. In nutrient rich environments, clonal growth allows offspring to stay in beneficial surroundings, while in nutrient poor conditions, sexual reproduction can allow escape and generation of new, potentially more fit offspring. I tested this hypothesis by comparing clonal and sexual reproductive allocation in *Penthorum sedoides* under differing nutrient levels over two generations**.** Genotypic and environmental influences on reproductive variation in *Lythrum salicaria* and *Penthorum sedoides* were separated by comparing clones within and between treatments. Allocation to fruits was higher in the control than the fertilized group, but only in the second year, providing partial support to an increase in sexual allocation in lower resource conditions. Allocation to spacer mass and ramet mass increased under high nutrients, while number of ramets did not, also providing limited support to the predictions of OPT. Genotype had little effect on sexual and clonal variation. Variation due to fertilizer was more influential, demonstrating plasticity in reproductive expression.

The two species differed in their reaction to nutrient levels, potentially a consequence of their differing clonal strategies.

Optimal Partitioning Theory also predicts that in situations detrimental to survival, such as herbivore attack, plants will invest less in sexual reproduction and more in clonal growth to aid tissue replacement and survival. I compared reproductive responses of three wetland species—*Eupatorium perfoliatum*, *L. salicaria* and *P. sedoides*—inflicted with simulated herbivory—leaf damage, root damage, both root and leaf damage and undamaged controls. Sexual reproduction in *P. sedoides* was reduced after root damage while it increased with root damage in *E. perfoliatum*, providing contradictory support for a shift away from sexual reproduction to increase survival. Increase in clonal growth under stressed conditions was seen in *E. perfoliatum* under root herbivory while clonality was unaffected in the other species. Support for OPT was therefore mixed and depended on species, year and trait measured.

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CHAPTER I

INTRODUCTION

Overview

The purpose of this dissertation is to explore how environmental variables alter the way plants distribute resources (measured as biomass), with emphasis on sexual and clonal reproduction. The overarching theory is that plants will change the distribution of their biomass in a way that optimizes their fitness in a given environment, which is the essence of Optimal Partitioning Theory (OPT) (Coleman et al. 1994, McConnaughay and Coleman 1999, Karlsson and Mendez 2005).

Clonal plants have two modes of reproduction, sexual and asexual, and each of these modes and the offspring they produce have different advantages (Jackson et al. 1985). It can therefore be predicted from OPT that plants will favor one mode over the other depending on environmental conditions (Gardner and Mangel 1999). To test this prediction, I compared the amount of clonal and sexual reproduction between plants in

contrasting environments by simulating root and leaf herbivory and manipulating nutrient levels Also addressed are the effects of plant size on reproductive allocation and the influence of genetic factors on variation in biomass distribution to both reproductive and vegetative traits. Genotypic control over variation of these traits may interfere with environmental responsiveness and ignoring size while investigating biomass partitioning may lead to misinterpretation of results. My general hypotheses were that 1. Simulated herbivory will cause an increase in clonal growth and a decrease in sexual reproduction. 2. Nutrient addition will cause an increase in clonal allocation and a decrease in sexual allocation. 3. Genotype influences the variation in clonal and sexual reproduction. Each of these hypotheses is discussed in more detail and the rationale behind them given in the description of the chapters below.

Organization, hypotheses and rationale

The dissertation is divided into six chapters:

Chapter I: Introduction to the dissertation

Chapter I presents an overview of the purpose of the dissertation and describes the organization and the hypotheses. Also discussed is the rationale for my choices of the species used in my research.

Chapter II: Background

In Chapter II, I provide background regarding the nature of clonal growth in plants. Clonal growth occurs in many plant species and provides a variety of fitness advantages to a plant, not the least of which is the production of new, genetically identical offspring. However, it is often ignored in the study of plant reproduction.

Chapter II also gives a brief overview of Optimal Partitioning Theory and its implications for plant growth and reproduction. I also discuss the importance of taking size into account when testing OPT.

Chapter III: Effect of simulated leaf and root herbivory on growth and sexual and clonal reproduction in three wetland species.

Under stressed and potentially life threatening conditions, a plant's priority should be survival and damage control, which, according to OPT, will be reflected in the way it partitions its resources. Sexual reproduction, which takes up a large amount of resources and does not aid in survival or repair should decrease under stressed conditions. In contrast, clonal growth leads to the production of more root and photosynthetic tissue, which can replace what was lost to herbivores. These predictions have been borne out by other studies, especially with regard to sexual reproduction (Reichman and Smith 1991, Parra-Tabla et al. 2004, Egan and Irwin 2008, Liu et al. 2009b), although there are few studies that look at the influence of herbivory on clonal growth, especially root herbivory. Additionally, the effect of size on response to herbivory has rarely been considered.

Chapter III describes an experiment testing the effect of simulated root and leaf herbivory on three wetland plant species, *Penthorum sedoides*, *Lythrum salicaria* and *Eupatorium perfoliatum*. I investigated the reactions of sexual and clonal reproduction and vegetative traits (such as leaf mass and number of branches) to these two types of herbivory, both independently and in combination.

Hypotheses of Chapter III:

1. Simulated root and leaf herbivory will decrease growth (height and biomass) of the three species.
2. The damage treatments will decrease sexual reproduction to increase resources available for repair and regrowth.
3. The damage treatment will lead to an increase in clonal growth structures as a means to re-grow lost tissue.

Chapter IV: Genetic effects on the partitioning of biomass to growth and sexual and clonal reproduction in *Lythrum salicaria* and *Penthorum sedoides*.

The traits expressed by a plant (its phenotype) are a product of its genes (genotype) and its environment (Falconer 1981). To determine the relative importance of genotype and environmental variability, identical clonal replicates in differing environments can be compared. The differences in phenotype between clones must be due to environmental differences, allowing the determination of the relative magnitude of genotypic and environmental influences on phenotypic variation. If genotype strongly controls a trait's phenotypic expression, it is less likely a plant will be able to alter aspects of the trait in response to environmental conditions. Traits with little genetic control over variation will be more plastic and better able to adapt to environmental changes. Genotypes may differ in the amount of plasticity they exhibit, meaning that phenotypic plasticity itself has a genetic component (Pigliucci 2005). This situation can be detected by an interaction between genotype and environment.

In Chapter IV, I investigated the genetic components of variation in plant growth and reproduction through the comparison of genetically identical clones raised in contrasting nutrient environments—fertilizer added and control. I compared two species with contrasting growth forms, tightly clumped *L. salicaria* and loosely aggregated *P. sedoides*. Since the clonal growth form of *P. sedoides* can be more variable than that of *L. salicaria*, I expected its variation to be under more environmental control and less genotypic control than *L. salicaria.* A comparison of genotypic influence over variation in clonal characteristics between species with opposite growth forms has not previously been done.

Hypotheses for Chapter IV:

1. The two species will differ in amount of resources (biomass) expended on clonal growth and sexual reproduction.
2. There will be interactions between soil nutrient levels and the genetic variation observed.
3. The species will differ in the amount of influence genetic variability has on variation in sexual and clonal reproduction;

Chapter V: Size-dependent analysis of allocation to sexual and clonal reproduction in *Penthorum sedoides* under contrasting nutrient levels.

From OPT, I predicted that in high resource environments, plants will allocate more of their resources to clonal growth as a means of producing as many offspring as possible in the good environment (Gardner and Mangel 1999). Under nutrient poor

conditions, sexual reproductive allocation would increase as a means for escape (seeds can usually travel farther than clonal offspring) and producing genetically diverse and potentially more fit offspring (Silander 1985, Gardner and Mangel 1999, van Kleunen et al. 2002). However, when testing for differences in biomass allocation between treatments, it is important to take into account the allometric nature of plant growth (McConnaughay and Coleman 1999). The way plants partition biomass is to a large extent a function of plant size. If a treatment, such as nutrient addition, increases overall plant size, the larger fertilized plants may appear to have more biomass in a trait than the smaller unfertilized plants. However, this difference could occur because big plants always allocate more to that trait than small plants. In such a situation, there is no direct effect of treatment on the trait and testing the results without considering size may be misleading.

In the studies of plant reproduction, two values are typically discussed. Reproductive output (RO) is the absolute amount of reproduction (e.g. total fruit or seed mass) while reproductive allocation (RA) is the proportion of resources put into reproduction (Bazzaz et al. 2000). Due to statistical problems, it is difficult to analyze RA directly. Instead, the best way to study RA is to examine the relationship between RO and plant size (Klinkhamer et al. 1992). Although these analytical methods are becoming more common for sexual reproduction, clonal reproduction is understudied in this respect.

In Chapter V, I use the results from the experiment described in Chapter IV to investigate the relationship between reproductive modes and plant size in *Penthorum sedoides*. The analysis of reproductive allocation was done using methods that test for

both a minimum size for reproduction and a non-linear relationship between reproduction and plant size (Klinkhamer et al., 1992). Although these methods had been used to investigate sexual reproductive allocation, they had not been applied to clonal reproduction. Weiner et al. (2009), in a review of studies investigating the relationship between size and reproduction, report that the most common result is that sexual reproduction increases with plant size while allocation to reproduction remains constant over plant sizes.

Hypotheses for Chapter V:

1. Reproductive output (total mass of fruits, stolons, number of ramets) will increase with size while RA will remain unchanged.
2. The relationship between plant size and allocation to the two modes reproduction will differ between the fertilizer and control group:
    a. The fertilized group will allocate more biomass to clonal reproduction.
    b. The control group will allocate more biomass to sexual reproduction.

Chapter VI: Conclusions

Chapter VI is a discussion of the results of my research and future research I wish to perform based on the results of this dissertation.

Species descriptions

The three wetland plant species studied were chosen for taxonomic breadth, their ability to reproduce both sexually and clonally and their varying growth forms. I also had a good deal of personal familiarity with the species, having grown them for a previous experiment.

Figure 1. *Penthorum sedoides*. (USDA-NRCS PLANTS Database / Britton and Brown. 1913).

Penthorum sedoides (Figure 1; ditch stone-crop) is a perennial, herbaceous obligate wetland plant native to the Eastern United States, including Northeast Ohio (Haskins and Hayden 1987, Chadde 1998). It is able to reproduce sexually through the production of flowers and fruits and clonally by sending out stolons that establish themselves as new ramets. The familial classification *P. sedoides* and *P. chinensis*, the only other species in this genus, is currently under debate. The genus *Penthorum* has been classified in Saxifragaceae, Crassulaceae and into its own monogeneric family, Penthoraceae, by various authors (Haskins and Hayden 1987). *Penthorum sedoides* grows to be 1-6 dm tall (Chadde 1998).

Lythrum salicaria (Figure 2; family Lythraceae; purple loosestrife), like *P. sedoides,* is a perennial herbaceous obligate wetland plant. However, *L. salicaria* was introduced from Europe and has become an invasive species in North America (Mal et al. 1992, Chadde 1998). It spreads clonally through root buds and exhibits tristyly making it self-incompatible (Mal et al. 1992). It grows to be about 6-15 dm tall (Chadde 1998).

Eupatorium perfoliatum (Figure 3; family Asteraceae; boneset) is a perennial, herbaceous, facultative wetland plant native to Northeast Ohio. It can reproduce clonally through short rhizomes that produce new shoots and sexually through windborne seeds (Chadde 1998). It reaches 3-15 dm in height (Chadde 1998).

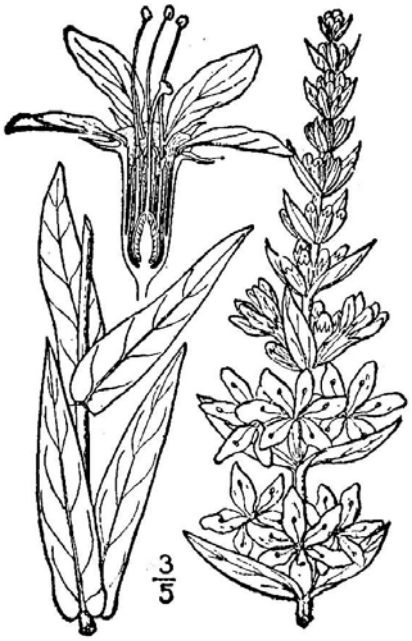

Figure 2. *Lythrum salicaria* (USDA-NRCS PLANTS Database / Britton and Brown. 1913).

All three species were used in my first experiment on simulated herbivory (Chapter III). However, due to time and resource constraints, only *Penthorum sedoides* and *Lythrum salicaria* were used in the nutrient addition experiments (Chapters IV and V) and only data from *P. sedoides* were used in the size dependant analysis of reproductive allocation due to low seed set in *L. salicaria*.

Figure 3. *Eupatorium perfoliatum*. (USDA-NRCS PLANTS Database / Britton and Brown. 1913).

I chose *Penthorum sedoides* and *Lythrum salicaria* for the nutrient addition experiments because of their individual reproductive characteristics and also the contrasts they exhibit. *Penthorum sedoides* is a highly clonal species. It begins producing spacers (i.e. stolons) at a very small size (well before flowering commences) and continues to create new stolons throughout the growing season. Many of these stolons

begin to grow upward to become new individuals (ramets), genetically identical to the parent plant. These ramets can be widely spaced depending on the length of the stolon from which they formed. *Penthorum sedoides* also produces multiple inflorescences (on the parent plant and the ramets) and fruits. I have recorded 600 fruits on a single individual of *P. sedoides,* all of which produced thousands of small seeds. This abundance of both clonal and sexual reproduction makes *P. sedoides* a good study species for investigating resource allocation to reproduction.

Lythrum salicaria also exhibits large reproductive capacities. It is estimated that a single plant of *L. salicaria* is able to produce over 2 million seeds and have as many as 30-50 stems (Mal et al. 1992). However, it differs from *P. sedoides* in several ways. *Lythrum salicaria* is a superior competitor, able to dominate entire wetlands (Mal et al. 1992). Its mode of clonal reproduction differs from *P. sedoides*; instead of producing long spacers, new ramets arise from buds formed on the root stock (root buds) (Chadde 1998). Therefore the ramets produced by *L. salicaria* are remain close to the parent plant and are tightly packed together. *Lythrum salicaria* begins reproducing clonally later in life and not as rapidly as *P. sedoides*.

CHAPTER II

BACKGROUND

Nature of Clonal Growth

Clonal plants spontaneously produce potentially independent offspring by means of vegetative growth (Jackson et al. 1985, Hutchings and Mogie 1990, van Groenendael et al. 1996). Clonal reproduction occurs without meiosis and syngamy; it only requires mitosis of the plant's somatic cells, similar to vegetative growth (Aarssen 2008). Because plant cells are totipotent (able to form other cell types even after differentiation), it is possible for shoot cells to give rise to roots and root cells to give rise to shoots (Schmid 1990, van Groenendael et al. 1996, Aarssen 2008). When this occurs, the structure produced is called a "rooted unit", a module of plant tissue that is able to photosynthesize and obtain nutrients and water from the substrate. Rooted units are therefore capable of life independent from the parent plant (Hutchings and Mogie 1990, Schmid 1990, Aarssen 2008). Such potentially independent portions of a clonal plant are referred to as ramets, and typically have all the traits and functions of the parent plant (Hutchings and Mogie 1990, Pan and Price 2002). In many clonal species, new ramets

are formed from and connected to each other (either permanently or temporarily) by specialized organs called spacers (e.g. stolons or rhizomes). Since no genetic recombination occurs in the formation of spacers and ramets, each ramet produced by a plant is genetically identical to the other ramets, including the parent plant, barring any somatic mutations that may have occurred in the tissue of the original plant during creation of the ramet or in the ramet itself (Jackson et al. 1985, Pan and Price 2002). This group of genetically identical ramets is called a genet which can be thought of as all the plant tissue that develops from a single zygote, whether it is contained in one "rooted unit", as would be the case for non-clonal plants, or spread out among a multitude of clonally produced independent ramets (Pan and Price 2002, Aarssen 2008). Since independent ramets can be produced through clonal growth, a distinction must be made between genetic and physiological individuals (Jackson et al. 1985). If connections remain intact, a genet is a physiologically integrated system, with some carbohydrates, minerals and water transported from one ramet to another through the spacers (Marshall 1990). However, even if the connections between ramets are still physically intact ramets can act as independent individuals (Marshall 1990).

Advantages of Clonal Reproduction

Clonal reproduction offers a plant many advantages over sexual reproduction. Most obviously, sex is not involved, which saves the plant the cost and risk of producing flowers, fruits and seed. Although genetic recombination from sexual reproduction is the only way new genotypes are produced, sex has drawbacks for the parental genotype. Sexual reproduction entails a "meiotic cost" for the parent plant (Williams 1975). Except in the case of selfing, sexually produced offspring carry only half the genes of

each parent while clonal offspring, formed through mitosis, carry all the genes of the original plant (Williams 1975). Asexual reproduction allows adaptive gene polymorphisms to remain intact in the individual's offspring, while sexual reproduction may break them up by genetic recombination (Silander 1985). If certain genotypes are well adapted to a particular environment, asexual reproduction allows for the spread of the most fit genotype (Silander 1985, Menges 1990).

An important outcome of clonal growth is persistence and longevity of the genet (Eriksson and Jerling 1990, Hutchings and Mogie 1990, van Groenendael et al. 1996). Because a genet is able to continuously produce new ramets, the genet itself may not be subject to senescence, although individual ramets may be (Sackville Hamilton et al. 1987). This constant addition of new ramets is an example of risk spreading, a strategy to avoid extinction of the genet by placing numerous offspring in variable environments (Eriksson and Jerling 1990). Genet mortality is related to the probability of all ramets of a genet dying (Harper 1985, Pan and Price 2002), and the production of new ramets decreases that risk, since it is unlikely mortality will befall multiple, wide-spread ramets at the same time (Hutchings and Mogie 1990). Theoretically, a clonal plant may be immortal (Hutchings and Mogie 1990, Hutchings and John 2004). As a ramet dies, others will replace it and the genotype can exist indefinitely. While immortality in plants cannot be proven, there is evidence of genotypes existing a very long time. For example, certain genets of the sedge *Carex curvula* are estimated to be 2000 years old (Steinger et al. 1996).

At any point in a plant's life, sexual reproduction may be impossible. It may not be the proper time in the growing season, the plant may not be large enough or have

enough resources to initiate reproduction (Menges 1990, Mendez and Obeso 1993), or environmental conditions may not be conducive to flowering and fruiting. At these times, clonal reproduction is especially important. Clonal growth is not as seasonally limited as sexual reproduction and can occur at almost any time during the growing season (Williams 1975). Compared to seed production, germination and seedling growth, the production and growth of spacers and ramets occurs much more quickly and is better able to change with temporal environmental conditions. This rapid response to environmental change allows a genet to take advantage of variable and unpredictable resource fluctuations (Williams 1975, Hutchings and Mogie 1990).

Clonal reproduction is less costly in terms of resources than sexual reproduction and typically does not have a minimum size requirement for initiation. (Schmid et al. 1995). However, flowering requires a minimum amount of resources to occur successfully. This means that clonal reproduction may occur under more adverse conditions, at smaller plant sizes and with fewer resources than sexual reproduction (Schmid et al. 1995). Clonal reproduction can compensate for lost offspring production in years when environmental conditions limit sexual reproduction, or in years following extensive sexual reproduction when a plant's reserves are low (Jones and Gliddon 1999, Ceplitis 2001b). Clonal growth is often maintained at a constant level from year to year while sexual reproduction fluctuates more, depending on resource availability (Fitter and Setters 1988). When seedling recruitment is restricted or varies greatly from year to year, sexual reproduction can be ineffective. Under such conditions, clonal reproduction is essential to genotype reproduction and is therefore very common (Menges 1990, Stocklin and Winkler 2004).

The ramets resulting from clonal growth have a variety of advantages over sexually produced seedlings. When ramets become independent from their parent plant, they have a larger initial size and are more mature than seedlings (Williams 1975, Menges 1990). They also have the capability of remaining integrated with the parent plant through the rhizome or stolon, allowing exchange of resources between ramets (Marshall 1990). The genet of a clonal plant is a population of ramets, some still interconnected, that vary in age, size and the environmental conditions to which they are exposed. It provides a support system to newly developed ramets or those growing in adverse conditions (Marshall 1990). In a review of studies on resource sharing between ramets using a radiotracer, ^{14}C, Marshall (1990) summarizes the general pattern found as a new ramet ages: Typically, young ramets import a great deal of resources from the mother ramet or other established ramets, but the amount imported decreases with ramet development. Eventually, the ramet becomes mostly or totally independent of the older ramets and can begin exporting resources it now acquires on its own (Marshall 1990). If adult ramets are damaged by defoliation or shading, they can again receive support from other ramets (Marshall 1990).

Since clonal offspring usually grow closer to the parent plant than seed-generated offspring, the offspring's environment and the optimal genotype are more predictable based on the parental environment (Williams 1975). Asexual reproduction allows the most fit genotype to spread in a particular environment (Silander 1985). The probability of offspring establishment in a good patch is higher for vegetatively formed offspring because spacers can search for favorable sites, while seed dispersal is random (Silander 1985, Sakai 1995). This placement of ramets in more favorable positions is called

"foraging" and serves to enhance the development of new ramets and increase resources available to the entire genet if ramets are still integrated (Macek and Leps 2003). These characteristics enable new clonal ramets to enjoy a lower mortality rate and more competitive advantages than sexually produced seedlings (Menges 1990, Singh and Singh 2002, Macek and Leps 2003).

Apart from reproduction, clonal growth offers a variety of other advantages to a plant. Clonal growth allows a plant to live in areas or conditions in which it would otherwise be unable to thrive. A comparison of two flood tolerant species, *Epilobium hirsutum* and *Mentha aquatica* indicates that *M. aquatica* exhibits more effective clonal growth in deeper water than *E. hirsutum. M. aquatica* is therefore better able to spread, store resources and persist than its less clonal competitor under flooded conditions (Lenssen et al. 2000). By increasing the quantity of root and photosynthetic tissue, and dispersing it through time and space, clonal growth results in an increase in the resource depletion zone of a genet and in the area inhabited (Hutchings and Mogie 1990). In a resource rich environment, overlapping resource depletion zones of closely packed ramets ensure that all resources in the genet's vicinity are monopolized by the genet (Harper 1985). Since plants are able to produce clonal ramets throughout the growing season and can grow very quickly, they can take advantage of fluctuating resources (Williams 1975). The ability of clonal growth to quickly and efficiently utilize resources favors high rates of clonal reproduction especially in high resource environments (Gardner and Mangel 1999).

Although clonal reproduction has many advantages over sexual reproduction, sex provides benefits that asexual reproduction cannot provide. The most important of these

is increased genetic diversity through meiosis and syngamy, resulting in new genotypes produced as seeds (Williams 1975, Silander 1985, Pan and Price 2002). Clonal growth can cause large clumps of genetically identical individuals that are susceptible to the rapid spread of disease and death of the genet (Harper 1985). Extensive clonal growth can also hinder evolution in an individual's descendents (Sackville Hamilton et al. 1987). If a genet produces only clonal offspring, its progeny will lack the genetic diversity required to survive future environmental changes (Sackville Hamilton et al. 1987). Sexually produced seeds tend to be smaller and more easily dispersed than clonal offspring (Silander 1985) and they tend to travel longer distances from the parental plant (Williams 1975). Models show that although vegetative reproduction is the optimal mode of reproduction, seeds may be maintained partly to offset the local density increases that can occur with exclusive clonal growth (Nishitani et al. 1999, Olejniczak 2001). The dispersal aspect of sexual reproduction also allows plants to colonize new areas and facilitate gene flow between plant populations (Olejniczak 2001, Pan and Price 2002). Since seeds can remain dormant in the soil for years, they allow a plant to "disperse through time" and potentially allow its offspring to grow when conditions are more favorable (Williams 1975, Silander 1985).

Optimal partitioning theory and Allometric growth

Theoretically, an organism has a limited amount of time and resources to perform all the activities it needs in order to survive, grow and produce offspring. Natural selection should favor organisms that allocate their resources in a way that maximizes their genetic representation in future generations (Karlsson and Mendez 2005). This idea,

called the Optimal Partitioning Theory (OPT), holds that plants alter the allocation of biomass or other resources to their organs in response to environmental conditions in order to maximize acquisition of nutrients and other resources necessary for growth, survival and reproduction and to enhance fitness (Coleman et al. 1994, McConnaughay and Coleman 1999, Karlsson and Mendez 2005). For example, in a dry environment an individual may allocate more biomass to roots to better absorb water, while in a moist but shady environment, individuals of the same species will allocate more resources to leaves to improve light harvesting for photosynthesis.

In the past, altered allocation patterns across variable environments were tested by comparing the proportion of resources (for example, biomass) to the various organs in different environments (Weiner 2004). For example, root/shoot ratio and the ratio of reproductive biomass total or vegetative mass are often compared between environments (McConnaughay and Coleman 1999, Wang et al. 2006). However, this proportional view of plant biomass allocation does not take into account that plant growth is allometric—meaning that some changes in allocation may simply be due to the nature of plant growth and development and not the plant's attempt to maximize the acquisition of needed resources (McConnaughay and Coleman 1999, Weiner 2004, Cheplick 2005). Plants proceed through predictable changes in biomass allocation (ontogentic drift) that depend on its needs at a given stage of development (McConnaughay and Coleman 1999). An example of this phenomenon is the change in the root-to-shoot ratio that occurs as a plant develops from a seedling to an adult. Because roots are essential in both nutrient and water acquisition and allow a seedling to remain securely in place, young plants allocate a very large proportion of their resources to roots (Harper and Ogden 1970). This high

allocation to roots decreases as the seedling becomes established and requires more leaves for photosynthesis as resources stored in the seed are depleted. Such changes are typical of the growth pattern all plants must go through, but can lead to what McConnaghay and Coleman (1999) call "apparent plasticity." The rate at which a plant grows varies based on the environmental conditions it experiences. A plant that grows slowly due to limited resources will have a more prolonged resource allocation stage similar to a younger plant than will a quickly growing plant in an enriched environment. Two plants of the same age, each grown in a different environment, may appear to allocate their resources differently, but the difference is just that the resource deprived plant is morphologically and metabolically "younger" (McConnaughay and Coleman 1999).

Due to the allometric nature of plant growth, factors that influence plant size will also affect allocation patterns (Weiner 2004). Assuming that changes in allocation are due only to environmental conditions can lead to misinterpretation of results and spurious support for OPT (McConnaughay and Coleman 1999). The alternative to apparent plasticity is "true plasticity", which is alterations in biomass patterns when size is taken into account, and independent of different growth rates (McConnaughay and Coleman 1999, Weiner 2004). Weiner (2004) suggests that when studying the effect of environmental conditions on plant resource allocation, the null hypothesis should not be that all plants will allocate their resources is the same proportions, but that plant growth is allometric and larger plants will allocate their resources differently from smaller plants. Therefore, plasticity in allocation should be viewed as changes in the allometric trajectory between plants of different environments (Weiner 2004).

Although some seemingly plastic differences between environments may be due to limitation on growth, it is also possible that these differences are adaptive (Sultan 2000). Such results have been found for intraspecific competition (Weiner and Fishman 1994), sowing date (Weiner 2004), light and nutrients (McConnaughay and Coleman 1999, Miao et al. 2008) and between different species of plants (Miao et al. 2008). In some cases, plants alter their allocation in ways that support OPT (McConnaughay and Coleman 1999). However, there have been many instances where only apparent plasticity was detected— environmental conditions affected plant size but not allocation patterns. McConnaughay and Coleman (1999) found that growth rates were altered by water availability but allocation patterns did not differ. Whether plants show true or apparent plasticity may depend on environmental conditions. For example, in one experiment, plants under un-crowded conditions exhibited simple allometry—plants at a given size always had the same pattern. However, under competition, plants differed in growth patterns due to asymmetric competition. Crowded plants, because of their need to be taller, were unable to reach the same shape as un-crowded plants even if they were able to achieve the same mass (Weiner and Fishman 1994).

Because of the potentially large influence size has on resource allocation and the testing of OPT, the statistical analyses of each of my experiments includes a measure of plant size and avoids the analysis of ratios. However, the relationship between plant size and reproductive allocation is the focus of Chapter V.

CHAPTER III

EFFECT OF LEAF AND ROOT HERBIVORY ON GROWTH AND SEXUAL AND CLONAL REPRODUCTION IN THREE WETLAND SPECIES

Abstract

Herbivory can potentially have detrimental effects on plant survival and reproduction. In this study, I simulated root and leaf herbivory on three wetland plant species, *Penthorum sedoides, Lythrum salicaria* and *Eupatorium perfoliatum*, to investigate the effects of damage on growth and sexual and clonal reproduction. My hypotheses were that simulated root and leaf herbivory would 1. decrease growth (height and biomass); 2. decrease sexual reproduction and 3. increase clonal growth structures as a means to compensate for the damage. I also investigated interactions between the two forms of damage and the effect of plant size on the responses to damage, which has been rarely studied. Many aspects of growth were unchanged by damage, although height decreased in *E. perfoliatum* in the leaf-damaged plants and both leaf and root damage decreased leaf biomass in *L. salicaria*. In other cases, plants seemed to compensate or over-compensate for the damage. Height and branch number in *L. salicaria* and

and *P. sedoides* and components of vegetative biomass increased in *L. salicaria* and *E. perfoliatum* after damage. Mass of clonal structures increased for damaged plants only in *E. perfoliatum*. *Penthorum sedoides* decreased fruit mass with root damage. However, *E. perfoliatum* increased fruit mass with root damage. Significant interactions occurred between plant size and the treatment effects for several of the measured traits, demonstrating the importance of testing for size-dependent effects when investigating responses to environmental variables such as herbivory.

Introduction

Herbivory is a ubiquitous stressor for many species of plants, at both individual and community levels. Aboveground (stems and leaves) and belowground structures (roots and storage organs) are susceptible to herbivore attack (Blossey and Hunt-Joshi 2003, Poveda et al. 2003, Hunt-Joshi and Blossey 2005, Hladun and Adler 2009). However, leaf herbivory has been the primary focus of research (Blossey and Hunt-Joshi 2003). Aboveground herbivory affects sugar production through photosynthesis both directly by the removal of photosynthetic tissue and indirectly by reducing the photosynthetic capacity of the remaining tissue (Nabity et al. 2009). The photosynthetic inhibition in the remaining tissue may be more detrimental to plant growth and survival than the actual removal of tissue and may be a result of the severing of vascular tissue and metabolic and physiological changes that can occur after herbivory (Nabity et al. 2009)

Direct and indirect effects of leaf herbivory have been shown to decrease plant growth and fitness, including total biomass, above ground biomass (stems and leaves), belowground biomass (roots and storage organs), seed and fruit biomass, height and

survival (Cain et al. 1991, Moron-Rios et al. 1997, Alpert 1999, Gutman et al. 2002, Throop 2005, Gonzalez-Teuber and Gianoli 2007, Zhao et al. 2008). However, there are cases where damaged plants did not differ from undamaged plants, or were superior to the undamaged plants, suggesting that individuals subjected to herbivory were able to compensate or even overcompensate for lost tissue (Karban and Strauss 1993, Meyer 2000, Parra-Tabla et al. 2004, King et al. 2008, Zhao et al. 2008, Hladun and Adler 2009, Liu et al. 2009b).

Even though root herbivory is as frequent and potentially damaging as shoot herbivory, experiments investigating root damage are less common. This is likely because shoot herbivory is easier to observe and manipulate (Blossey and Hunt-Joshi 2003). Root damage can affect water and nutrient absorption, carbohydrate storage, synthesis of hormones and secondary compounds and can have an indirect negative impact because energy and other resources must be diverted from other functions to repair or replace roots (reviewed by Blossey and Hunt-Joshi, 2003). Root herbivory negatively affects height and total, reproductive, aboveground and/or belowground biomass (Reichman and Smith 1991, Houle and Simard 1996, Moron-Rios et al. 1997, Notzold et al. 1998, Murray et al. 2002, Barber et al. 2011), as well as increasing mortality (Reichman and Smith 1991, Moron-Rios et al. 1997, Maron 1998). As with shoot herbivory, some plants seem able to compensate or over-compensate for root damage (Dunn and Frommelt 1998, Hladun and Adler 2009).

With a few exceptions (Dunn and Frommelt 1998, Johnson and Lincoln 2000, Meyer 2000, Poveda et al. 2003, Egan and Irwin 2008), root and shoot herbivory reduce sexual reproduction. Damage to roots and/or shoots can delay and shorten flowering

time, reduce the number of flowers and the proportion of plants that flower, decrease seed production (number and mass of seeds produced), and the portion of biomass allocated to fruits or seeds (Reichman and Smith 1991, Wise and Sacchi 1996, Gutman et al. 2002, Poveda et al. 2003, Parra-Tabla et al. 2004, Throop 2005, Milbrath 2008). Reductions in reproductive characteristics can occur even if the plant compensates for the lost tissue through the regrowth of roots and/or shoots (Milbrath 2008).

Although many aspects of plant growth and sexual reproduction are detrimentally affected by leaf herbivory, clonal growth generally increases. Clonal growth characteristics such as the ramet number (Gutman et al. 2002, Gonzalez-Teuber and Gianoli 2007, Egan and Irwin 2008, Zhao et al. 2008, Liu et al. 2009b) and rhizome size and number (Pucheta et al. 2004, Wise et al. 2006) tend to increase following shoot damage or herbivory. Increased clonal growth generates more photosynthetic tissue, which can replace that lost due to herbivory. Rarely does shoot herbivory reduce clonal growth, and when it does, it may not greatly affect the overall ability of the genet to expand clonally (Cain et al. 1991). Most work on the impact of herbivory on clonal growth has focused on shoot damage rather than root damage, with the exception of Saner and Muller-Scharer (1994), who found that root borers increased the number of shoots of *Linaria vulgaris* soon after herbivory occurred and again after the damaged plants over-wintered.

Given that few studies focused on root herbivory, even fewer investigated the potential interactions between root and shoot herbivory. An interactive effect could occur if root and leaf herbivory together cause more harm to a plant than either root or leaf herbivory alone. For example, leaf and root damaged plants may be unable to produce

enough sugar to repair lost roots and would therefore be unable to provide water and nutrients to maintain and repair the leaves. Results from studies looking into this phenomenon varied, with some showing interactions between the two forms of herbivory (Houle and Simard 1996, Moron-Rios et al. 1997, Poveda et al. 2003), while others found only additive effects (Reichman and Smith 1991, Maron 1998, Hladun and Adler 2009). The interactive nature of root and shoot herbivory may depend on the type of measurement (biomass vs. growth rate) (Houle and Simard 1996) or the intensity of the herbivory involved (Moron-Rios et al. 1997).

Whenever a treatment affects plant size, as has been shown for herbivory, (Gutman et al. 2002, Gonzalez-Teuber and Gianoli 2007), this difference can be reflected in other traits. Changes in plant characteristics may be related to size rather than to direct treatment effects (Weiner 2004). For example, if herbivory decreases the height of a plant, there will be fewer nodes for leaf formation and therefore fewer leaves. Leaf number will be lower in the treated group because of herbivory-induced changes in height rather than from any direct influence of damage on leaf number. This concept can be expanded to include biomass allocation, with plants of different sizes allocating biomass differently regardless of environment, potentially making the effect of size on resource allocation more important than direct treatment effects. Although treatment-induced size effects on growth have been considered for other environmental variables (e.g. plant density, competition, resource availability and light levels; Weiner and Fishman 1994, Wang et al. 2006, Bonser and Aarssen 2009), indirect effects due to size havr rarely been addressed with herbivory.

In this study, I investigated the effects of simulated root and leaf herbivory on growth and sexual and clonal reproduction of three perennial wetland species, *Penthorum sedoides, Lythrum salicaria* and *Eupatorium perfoliatum.* Each of these species exhibits clonal growth in addition to sexual reproduction. I focused on some of the less frequently studied aspects of herbivory, such as the effects of root herbivory, especially on clonal reproduction, the interaction between root and shoot herbivory, and whether plant size has an influences possible responses to herbivory. The hypotheses I tested include: 1. That simulated root and leaf herbivory will decrease growth (height and biomass) of the three species 2. That damage treatments will decrease sexual reproduction as survival becomes more important and 3. That damage treatments will increase in clonal growth structures to compensate for lost roots and photosynthetic tissue. I also tested for potential interactions between root and leaf damage and the effect of plant size on the responses to damage.

Methods

Individuals of *Penthorum sedoides, Lythrum salicaria* and *Eupatorium perfoliatum,* were grown indoors under artificial lights with a 16:8 light/dark schedule. In March 2004, seeds of each species were germinated in Petri dishes on moist filter paper and seedlings were then transplanted to small pots. I treated the plants during the third week of May 2004. Prior to the administration of treatments, the height of each plant was measured. There were four treatments to simulate herbivory: leaf damage (L), in which I made a hole (5 mm diameter) every 2 cm around edge of each leaf with a hole punch; root damage (R), in which I removed approximately 1/3 of the rhizosphere by making two longitudinal cuts through the pot and soil of each plant and removing the soil and

roots between the cuts; combined leaf and root damage (LR); and an undamaged control (C). Each species had at least 12 replicates of each treatment, although more were assigned in case of mortality. I repotted the plants into larger containers immediately following treatment (controls were only repotted) and moved them to the Outdoor Ecological Research Area on the Cleveland State University campus, Cleveland, OH (41.47° N, 81.68° W). Once treatments were applied, I measured height and number of branches biweekly (25 June, 5 July , 20 July, 1 August, 16 August, 2 September and 16 September). I refer to these measurement dates as the number of days after treatment (Days 39, 49, 64, 76, 91, 107 and 121, respectively). These measurements were also taken prior to harvest in October 2004 when I separated the plants into stems, branches, leaves, clonal structures and fruits. Each of these parts was placed in a brown paper bag and, except for the fruits, dried in an oven at 60°C for at least 48h. I weighed each plant part to the nearest hundredth of a gram and converted the mass to milligrams for ease of calculations and transformation.

Data analysis

I carried out all data analysis in R (R Development Core Team 2010). To determine the affect of treatment on the height and vegetative biomass, I performed two way factorial ANOVA with root and leaf treatments as fixed effects. Each species was analyzed independently. Height and branch number at the different measurement points were analyzed separately from each other. When a variable was potentially dependent on plant size, ANCOVA was performed with a measurement of size as the covariate. For fruit mass, ramet mass, clonal growth mass and number of ramets at the end of the experiment, the covariates were total biomass of the plant at harvest minus the variable

being tested. Total biomass minus fruit mass is referred to as vegetative mass, total biomass minus ramet mass is called non-ramet mass and total biomass minus clonal mass is non-clonal mass. For mass of branches and leaves, the height at harvest was used as a covariate, since the length of the main stem directly influences the number of these organs. For the number branches tested over two week intervals, the height of the plants at the measurement interval was used. I originally included the effect of the covariate, leaf and root treatment and interactions between treatments and/or the covariate in the model. Using the "step" function in R, I dropped any non-significant factors and re-ran the analysis (Crawley 2007). If interactions occurred, the four treatments (root, leaf, both root and leaf, and control) were analyzed separately and compared using the Tukey-Kramer test (Sokal and Rolf 1995, Lau 2009). Data were log transformed when necessary to improve normality.

Results

Biomass

The three species differed in response to damage in terms of biomass. They also differed in the effect of size and interactions between treatments and/or size (Table I).

Penthorum sedoides

Few of the measured biomass components of *Penthorum sedoides* were affected by simulated herbivory. Neither root nor leaf damage affected vegetative mass ($F_{3, 40}$=0.36, P=0.78) and *P. sedoides* did not have enough individuals with main stem leaves

Table I. Overview of the responses of *Penthorum sedoides*, *Lythrum salicaria*, and *Eupatorium perfoliatum* to simulated herbivory treatment. "Root" and "leaf" refer to the effect of root damage and leaf damage respectively. "R*L" indicates the interaction between root damage and leaf damage. "Size" indicates the effect of size on the measurement and "size*treat" indicates whether there is an interaction between size and one or more of the treatments. "Veg mass" refers to vegetative mass. "+" means that there was an increase with the treatment or size while "-" indicates a decrease and "0" indicates no effect. "(m)" indicates the effect was marginally significant (P<0.1). Otherwise effects are significant at a P<0.05 level. "y" in the interaction columns (R*L and Size*treat) indicated there was an interaction between the factors. "NA" indicates that either the test was not appropriate (i.e. for size for vegetative mass) or that insufficient data were available (e.g. due to low fruiting).

	Species														
	Penthorum sedoides					*Lythrum salicaria*					*Eupatorium perfoliatum*				
Variable	Root	Leaf	R*L	Size	Size* treat	Root	Leaf	R*L	Size	Size *treat	Root	Leaf	R*L	Size	Size *treat
Veg mass	0	0	0	NA	NA	0	+	0	NA	NA	+(m)	0	0	NA	NA
Leaf mass	NA	NA	NA	NA	NA	-	-	y(m)	+	0	+(m)	0	0	+	0
Stem mass	0	0	0	+	0	0	-	0	+	y	0	0	0	+	0
Branch mass	0	0	0	0	0	+	+	y	-	0	+	0	0	0	0
Ramet mass	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Clonal mass	0	0	0	0	y	0	0	0	+	0	+(m)	0	y	0	y
Fruit mass	-	0	0	0	0	NA	NA	NA	NA	NA	+	0	0	+	y

Table II. Analysis of covariance results summarizing significant effects of root and leaf damage, plant size and interactions on biomass measurements of *P. sedoides*.

Trait	Source	DF	Mean Square	F	P
Clonal Mass	Root damage	1	0.92	1.63	0.21
w/ outliers	Non-clonal mass	1	1.14	2.47	0.12
	Root*Non-clonal	1	2.86	5.04	0.032
	Error	30			
Fruit Mass	Root Damage	1	695175	4.18	0.047
	Error	44	166153		

to perform statistical tests. Stem mass did not vary between the treatments, although it increased significantly with height (ANCOVA, $F_{1,45}$=30.92, P<0.0001, r^2=0.39). Neither height at harvest nor treatment affected the mass of branches collected (ANCOVA, $F_{1,34}$=0.051, P=0.82). For ramet mass in *P. sedoides* there were no differences among treatments and ramet mass was not affected by size (ANCOVA, $F_{1,29}$=0.22, P=0.639). There was a significant interaction between root treatment and non-clonal mass; clonal mass decreased slightly with non-clonal mass for plants treated with root damage and increased slightly for plants without root treatment (Table II; Figure 4; ANCOVA, $F_{3,30}$=3.04, P=0.044, r^2=0.16). However, when two outliers were removed, this relationship was no longer significant; neither treatment nor non-clonal mass affected clonal mass (ANCOVA, $F_{1,30}$=0.061, P=0.81. The vegetative mass of individuals of *P. sedoides* did not influence the mass of fruits, although root damaged plants produced fewer fruits than plants without root damage (Table II; Figure 5;ANOVA $F_{1,44}$=4.18 P=0.047).

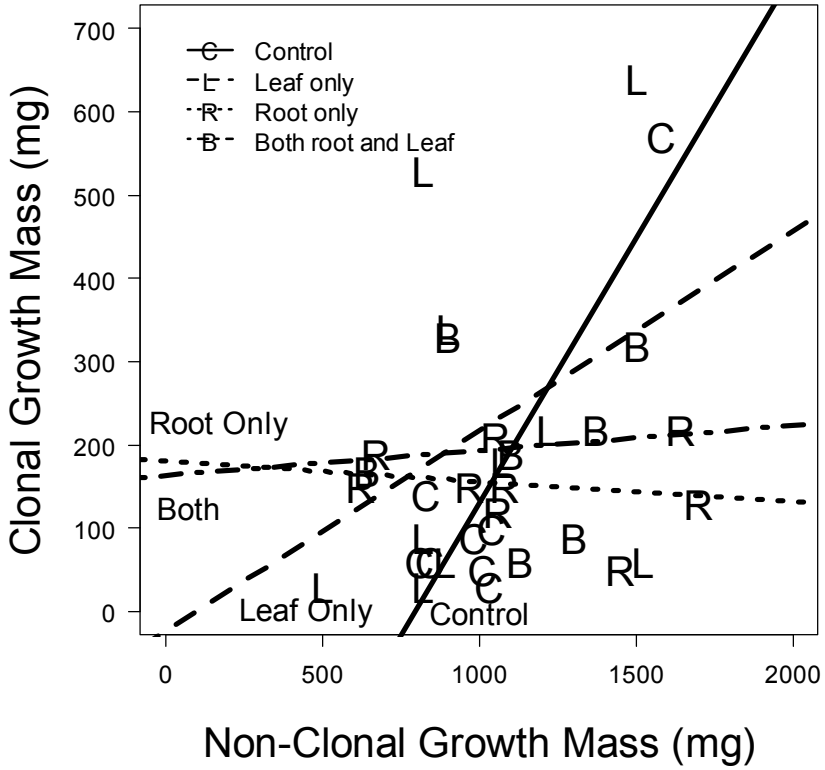

Figure 4. Effect of non-clonal mass and simulated herbivory on clonal mass of *P. sedoides*. Letters are data points symbolizing damage treatment (C=control, R=root only, L=leaf only and B=both root and leaf damage). R^2 for ANCOVA is 0.15 (P=0.04). The relationship is no longer significant after the removal of the two outliers.

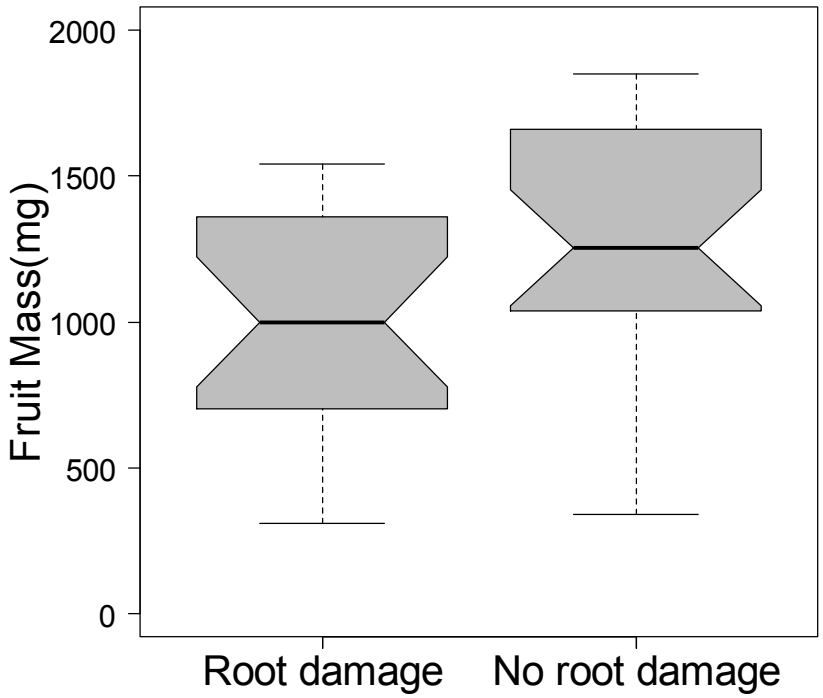

Figure 5. Effect of simulated root herbivory on fruit mass of *Penthorum sedoides*. "Root damage" refers to the treatments with root damage ("root only" and "both") and "No root damage" refers to treatments without root damage ("leaf only" and "control"). Treatments differ significantly (P=0.047).

Table III. Analysis of covariance results summarizing significant effects of root and leaf damage, plant size and interactions on biomass measurements of *L.*

Trait	Source	DF	Mean Square	F	P
Vegetative mass	Leaf damage	1	3006531	8.27	0.006
	Error	49	363365		
Leaf mass	Root damage	1	6.74	4.52	0.04
	Leaf damage	1	7.13	4.79	0.03
	Height	1	7.91	5.31	0.03
	Root*leaf	1	4.46	3.0	0.09
	Error	39			
Stem mass	Leaf damage	1	1314249	17.37	0.0001
	Height	1	7285323	96.28	<0.0001
	Leaf*height	1	1008998	13.33	0.0009
	Error	45	75669		
Branch mass	Root damage	1	665715	24.0	<0.0001
	Leaf damage	1	75608	2.72	0.11
	Height	1	569050	20.51	<0.0001
	Root*leaf	1	153516	5.53	0.023
	Error	44	27746		

Lythrum salicaria

Lythrum salicaria leaf damaged plants had more vegetative mass than plants with intact leaves (Table III, Figure 6; ANOVA $F_{1, 49}$=8.27, P=0.006). Leaf mass showed a positive relationship with height at harvest and treatments differed from each other even when size was taken into account. Marginally significant interactions occurred, with the control treatment having significantly more leaf mass than the LR treatment and marginally more leaf mass than the R and L treatments (ANCOVA, $F_{4,39}$=4.403; P=0.005, r^2=0.24). Stem mass increased with height in *L. salicaria* and was affected by leaf damage. The slope was greater in the leaf damaged treatments than when leaves

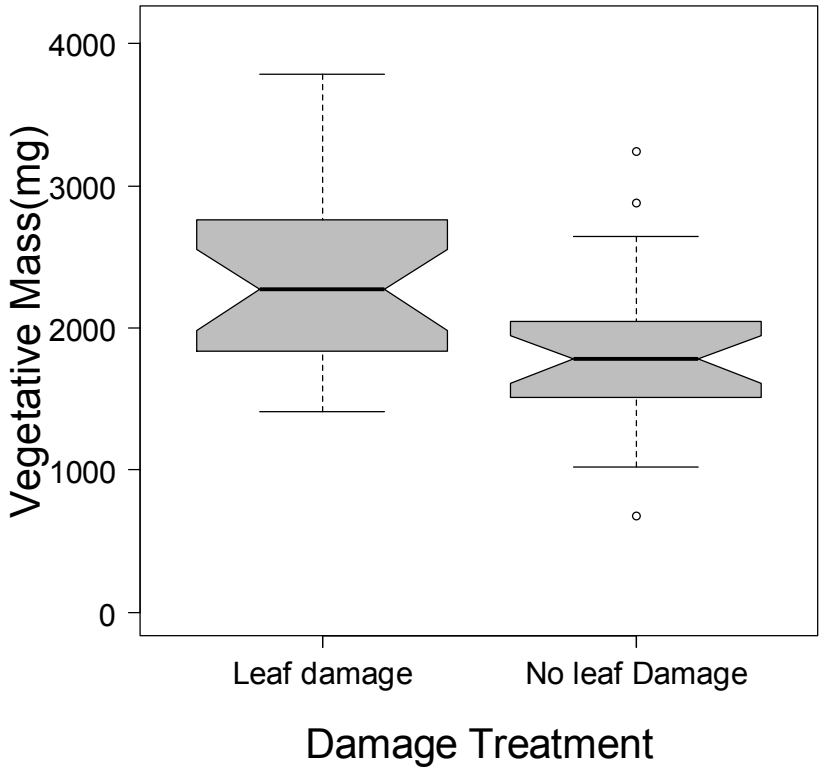

Figure 6. Effect of simulated leaf herbivory on vegetative mass of *Lythrum salicaria*. "Leaf damage" refers to the treatments with leaf damage ("leaf only" and "both") and "No leaf damage" refers to treatments without leaf damage ("root only" and "control").. Treatment differ significantly (P=0.006).

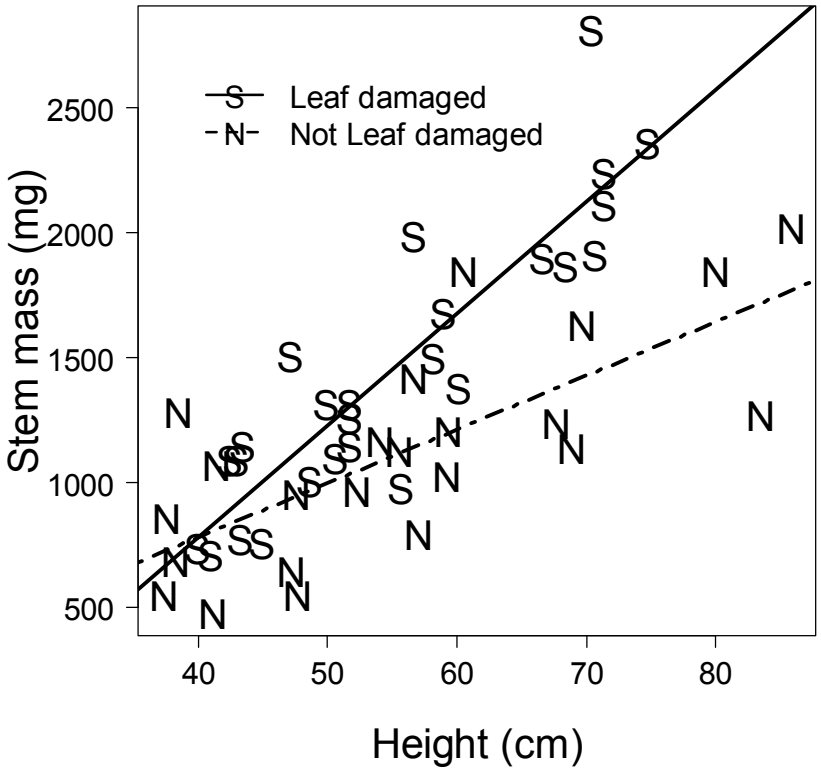

Figure 7. Effect of height and simulated herbivory treatment on stem mass of *L. salicaria*. "Leaf damaged" includes the "leaf only" and "both root and leaf" treatments and "not leaf damaged" includes the control and the "root only" treatments. The intercepts and slopes differ significantly. R for the ANCOVA was 0.72 (P<0.0001).

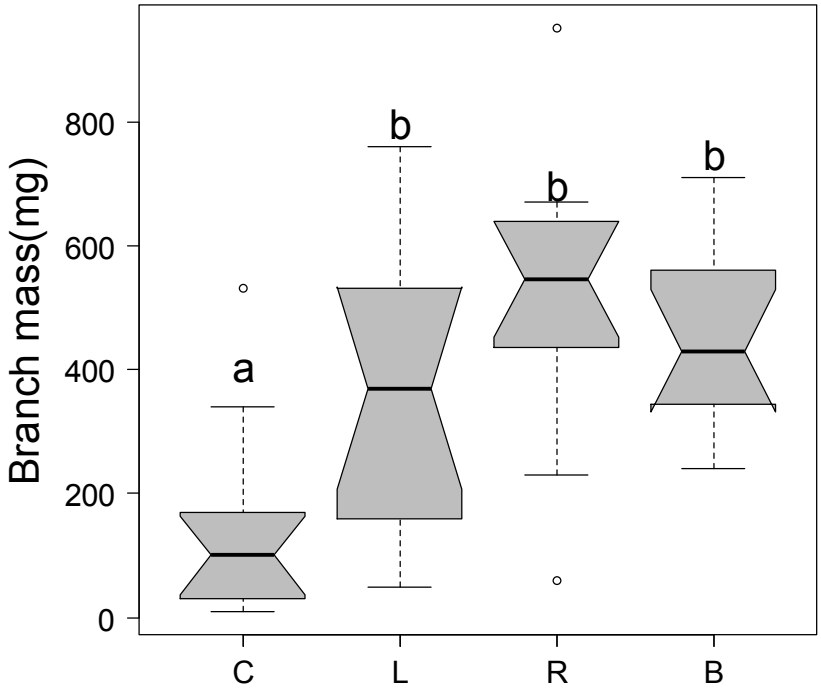

Figure 8. Effect of simulated herbivory on branch mass of *L. salicaria.* C refers to the control, L refers to the leaf damage only treatment, R stands for the root damage only treatment and B refers to the treatment with both root and shoot damage. Boxes that do not share a letter are significantly different from each other.

were undamaged, although leaf damaged plants had a significantly lower intercept (Figure 7; ANCOVA, $F_{3,45}$=42.33, P<0.0001, r^2=0.72). Branch mass decreased with height at harvest in *L. salicaria* and treatment means differed when this size relationship was taken into account with the control having lower branch mass than the two root damaged treatments (R and LR) and the L treatment (Figure 8; ANCOVA, $F_{4,44}$=13.19, P<0.001. r^2=0.5). An insufficient number of *L. salicaria* produced ramets or set fruits to perform ANOVA or regression analysis on ramet or fruit biomass.

Table IV. Analysis of covariance results summarizing significant effects of root and leaf damage, plant size and interactions on biomass measurements of *E. perfoliatum*

	Source	DF	Mean Square	F	P
Vegetative mass	Leaf damage	1	1457700	3.54	0.07
	Error	45	18523670		
Branch mass	Root damage	1	10.49	9.0	0.0049
	Error	36	1.16		
Clonal mass	Root damage	1	1.4	3.9	0.06
	Leaf damage	1	0.014	0.038	0.85
	Non-clonal mass	1	0.08	0.23	0.63
	Root*leaf	1	4.2	11.74	0.002
	Leaf*non-clonal	1	6.19	17.32	0.0002
	Error	30	0.36		
Fruit mass	Root damage	1	45204	8.89	0.005
	Leaf damage	1	1034	0.2	0.65
	Veg. mass	1	57584	11.32	0.0018
	Leaf*veg	1	3302	6.49	0.015
	Error	36	5087		

Although the mass of clonal growth organs (root buds) in *L. salicaria* increased with non-clonal biomass, treatments had no effect (ANCOVA, $F_{1,30}$=7.56, P=0.01. r^2=0.17).

Eupatorium perfoliatum

Root damage led to a marginally significant increase in the mass of vegetative structures in *E. perfoliatum*, (Table IV; ANOVA $F_{1, 45}$=3.54, P=0.07). Leaf mass increased significantly with final height at harvest for all treatments and root treatments had marginally significantly more leaf mass (ANCOVA, $F_{1,39}$=8.76. P=0.0052. r^2=0.162). Stem mass did not vary between the treatments although it increased significantly with height (ANCOVA, $F_{1,37}$=34, P<0.0001, r^2=0.46, respectively). In *E. perfoliatum*, branch mass did not change with height, although root damage led to greater mass of branches (Figure 9; $F_{1,36}$=9.00. P=0.005). An insufficient number of plants of this species produced ramets to perform ANOVA or regression analysis on ramet biomass. The

effect of non-clonal mass on the mass of clonal structures depended on the treatments applied (Figure 10). The mass of clonal structures did not change with the mass of the plants for the L and LR treatments, but it decreased with plant size for the control and R treatments. Neither the slopes or intercepts of the L and LR treatments differed (Table IV; ANCOVA, $F_{5,30}$=6.64, P<0.0003. r^2=0.45). There was an increase in fruit mass with vegetative mass, and root treatment led to a significant increase in fruit mass (Figure 11; Figure 12). There was an interaction between leaf treatment and vegetative mass; treatments with leaf damage had a less steep slope than treatments without leaf damage (Table IV; Figure 11; ANCOVA, $F_{4,39}$=6.64 P=0.0001, r^2=0.36).

Height

Prior to the administration of treatments, plants assigned to the different treatments did not differ in height. In *P. sedoides*, root damaged plants (R and LR) were significantly taller than plants without root damage (L and control) for all measurement points following damage (Table V; Figure 13 a; Day 39, $F_{1,49}$=38.19, P<0.0001; day 49, $F_{1, 48}$=32.59, P<0.0001; day 64, $F_{1, 46}$=21.33, P<0.0001; day 76, $F_{1,47}$=10.9, P=0.002; day 91, $F_{3,43}$=7.31, P=0.01; day 107, $F_{1 ,43}$=7.67, P=0.008 and day 121, $F_{3, 41}$=7.67, P=0.008). For *Lythrum salicaria*, height of plants in different treatments differed significantly for the first two measurement points, days 39 and 49 after treatment application (Figure 13 b). On day 39, there was a significant interaction between root and leaf damage treatments, the presence of root damage lessened the negative effect of

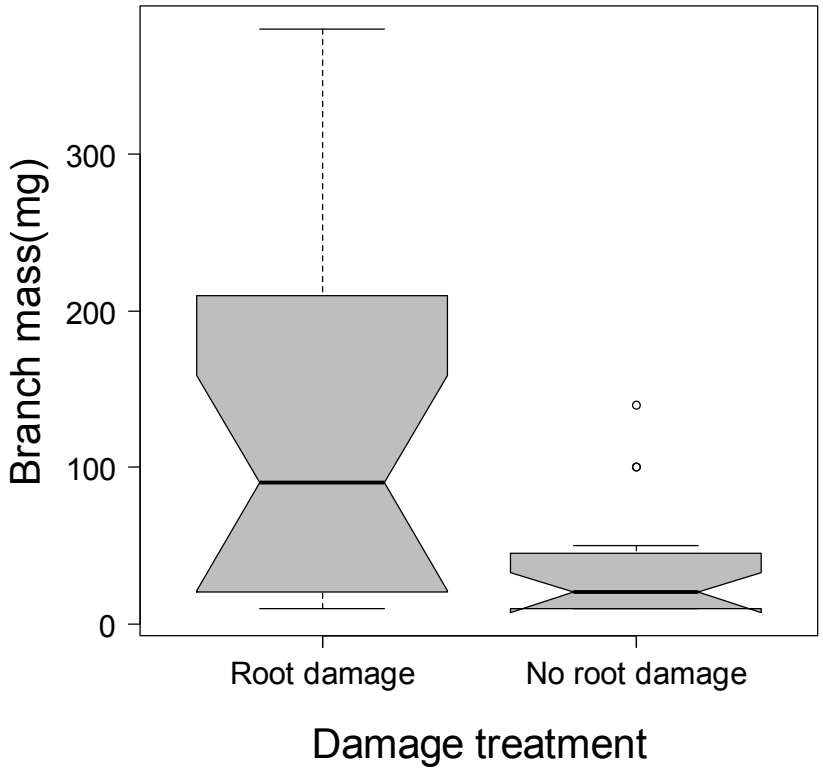

Figure 9. Effect of simulated herbivory on branch mass of *E. perfoliatum.* "Root damage" refers to the treatments with root damage ("root only " and "both") and "No root damage" refers to treatments without root damage ("leaf only" and "control"). The two treatments differ significantly from each other (P=0.0049)

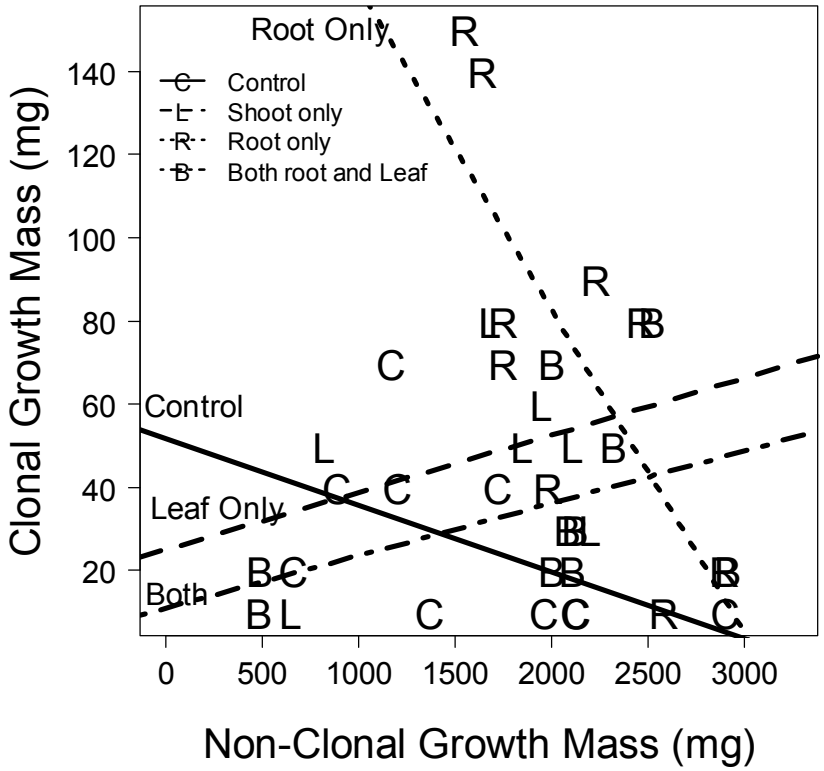

Figure 10. Effect of non-clonal mass and treatment on clonal mass of *E. perfoliatum* Letters are data points symbolizing damage treatment (C=control, R=root damage only , S=Leaf damage and B=both root and leaf damage). R^2 for ANCOVA was 0.45 (P=0.0003).

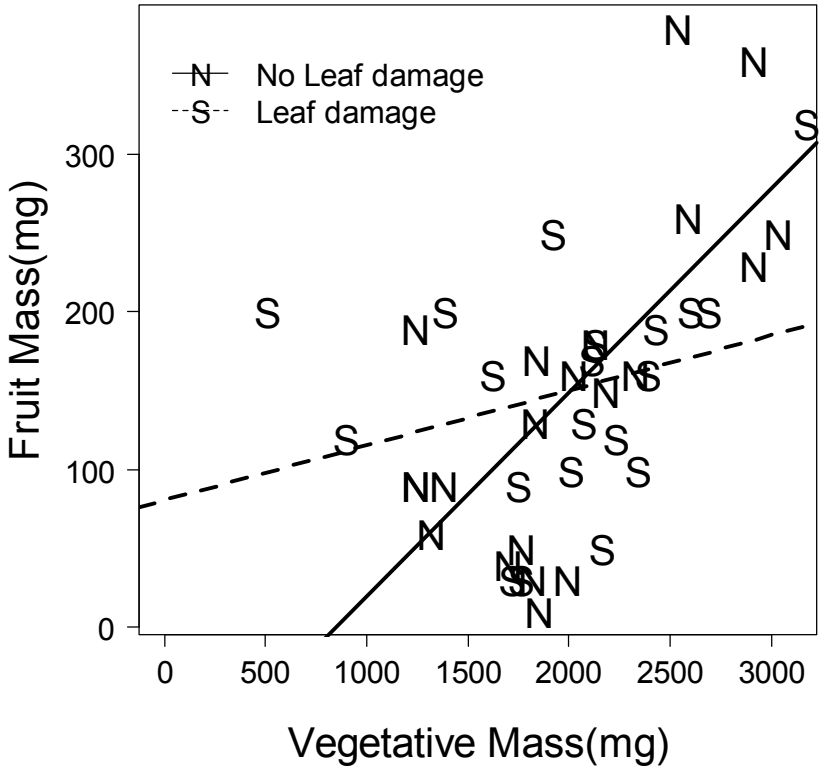

Figure 11. Effect of vegetative mass and simulated leaf herbivory on the fruit mass of *Eupatorium perfoliatum.* "Leaf damaged" includes the "leaf only" and "both root and leaf" treatments while "not leaf damaged" includes the control and the "root only" treatments. Both the intercepts and slopes of the regression lines differ significantly.

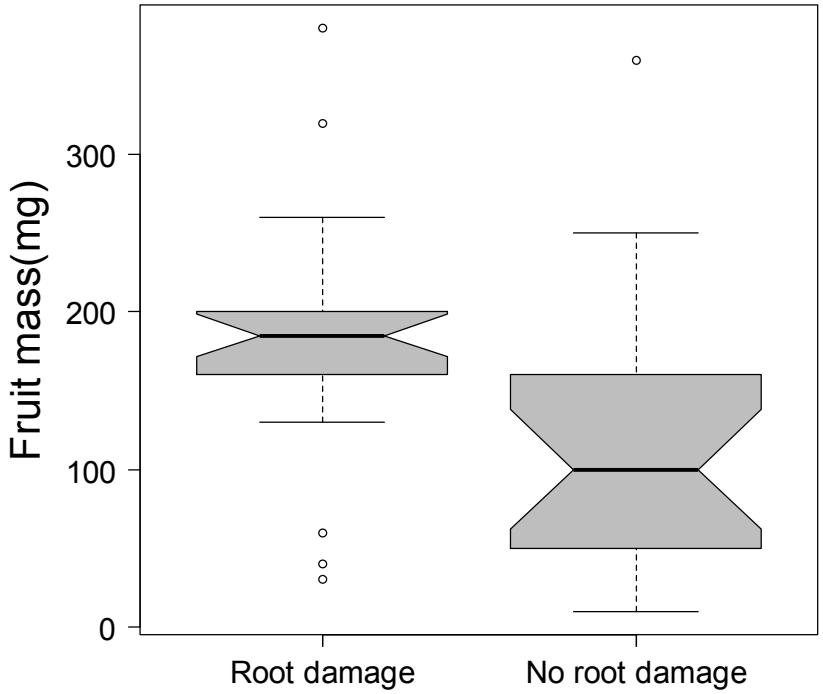

Figure 12. Effect of simulated root herbivory on fruit mass of *E. perfoliatum*. "Root damage" refers to the treatments with root damage ("root only" and "both") and "No root damage" refers to treatments without root damage ("leaf only" and "control"). Treatment differ significantly from each other (P=0.005).

Table V. Analysis of covariance results summarizing significant effects of root and leaf damage and interactions on height of *P. sedoides, L. salicaria* and *E. perfoliatum*

Measurement Date	Species	Source	DF	Mean squares	F	P
Day 39	*P. sedoides*	Root Damage	1	286.46	38.19	<0.0001
		Error	49			
	L. salicaria	Root Damage	1	234.24	6.5	0.01
		Leaf Damage	1	241.16	6.69	0.01
		Root*Leaf Damage	1	205.19	5.7	0.02
		Error	47	36.02		
Day 49	*P. sedoides*	Root Damage	1	188.36	32.59	<0.0001
		Error	48	5.78		
	L. salicaria	Root Damage	1	188.75	3.133	0.08
		Shoot Damage	1	578.32	9.6	0.003
		Error	49	60.23		
Day 64	*P.sedoides*	Root Damage	1	144.48	21.33	<0.0001
		Error	46	6.88		
Day 76	*P. sedoides*	Root Damage	1	114.96	10.9	0.002
		Error	47	10.54		
	E. perfoliatum	Leaf Damage	1	822.6	6.25	0.02
		Error	48	131.59		
Day 91	*P.sedoides*	Root Damage	1	56.07	7.31	0.01
		Error	43			
	E. perfoliatum	Leaf damage	1	575.9	4.66	0.04
		Error	46	123.5		
Day 107	*P. sedoides*	Root Damage	1	52.4	7.67	0.008
		Error	43	6.84		
	E. perfoliatum	Leaf Damage	1	847.1	4.45	0.04
		Error	48	190.31		
Day 121	*P. sedoides*	Root Damage	1	52.4	7.67	0.008
		Error	43	6.84		
	E. perfoliatum	Leaf Damage	1	847.1	4.45	0.04
		Error	48	190.31		

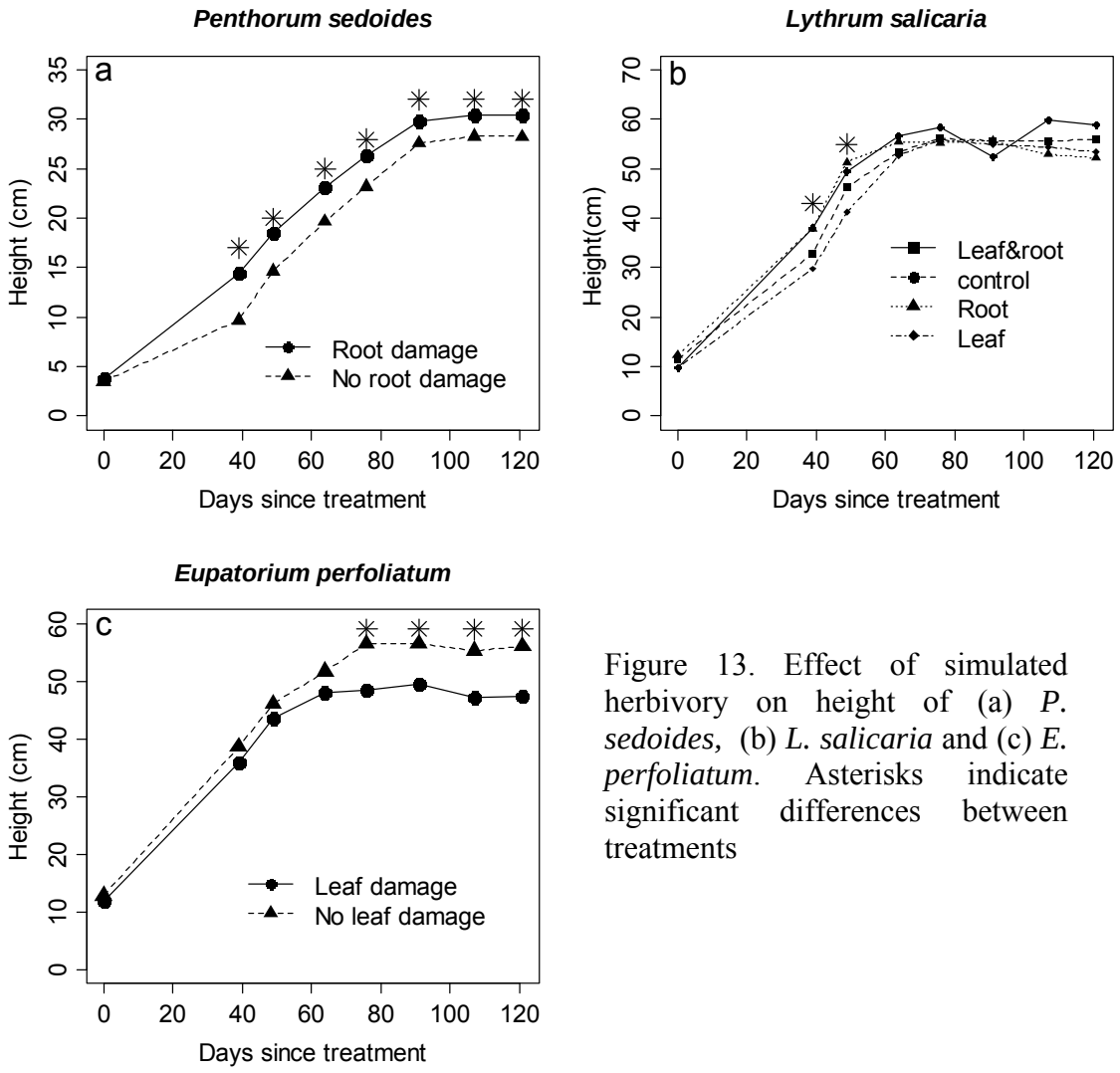

Figure 13. Effect of simulated herbivory on height of (a) *P. sedoides,* (b) *L. salicaria* and (c) *E. perfoliatum*. Asterisks indicate significant differences between treatments

leaf damage; height was significantly less in the L treatment than all the other treatments (control, R, and LR; $F_{3, 47}$=6.3; P=0.001). On day 49, interaction between the treatments no longer occurred and leaf treated plants were shorter than the non-leaf treated plants and root treated plants were marginally taller than the non-root treated plants ($F_{2,49}$=6.37; P=0.003). For the later measurement dates, damage did not affect height of *L. salicaria.* For *E. perfoliatum*, height did not differ between treatments for the first three measurement points following herbivory (Figure 13 c; days 39, 49 and 64). For the dates after this, plants that received leaf herbivory were significantly shorter than those with

intact leaves (day 76, $F_{1,48}$=6.25, P=0.02; day 91, $F_{1,46}$=4.66, P=0.04; day 107, $F_{1,48}$=4.45, P=0.04; day 121, $F_{1,48}$=4.45, 0.04).

Number of Branches

Prior to treatment, axillary branches were not observed on the plants. On day 39 *Penthorum sedoides* exhibited differences between treatments, with the root treatments having more branches than treatments without root damage (Table VI; Figure 14 a; ANOVA $F_{1,49}$=26.68, P<0.0001). On day 49, the number of branches increased significantly with height, but treatment had no effect (ANCOVA $F_{1,48}$=7.34, P<0.009, r^2=0.11). On days 64 and 121, neither height nor treatments affected the number of branches On day 39, root treatment led to a significant increase in the number of branches in *Lythrum salicaria* and branch number increased significantly with height (Figure 14 b; ANCOVA $F_{2,48}$=12.48, P<0.001, r^2=0.31). On day 49 and day 64, branch number did not increase with height for *L. salicaria*, and the treatments with root damage had more branches than those without root damage ($F_{1,50}$=19.01, P<0.0001 for day 49 and $F_{1,49}$=13.15, P=0.0005 for day 64). At the final branch measurement point, neither height nor treatment affected number of branches in *L. salicaria* (ANCOVA $F_{1,48}$=1.44, P=0.23). For all the measurement points of *Eupatorium perfoliatum*, the number of branches on each plant did not differ, although branch number was significantly affected by height on day 39 (Table VI; ANCOVA $F_{1,52}$=4.96, P=0.03, r^2=0.07) and marginally affected by height on day 49 (ANCOVA $F_{1,51}$=4.03, P=0.05, r^2=0.055).

Table VI. Analysis of covariance results summarizing significant effects of root and leaf damage, plant size and interactions on branch number of *Penthorum sedoides, Lythrum salicaria* and *Eupatorium perfoliatum*. "ns" signifies none of the effects were significant for that species at that measurement point.

Measurement Date	Species	Source	DF	Mean squares	F	P
Day 39	*P. sedoides*	Root Damage	1	256.5	26.68	<0.0001
		Error	49			
	L. salicaria	Root damage	1	309.48	14.82	<0.0001
		Height	1	212.49	10.18	0.0025
		Error	48	20.88		
	E. perfoliatum	Height	1	47.28	4.96	0.03
		Error	52	9.52		
Day 49	*P. sedoides*	Root Damage	1	2.31	7.2	0.007
		Height	1	110.11	0.685	0.68
		Root*Height	1	78.17	5.62	0.022
		Error	46	13.9		
		Error	50	26.27		
	L. salicaria	Root Damage	1	499.46	19.01	<0.0001
		Error	50	26.27		
	E. perfoliatum	Height	1	76.89	4.03	0.05
		Error	51	19.07		
Day 64	*P. sedoides*	ns				
	L.salicaria	Root Damage	1	326.53	13.15	0.0005
		Error	49	24.17		
	E. perfoliatum	Root damage	1	32.29	3.016	0.09
		Error	51	10.7		
Day 121	*P. sedoides*	ns				
	L.salicaria	ns				
	E. perfoliatum	ns				

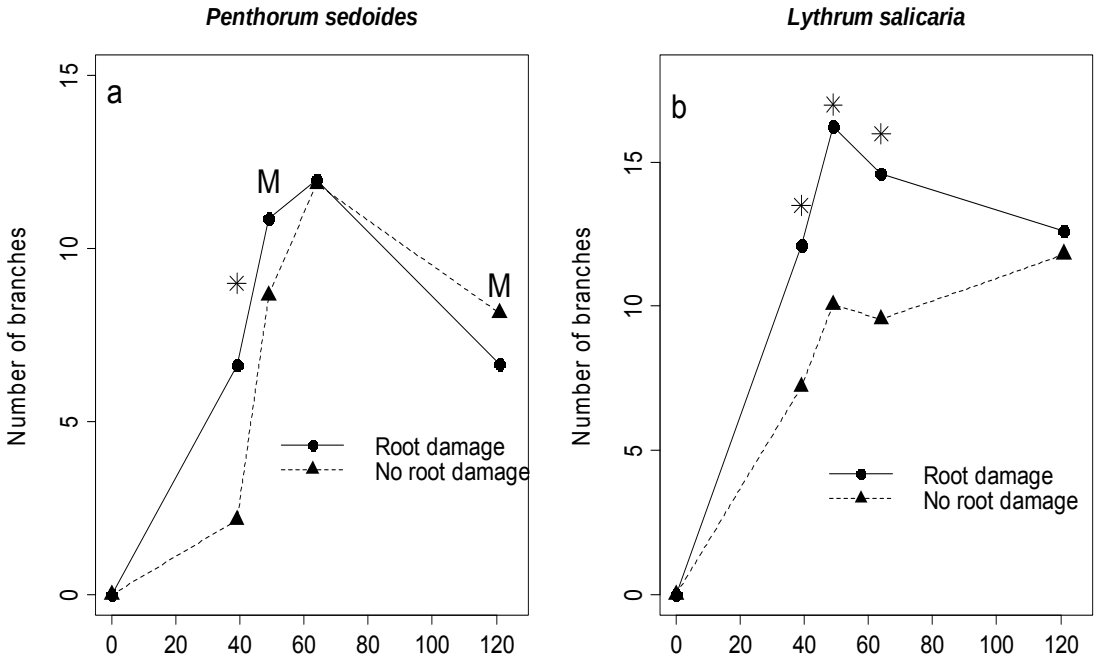

Figure 14. Effect of root damage on the branch number of a. *Penthorum sedoides* and b. *Lythrum salicaria*. Asterisks indicate significant differences between treatment. "M" indicates marginal differences between treatments.

Discussion

Many aspects of growth were not influenced by the removal of root or leaf tissue in the three study species. Lack of a detrimental effect of herbivory has been reported previously and indicates that plants are able to compensate for tissue loss (Karban and Strauss 1993, Throop 2005, Zhao et al. 2008). The study species differed in their reaction to the damage inflicted on them. The variation in response to herbivory among them may be a function of differences in morphology and physiology and past interactions with herbivory. Overall, little is known about the reactions and potential

resistances of *P. sedoides* and *E. perfoliatum* to herbivory. *Lythrum salicaria* is known to be susceptible to predation based on research on herbivory and *Lythrum salicaria* that deals with developing a biological control program to combat the spread of this invasive plant in North America (Hunt-Joshi et al. 2004, Hunt-Joshi and Blossey 2005). Although no formal studies have been performed on *P. sedoides*, in the field its leaves are often found covered by holes created by herbivores (personal observation), which implies that it may have mechanisms for dealing with tissue loss, although it is not herbivore resistant.

Penthorum sedoides

Penthorum sedoides was the least affected of the three test species, and the few herbivory induced changes that occurred were positive. The only measured trait that showed a detrimental response was sexual reproduction; root damage led to a decrease in fruit mass. Reductions in reproductive output following herbivory are commonly observed in other species (Reichman and Smith 1991, Wise and Sacchi 1996, Gutman et al. 2002, Poveda et al. 2003, Parra-Tabla et al. 2004, Throop 2005, Milbrath 2008, Barber et al. 2011), so the reaction of *P. sedoides* is not surprising. It is interesting to note, however, that fruit mass was the only measured variable that showed a decrease following root damage; all other aspects of growth in this species showed compensation or overcompensation. Compensation in *P. sedoides* came at a cost to sexual reproduction, which in the long run may most adversely affect the fitness on the genotype (Saner and Muller-Scharer 1994, Dunn and Frommelt 1998, Johnson and Lincoln 2000). As *Penthorum sedoides* is a perennial plant and compensation for fitness lost due to reduced sexual reproduction can occur in subsequent years, especially if clonal expansion is unaffected.

Surprisingly, height of *Penthorum sedoides* increased in response to root damage. The effect of root herbivory on plant height has rarely been, but in cases where it has, height has either decreased or remained unchanged following root herbivory (Muller-Scharer 1991, Notzold et al. 1998, Poveda et al. 2003). The reaction of *P. sedoides* is the only case of which I am aware where height increased in the presence of root damage. Increased height may be a way for the plant to increase photosynthetic surface area to increase resources available for root growth and repair.

In addition to an increase in height with root herbivory, *P. sedoides* inflicted with root damage also had significantly more branches than those without root damage, although the treatments only differed from each other on day 39. Increased branching with shoot damage has been reported, usually in association with damage to the apical meristem, resulting in release of lateral buds from apical dominance (Strauss and Agrawal 1999, King et al. 2008, Milbrath 2008), but it has also been shown to occur with the removal of leaves in the absence of shoot tip damage (Milbrath 2008). I found no other reports in the literature of root damage leading to an increase in the number of branches aboveground as was seen in *P. sedoides*. One possible explanation is that damage to the root crown may cause a release from apical dominance, allowing axillary buds to mature into branches (Saner and Muller-Scharer 1994).

Increased branching with root damage may also be related to asexual expansion (Bach 1998). In *P. sedoides*, branches, especially the lower ones, may grow towards the ground. Once portions of the stem make contact with the soil, roots arise from the stem tissue of the branch, which continues to grow against the ground, essentially becoming a stolon (personal observation). An increase in clonal growth is often seen following

herbivory (Gutman et al. 2002, Pucheta et al. 2004, Egan and Irwin 2008), and the increased branching seen in *P. sedoides* may be a modification of this pattern. Clonal spread through branches contacting the ground would be safer in areas of root herbivory because branches generate and lengthen above ground. This is in contrast to the more usual clonal structures, root buds and stolons, which originate underground or at ground level and could be attacked by root herbivores while developing and growing.

Stolon mass (*P. sedoides*' method of clonal reproduction) did not differ between treatments. This result does not support my hypothesis that clonal growth would increase following herbivory as a compensation method and is contrary to the findings of other studies. Shoot herbivory typically increases clonal growth and expansion (Saner and Muller-Scharer 1994, Gutman et al. 2002, Pucheta et al. 2004, Wise et al. 2006, Gonzales et al. 2007, Egan and Irwin 2008, Zhao et al. 2008, Liu et al. 2009b). A limited number of studies have investigated the influence of root herbivory on clonal growth characteristics with the exception of Saner and Muller-Scharer (1994), who found an increase in number of stems (ramets) produced temporarily and also in the next growing season after over-wintering. However, since branches (in addition to stolons) can act as clonal organs in *P. sedoides*, the increase in branch number seen in root damage treatments may be a mechanism to increase clonal growth without exposing stolons to underground herbivores.

Lythrum salicaria

Any type of damage to *L. salicaria* decreased leaf mass compared to the control (LR treatment significantly and L and R treatments marginally). Leaf mass loss following

damage has been reported for plants affected by both shoot and root herbivory (Reichman and Smith 1991, Meyer 1993, 2000, Blossey and Schat 1997) and *L. salicaria* reacted similarly in this study. Other aspects of shoot growth increased in the presence of herbivory, implying that *L. salicaria* has mechanisms for dealing with herbivore damage other than increasing leaf mass. For example, following herbivory, *L. salicaria* exhibited an increase in the branch number and ultimately compensated for damage in terms of height. The increase in branch number also led to an increase in branch mass for all of the damage treatments relative to the control. Stem mass of the leaf damaged plants increased at a faster rate with height than plants without leaf damage, meaning that stem mass in the leaf damaged treatments increased more than was accounted for by the increase in height. Overall, *L. salicaria* compensated for lost tissue despite the decrease in leaf mass; vegetative biomass of leaf damaged plants was greater than plants without leaf damage. This increase in shoot biomass is unusual; most studies report either a decrease in shoot or aboveground biomass (Moron-Rios et al. 1997, Meyer 2000, Throop 2005) or no change in these measurements (Karban and Strauss 1993, Throop 2005) following shoot herbivory.

Although *Lythrum salicaria* showed a decrease in height with leaf herbivory, it occurred only at the first two measurement points after treatment (days 39 and 49). From these results it appears than *L. salicaria* was able to compensate for lost biomass due to leaf damage over time. An interaction between root and leaf damage occurred in *L. salicaria* on day 39, implying that leaf herbivory in the presence of root herbivory is less damaging than leaf herbivory alone. A potential explanation is that while leaf herbivory decreases height, root herbivory may cause a slight increase in height, ameliorating the

effect of leaf herbivory. In fact, root damage only plants were taller than control plants at this point, although this relationship was not significant, and root damaged plants were marginally taller on the following measurement (day 49). Shoot herbivory is commonly reported to decrease the height of plants (Poveda et al. 2003, Wise et al. 2006, Gonzales et al. 2007), so my observation of decreased height in the leaf treatments in *L. salicaria* is not surprising. In the few cases where effect of root herbivory on height has been reported, height has either decreased or remained unchanged when root herbivory occurs (Muller-Scharer 1991, Notzold et al. 1998, Poveda et al. 2003).

Eupatorium perfoliatum

In *E. perfoliatum*, root damaged plants had marginally more leaf mass than plants without root damage. This consequence of herbivory is not often seen, although Johnson and Lincoln (2000) found an increase in leaf mass relative to shoot mass following artificial defoliation in *Heterotheca subaxillaris*. Root-damage induced increase in leaf mass could be a mechanism for replacing lost leaf tissue and increasing the above ground surface area for photosynthesis to replace root tissue. The increased leaf mass must have been due to an increase leaf size rather than the number of leaves since leaf number at harvest did not differ between treatments in this species

Fruit mass increased in plants subjected to root damage in *E. perfoliatum*, contrary to what is commonly observed; typically, herbivory decreases sexual reproductive characteristics (Maron 1998, Parra-Tabla et al. 2004, Throop 2005, Milbrath 2008, Hladun and Adler 2009), although there are some exceptions. For example, Egan and Irwin (2008) found that shoot damage led to an increase in number of flowers and

expanded fruits and Saner and Muller-Scharer (1994) reported that root herbivory led to an increase in length of flowering period and number of fruits, although due to fruit abortion, this difference did not influence the number and mass of seeds. In my experiment, the increase in fruit mass in *E. perfoliatum* could be a mechanism to escape from a detrimental situation. The fruits of *E. perfoliatum* are light and easily airborne, able to travel long distances. An increase in seed production would lead to more offspring that could find new habitats away from the root herbivory experienced by the parent plant.

Root damage increased branch mass in *E. perfoliatum*, as was the case for *L. salicaria* and for branch number in *P. sedoides*. This may be an attempt to increase clonal expansion. However, given that fruit mass increased with root herbivory as well, increased branching could be a means to increase the number of inflorescences and therefore fruits and seeds produced. In *E. perfoliatum*, as in my other two species, each branch terminates in one or more inflorescences. Therefore, more and larger branches mean more and larger inflorescences and fruits.

Eupatorium perfoliatum was the only species of the three tested that directly supported my hypothesis that herbivory would have a positive effect on clonal growth. In this species, herbivory altered not only the mass of clonal growth structures but also changed the relationship between plant size and clonal structure mass (i.e. there was a significant interaction between plant size and treatment). In the absence of leaf damage (the control and root only treatments), clonal growth of *E. perfoliatum* decreases with size. Despite the decrease, plants with root damage alone had more clonal biomass than the control over the observed size range. These results provide new support for the

importance of clonal growth when a plant is sustaining root damage. Clonal growth mass was unrelated to size for leaf damaged *E. perfoliatum*, supporting the importance of clonal growth under these conditions since it is maintained across all plant sizes, potentially at the cost of other functions. The tendency of plants to increase clonal growth with herbivore damage may help compensate for damage through the formation of new biomass, potentially away from the source of damage or may be due to release of apical dominance resulting from apical meristem damage (King et al. 2008, Liu et al. 2009b). Clonal growth under herbivory can allow the transfer of resources from damaged to undamaged ramets or the storage organs of the genet (Schmid et al. 1990, Buschmann et al. 2006, Liu et al. 2009b) and can offset decreases in reproduction commonly seen following tissue damage.

Height in *Eupatorium perfoliatum* decreased with leaf herbivory, although not until the last three measurement points (days 121, 107 and 91). This delayed effect of herbivory may have involved the use of stored carbohydrates by damaged *E. perfoliatum*. These resources could be used for tissue growth and repair following damage induced loss of photosynthetic capability, leading to temporary compensation. However, if the stored resources ran out before photosynthetic ability was returned to normal, growth would then suffer, explaining the reduction in height at the later measurement points.

Conclusions

Costs of root and leaf herbivory were more limited than anticipated. Apart from height in *E. perfoliatum*, leaf and stem mass in *L. salicaria* and fruit mass in *P. sedoides*, all other measured biomass traits remained unchanged or improved after damage to roots and/or leaves. It is possible that the treatments applied were not severe enough and

perhaps repeating them would have been more realistic than a single treatment. However, my results demonstrated contrasts in how these three species react to tissue damage and their potential ability to compensate or over-compensate for herbivory. One factor all three species had in common was an increase in number and/or mass of branches after simulated root herbivory. This may have been a means of increasing photosynthetic capacity by generating more leaves or a means for potential future reproduction, either clonal or sexual. This effect of root damage on branching has not previously been reported. *Penthorum sedoides*'s biomass was unchanged under leaf damage and under root damage and its non-sexual biomass was maintained, but at a possible cost of fruit reproduction. *Lythrum salicaria* suffered the most from herbivory, although it showed an increase in branch production after root damage that was more long lasting than that of *P. sedoides*. *Eupatorium perfoliatum* increased in almost all the biomass measures, including clonal growth and fruit production, but height was reduced, so compensation was not complete. Interactions between root and leaf herbivory and size dependence relationships did occur, although only in a limited number of traits.

CHAPTER IV

GENETIC EFFECTS ON THE PARTITIONING OF BIOMASS TO GROWTH AND SEXUAL AND CLONAL REPRODUCTION IN *LYTHRUM SALICARIA* AND *PENTHORUM SEDOIDES*.

Abstract

The phenotype exhibited by a plant has two sources of variation, environment and genotype. By comparing genetically identical clones, it is possible to determine the relative influence of these sources of variation on a trait. Using two clonal species with contrasting clonal growth forms—*Penthorum sedoides*, a "guerilla" species and *Lythrum salicaria*, a "phalanx" species—I investigated whether clonal reproduction would have more environmental and less genetic influence than sexual traits and whether the phalanx species would have more genotypic differences and less environmental influence than the guerilla, especially in regards to clonal growth. Genotypes of the two species were cloned to create genetically identical groups and environmental heterogeneity was created

through the application of fertilizer. The experiment was performed over two years for *P. sedoides*, with the plants of the first year acting as parents for the plants of the second year. Few traits exhibited genetic variation, but fertilizer addition had a strong effect. Clonal reproduction tended to vary more between treatments than did sexual mass, although in *P. sedoides* genetic control over the variation of these traits differed between years. In the first year, genotypes differed in fruit mass while in the second year, clonal traits were influenced more by genotype As predicted, *P. sedoides* demonstrated more plasticity in clonal structures than did *L. salicaria*, although clonal variation in *L. salicaria* was not influenced more by genetics than in *P. sedoides*.

Introduction

Clonal plants are able to produce genetically identical new individuals called ramets (Jackson et al. 1985). Commonly, ramets are formed when the parent plant produces spacers, such as stolons, root buds or rhizomes, at the end of which a new plant grows. Variation in the length of clonal spacers can lead to differences in clonal architecture; plants with short spacers will have tightly packed ramets while plants with long spacers will have widespread ramets. Lovett Doust (1981) referred to these contrasting clonal growth forms as "phalanx" (clumped ramets) and "guerilla" (widespread ramets). The ecological advantages and costs of the two strategies have been demonstrated--phalanx plants are able to monopolize large resource patches by excluding competitors from the area within the phalanx while guerilla plants' ramets are mobile and able to forage for smaller and more widespread resource patches (Harper 1985, Humphrey and Pyke 1998, Ye et al. 2006). Although the relative plasticity of

plants exhibiting phalanx or guerilla growth forms has been investigated, these studies did not compare the amount of genetic control on variation in sexual and clonal reproduction or on vegetative traits between phalanx and guerilla species (Schmid 1985, Schmid and Bazzaz 1992, He et al. 2007).

All of the clones produced by a parent plant (the genet) can be expected to be phenotypically similar because of their identical genetic bases (i.e. they gave the same genotype). The tendency for close genetic relatives to resemble each other is referred to as broad sense heritability (Falconer 1981). This resemblance may be lessened by the different environmental conditions to which the ramets are exposed. By comparing the growth and reproduction of clones or other closely related individuals across variable conditions, it is possible to estimate the importance of genotype (broad sense heritability) relative to environmental influences.

Variation in many components of plant growth and reproduction are under genetic influence. Many sexual characters are heritable in the broad sense, including number and mass of flowers (Goldberg 1988, Prati and Schmid 2000, Ronsheim and Bever 2000, Torang et al. 2010), number and mass of seeds (Aarssen and Clauss 1992, Biere 1995, Cheplick 1995), timing of reproduction (Biere 1995, van Kleunen 2007, Torang et al. 2010) and allocation to sexual reproduction, usually described as the proportion of flowering nodes (Biere 1995, Reekie 1998, Sugiyama and Bazzaz 1998, Prati and Schmid 2000, van Kleunen et al. 2002, 2005, Torang et al. 2010). These estimates of broad-sense heritability are sometimes rather large, with genotype or family accounting for more than 50% of the total phenotypic variances in some cases (Ronsheim and Bever 2000, Toker 2004). Few published studies have shown no broad sense heritability for

reproductive traits; van Kleunen (2007) found that maternal families of *Mimulus guttatus* did not differ in the floral traits measured and Cheplick (2001) found that inbred line and family within inbred line lacked variation in seed mass in *Amaranthus albus*.

Genetic influences on clonal characteristics are less frequently investigated than sexual traits. Nevertheless, some clonal traits have also shown broad sense heritability, including the number of new ramets produced (Cheplick and Gutierrez 2000, van Kleunen et al. 2005), number and mass of asexual bulbils (Ronsheim and Bever 2000, Fischer et al. 2004, Thompson and Eckert 2004), spacing of ramets and spread of the genet (Cheplick 1997, Skalova et al. 1997, Cheplick and Gutierrez 2000, van Kleunen 2007) and rhizome mass (Goldberg 1988, Cheplick 1995). However, lack of heritability for clonal traits such as bulbil production and stolon length has also been reported (Tworkoski et al. 2001, Ceplitis and Bengtsson 2004). There has only been limited investigation of the genetics of variation in spacer biomass such as stolons and root buds, as are found in the wetland plants *Penthorum sedoides* and *Lythrum salicaria*, respectively, especially with reference to nutrient levels in the soil. Spacers and ramets are longer-lived and interact more with the environment, particularly the potentially very heterogeneous soil, than do flowers and fruits. I therefore predict that phenotype of clonal traits will be determined more by environmental conditions and the variation of these traits will be under less genetic influence than sexually reproductive traits. The phenotype of a guerilla species, *Penthorum sedoides* should exhibit more environmental influence and be under less genotypic control than a phalanx species such as *Lythrum salicaria*. I expect this to be especially true for the clonal characteristics, as the stolons of *P. sedoides* require flexibility to have advantageous placement of its ramets.

In this study, I compared the genetic and environmental components of variation in plant growth characteristics, sexual and clonal reproduction by investigating the reactions of clones of 30 genotypes to nutrient addition. I used two unrelated wetland plants as my study species: *Penthorum sedoides*, a native species with extensive fruit production and aggressive, wide spread clonal growth through stolons (guerilla growth form), and *Lythrum salicaria*, an invasive species with more limited, compact clonal growth through root buds (phalanx growth form). The questions addressed include 1. Do the two species differ in amount of resources (biomass) expended on clonal growth and sexual reproduction; 2. Are there interactions between soil nutrient levels and the genetic variation observed; 3. How different are genetic influences on variation in sexual and clonal reproduction between *Penthorum sedoides* and *Lythrum salicaria*. I predicted that variation in clonal characteristics is under more environmental influence and less genetic influence than sexual characteristics. I also predicted that variation in the more widespread guerilla species *Penthorum sedoides* will show more environmental influence and be under less genetic control than the compact phalanx species *Lythrum salicaria*.

Methods

First generation

The first generation was raised in 2004. Seeds of *Penthorum sedoides* and *Lythrum salicaria* obtained from Ernst Conservation Seeds (Meadville, PA) were germinated on moist filter paper in Petri dishes in growth chambers on 24 January, 2004. Seedlings were transplanted to small pots on 23 February and allowed to grow under grow lamps (16:8 light dark cycle) until they were large enough to be cloned. Throughout the experiment, I watered the plants every other day as needed. Beginning in early June,

I randomly chose thirty plants (genotypes) of each species and cloned them by cutting the plant between the nodes and placing the cuttings in water until roots formed. I planted each cutting (clone) into a small pot. Using this procedure, I created six clones per genotype that were genetically identical to the parental genotype and each other. For each genotype, I randomly assigned three of the clones to the fertilizer treatment while the other three served as untreated controls. In early July, two weeks after cloning was completed, I moved the plants to the outdoor ecological research area on the Cleveland State University campus (Cleveland OH) where they were transplanted into the larger pots. After initial measurements of height, I applied the fertilizer treatments beginning 21 July. For the fertilizer group, I added commercial fertilizer (Miracle-Gro®) to the pots following the manufacturer's instructions. The control group received water without fertilizer added. Treatments were applied four times at two week intervals. At the end of the experiment in early October, I again measured the height of all plants and counted the number of ramets. When harvesting the plants, I divided them into stem, leaves, stolons, ramets and fruits and placed these parts into individual paper bags. I then dried the harvested tissue, except the fruits, in an oven at 60 degrees C for at least 48 hours and weighed it to the nearest tenth of a gram.

Second generation

Because most plants in the first generation of *L. salicaria* failed to set viable seeds, this species was excluded from the second generation of the experiment. In 2006, eight genotypes of *P. sedoides* from the 2004 experiment were randomly chosen to be the maternal parents of the second generation. Seeds were taken from plants raised in the high nutrient treatment. On 15 December, 2006, I germinated the seeds and grew the

plants in a manner similar to the first generation. When the plants were large enough (in early to mid May 2007), three young plants from each of the eight parents were cloned as described for the first generation experiment. Six clones were generated from each of the young plants (genotypes) with three randomly assigned to the added nutrient treatment and three to be untreated controls. This gave rise to 144 plants (24 genotypes with 6 clones each). After the clones were established, they were transplanted to large pots in the outdoor experimental garden on the Cleveland State University campus in early July. Initial measurements of height were recorded for each plant. Starting 15 August, plants assigned to the fertilizer group were treated using commercial fertilizer following the company's instructions. Treatments were applied three times at 2 week intervals. Plants designated as control received only water at all times. After fertilizer treatments were applied, I measured the height of plants at two week intervals. Beginning 22 September, final measurements of height were taken and ramets were counted. During harvest, each plant was divided into main stem, fruits, ramets, stolons and roots and each of these parts were placed in separate paper bags and, except for the fruits, were dried in an oven at 60°C for at least 48 hr and weighed to the nearest tenth of a gram.

Statistical Analysis

For the analysis, fertilizer treatment was considered a fixed effect while genotype and parent were random effects. For *P. sedoides* in the second generation, genotype was nested in parent in the analysis. Due to oven malfunction, root and stolon biomass were lost for some plants, leading to an unbalanced data set. Because of this, I utilized mixed methods using Maximum likelihood (ML) to estimate variance in lieu of the more

traditional nested ANOVA techniques, since ANOVA is more sensitive to unbalanced data than ML (Littell et al. 2002).

Using PROC MIXED in the SAS statistical package, which utilizes ML methodology, I analyzed the effect of parent, genotype, fertilizer treatment and the interactions between these factors on components of plant reproduction and growth. When appropriate, I also included a measure of plant size as a covariate to account for potential size dependence. Different covariates were applied to the traits studied; for fruit characteristics, the covariate was vegetative mass (total biomass minus fruit mass); for stolon mass, it was "non-stolon mass" (vegetative mass minus stolon mass); for the ramet characteristics, it was "non-ramet mass" (vegetative mass minus ramet mass); and for root bud mass, it was "non-root bud mass" (vegetative mass minus root bud mass). These variables were used as the covariate instead of total biomass to prevent autocorrelation between the two variables (e.g. fruit mass would be present in both the dependent and independent variables) (Samson and Werk 1986). I performed the analysis by taking the full model including all variables and interactions, and removing factors one at a time in a step-wise manner. Order of removal was determined by the variance components of the factors (smallest removed first) and interactions were taken out before the other factors. After each step, models were compared using the likelihood ratio test to determine whether removal of the component significantly reduced the fit of the model. The likelihood ratio can be calculated as the difference between -2 x the log likelihood of the two models and approximates a χ^2 distribution with one degree of freedom (Littell et al. 2002, Bolker 2008). If the removal of the variable or interaction significantly decreased the fit of the model (significantly increased the value of -2 x the log

likelihood), it was kept in the model, otherwise the variable was removed before the next model was run until only significant sources of variation remained in the model.

Results

Lythrum salicaria

All height measurements of *Lythrum salicaria* were affected by genotype and height increased for treated plants (Table VII, Figure 15 a; Figure 16; on 24 August mean for control was 24.71 cm ± 0.87, for fertilizer treatment 30.27 cm ± 0.87; on 17 October mean for control was 33.45 cm ± 0.93, for fertilizer treatment 51.11 cm ± 1.56). Mass of main stems varied by genotype and increased with fertilizer treatment (Table VIII; Figure 17); mean for control was 2.51 g ± 0.15, mean for fertilized plants was 8.64 g ± 0.46). Root bud mass was unaffected by any of the factors tested but was correlated with non-root bud mass (Table VIII). Ramet mass increased with fertilizer treatment (Table IX; 0.88 ± 0.13 g for control, 2.63 g ± 0.31 for fertilized treatment), did not vary by genotype and was not correlated with non-ramet mass. Ramet number increased with non-ramet mass but was no influenced by genotype or fertilizer treatment (Table IX). Due to extremely low fruit set in *L. salicaria*, fruit data could not be analyzed in that species

Penthorum sedoides--First generation

Genotype affected all measurements of height and after nutrient application, height differed between fertilizer treatments in the first generation of *P. sedoides* (Table X; Figure 15 b; Figure 18; mean for control was 17.61 g ± 0.67, mean for fertilized was 20.17 g ± 0.81 on 24 August and mean for control was 27.66 g ± 0.70, mean for fertilized was 31.39 g ± 1.12 on 7 October). Main stem mass varied based on fertilizer treatment

Table VII. Maximum likelihood estimates and results of the likelihood ratio test for height in *Lythrum salicaria*. " Fert" stands for fertilizer treatment and "LR" for Likelihood ratio.

Trait	Model	2*Negative log likelihood	Compared to	LR	P	Conclusion
Height before treatment						
15 July	1. Full Model	1967.5				
	2. Minus fert*genotype	1967.5	Model 1	0	1	Drop fert*genotype
	3. Minus fert	1967.6	Model 2	0.1	0.75	Drop fert
	4. Minus genotype	1986.8	Model 3	19.2	<0.0001	Retain genotype
Height after treatment						
24 August						
	1. Full Model	1222.8				
	2. Minus fert*genotype	1223.0	Model 1	0.2	0.65	Drop fert*genotype
	3. Minus genotype	1269.7	Model 2	46.7	<0.0001	Retain genotype
	4. Minus fert	1253.4	Model 2	30.4	<0.0001	Retain fert
17 October						
	1. Full Model	512.0				
	2. Minus fert*genotype	512.3	Model 1	0.3	0.58	Drop fert*genotype
	3. Minus genotype	556.8	Model 2	44.5	<0.0001	Retain genotype
	4. Minus fert	592.5	Model 2	80.2	<0.0001	Retain fert

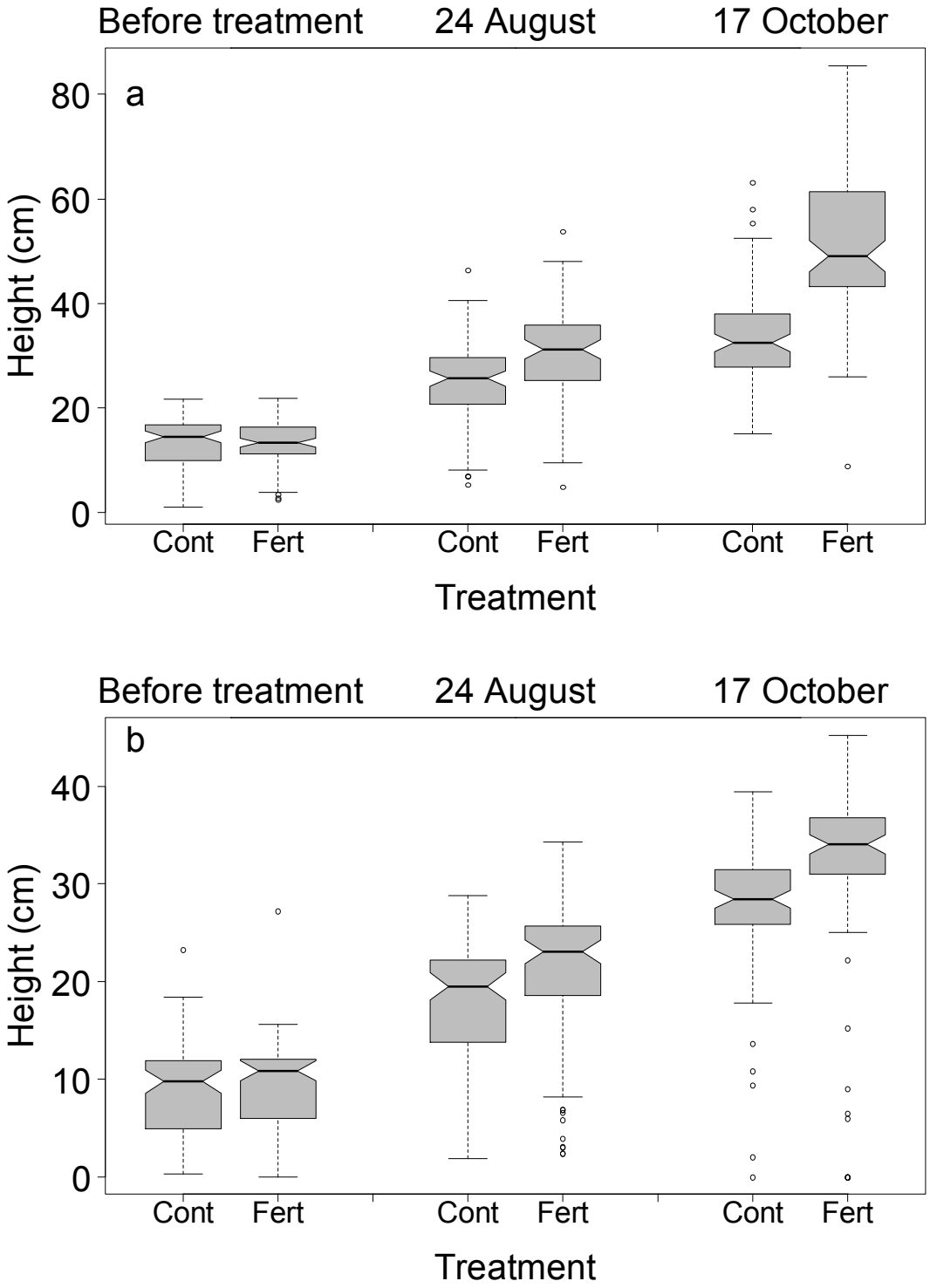

Figure 15. Effect of fertilizer treatment on height in a. *Lythrum salicaria* and b. *Penthorum sedoides* in the first generation. "Cont" stands for the control and "Fert" refers to the fertilizer added treatment.

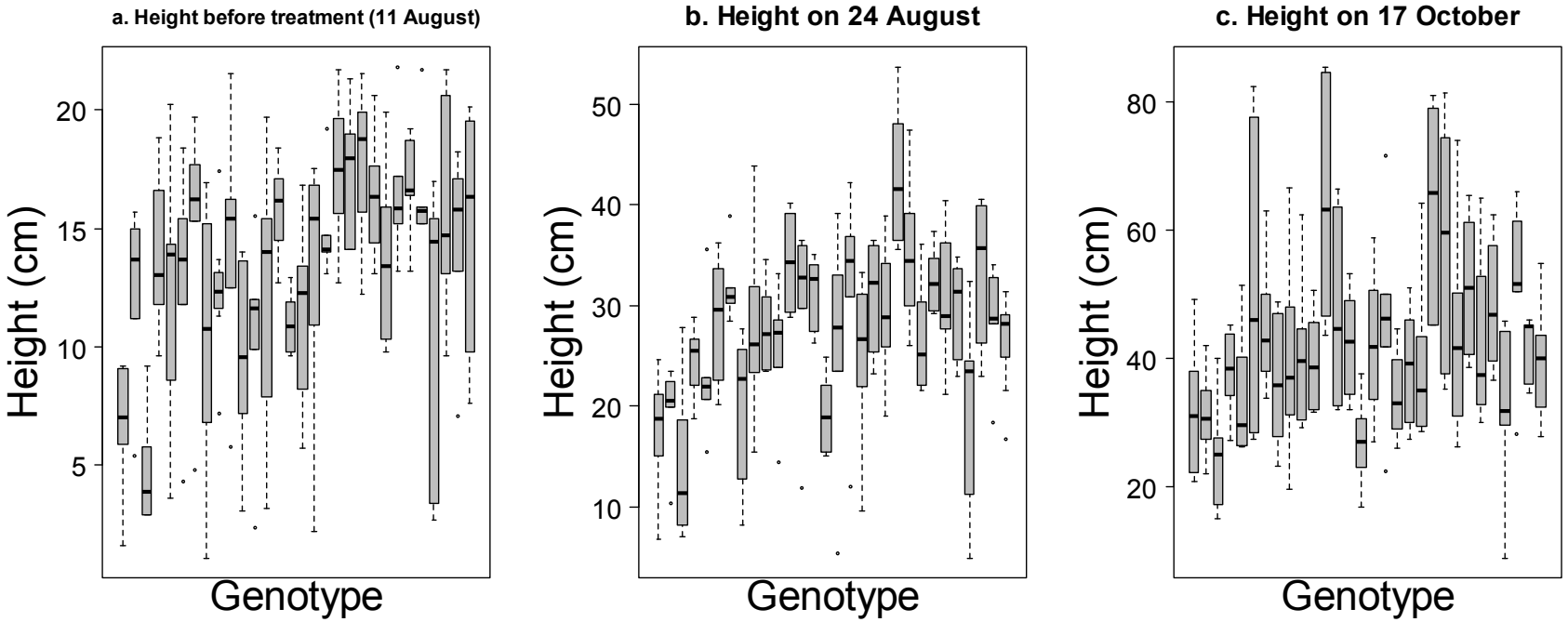

Figure 16. Genotypic differences in the height of *Lythrum salicaria*. Each bar represent an individual genotype (30 genotypes total).

Table VIII. Maximum likelihood estimates and results of the likelihood ratio test for main stem and root bud mass in *Lythrum salicaria*. "Fert" stands for the fertilizer treatment and "LR" stands for likelihood ratio.

Trait	Model	2*Negative log likelihood	Compared to	LR	P	Conclusion
Main stem mass						
	1. Full Model	365.2				
	2. Minus fert*genotype	365.2	Model 1	0	1	Drop fert*genotype
	3. Minus genotype	372.5	Model 2	7.3	0.0068	Retain genotype
	4. Minus fert	511.9	Model 2	146.7	<0.0001	Retain fert
Root bud mass						
	1. Total	182.8				
	2. Minus non-root bud*fert*genotype	182.8	Model 1	0	1	Drop non-root bud*fert*genotype
	3. Minus fert*genotype	182.8	Model 2	0	1	Drop fert*genotype
	4. Minus non-root bud genotype	183.5	Model 3	0.7	0.4	Drop non-root bud* genotype
	5. Minus non-root bud*fert	183.6	Model 4	0.1	0.75	Drop non-root bud*fert
	6. Minus genotype	183.6	Model 5	0	1	Drop genotype
	7. Minus fert	183.6	Model 6	0	1	Drop fert
	8. Minus non-root bud	224.0	Model 7	40.4	<0.0001	Retain non-root bud

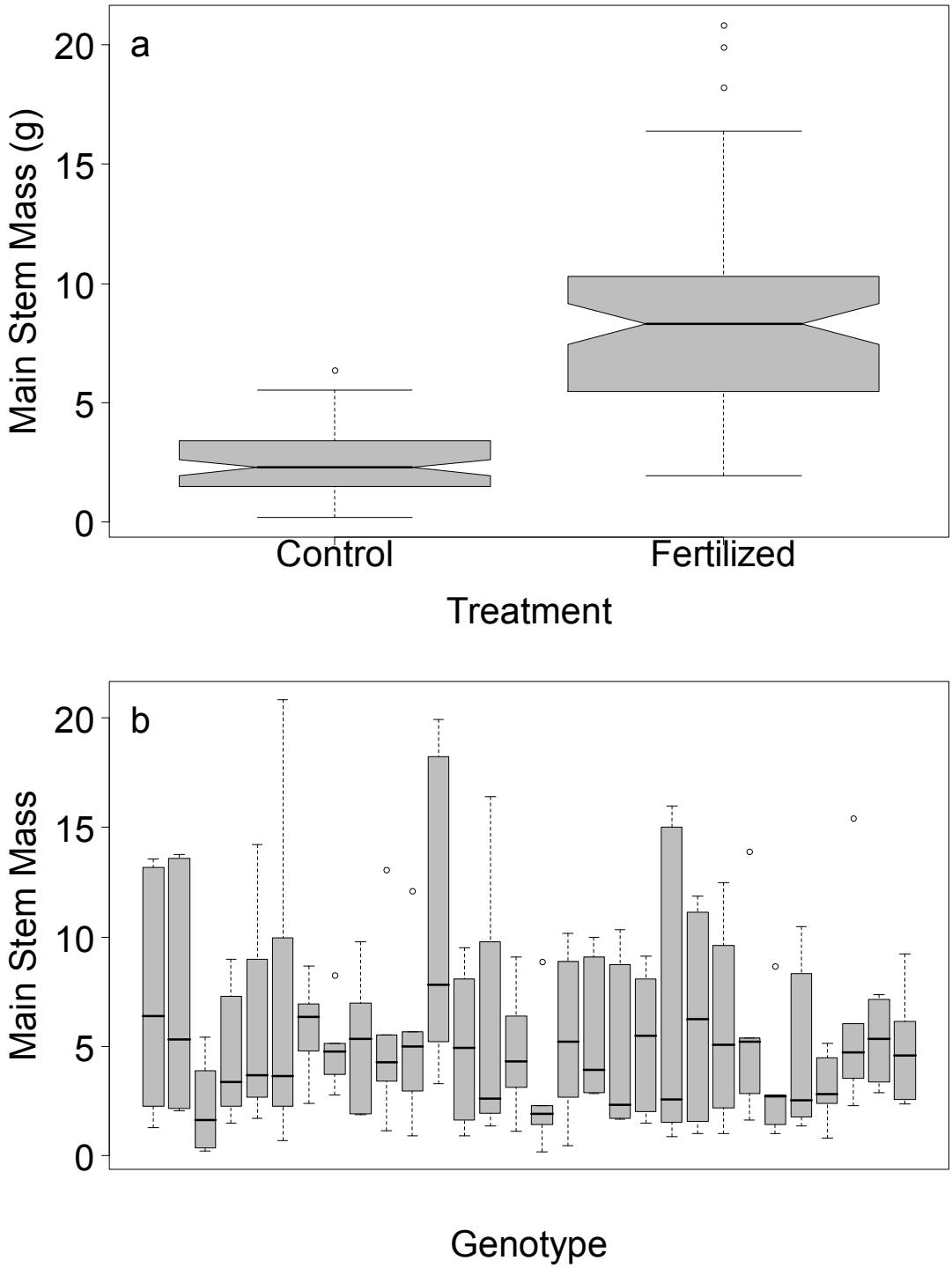

Figure 17. Effect of a. treatment and b. genotype on main stem mass in *Lythrum salicaria*. In b, each bar represents an individual genotype (30 genotypes total).

Table IX. Maximum likelihood estimates and results of the likelihood ratio test for ramet mass and number in *Lythrum salicaria*. "Fert" stands for the fertilizer treatment and "LR" stands for likelihood ratio.

Trait	Model	2*Negative log likelihood	Compared to	LR	P	Conclusion
Ramet mass						
	1. Full model	247.7				
	2. Minus non-ramet*fert*genotype	248.1	Model 1	0.4	0.53	Drop non-ramet*fert*genotype
	3. Minus genotype*fert	248.1	Model 2	0	1	Drop genotype*fert
	4. Minus non-ramet*genotype	248.2	Model 3	0.1	0.75	Drop non-ramet*genotype
	5. Minus non-ramet*fert	248.2	Model 4	0	1	Drop non-ramet*fert
	6. Minus genotype	248.2	Model 5	0	1	Drop genotype
	7. Minus non-ramet	261.7	Model 6	13.5	0.0002	Retain non-ramet
	8. Minus fert	276	Model 6	27.8	<0.0001	Retain fert
Ramet number						
	1. Full model					
	2. Minus non-ramet*fert*genotype	436.7	Model 1	0	1	Drop non-ramet*fert*genotype
	3. Minus non-ramet*genotype	436.7	Model 2	0	1	Drop non-ramet*genotype
	4. Minus genotype*fert	436.7	Model 3	0	1	Drop genotype*fert
	5. Minus non-ramet*fert	439.1	Model 4	2.4	0.12	Drop non-ramet*fert
	6. Minus genotype	439.3	Model 5	0.2	0.65	Drop non-ramet*fert
	7. Minus non-ramet	467.3	Model 6	28.0	<0.0001	Retain non-ramet
	8. Minus fert	439.4	Model 6	0.1	0.75	Drop fert

(Table XI; control mean was 2.02 g ± 0.13, fertilized mean was 4.54 g ± 0.38) but not genotype. Stolon mass and ramet mass increased after fertilizer treatment (mean for control was 7.56 g ± 0.48 and mean for fertilized was 17.93 g ± 1.36 for stolon mass; mean for control was 1.09 g ± 0.18 and mean for fertilized was 4.02 g ± 0.43 for ramet mass) and stolon and ramet mass was less in larger plants that smaller plants (Table XI; Figure 19 a and b). Number of ramets increased after fertilizer treatment and increased with non-ramet mass (Table XII; Figure 19 c; for control the mean was 2.62 ± 0.2 and for the fertilizer treatment, 4.47 ± 0.26). Fruit mass varied among genotypes (Figure 20) and correlated positively with vegetative mass but not fertilizer treatment (Figure 19 d). There were no interactions between factors in the first generation of *Penthorum sedoides*.

Penthorum sedoides—Second generation

Height of the plants assigned to the two treatment groups did not differ prior to the administration of fertilizer treatments and genotype nested in parent was only marginally significant at that time (Table XIII; Figure 21; P=0.051). On 23 August, following treatment, plants that received fertilizer were marginally taller (P=0.06) than the control plants (mean for control was 16.75 cm ± 0.63 and for fertilized treatment, 18.42 cm ± 0.64). On 7 September, fertilizer treated plants were again taller than the control (Table XIV; mean for control was 22.97 cm ± 0.69 and mean for treated plants was 26.68 cm ± 0.66). At harvest, height of the nutrient enriched plants increased relative to the control (mean for control was 25.78 cm ± 0.61 and for fertilized was 30.31 ± 0.58) without a genetic effect. For the main stem mass, only fertilizer treatment had a

Table X. Maximum likelihood estimates and results of the likelihood ratio test for height for *Penthorum sedoides* in the first generation. "Fert" stands for the fertilizer treatment and "LR" stands for likelihood ratio.

Trait	Model	2*Negative log likelihood	Compared to	LR	P	Conclusion
Height before treatment						
15 July	1. Full model	1003.3				
	2. Minus fert*genotype	1003.9	Model 1	0.6	0.44	Drop fert*genotype
	3. Minus fert	1004.6	Model 2	0.7	0.4	Drop Fertilizer
	4. Minus genotype	1010.3	Model 3	5.7	0.017	Retain genotype
Height after treatment						
24 August						
	1. Full model	1164.4				
	2. Minus fert*genotype	1164.4	Model 1	0	1	Drop fert*genotype
	3. Minus genotype	1172.3	Model 2	8.2	0.004	Retain genotype
	4. Minus fert	1174.4	Model 2	10.3	0.0013	Retain fertilizer
17 October						
	1. Full model	1267.0				
	2. Minus fert*genotype	1267.2	Model 1	0.2	0.65	Drop fert*genotype
	3. Minus genotype	1273.8	Model 2	6.6	0.01	Retain genotype
	4. Minus fert	1276.8	Model 2	9.6	0.0019	Retain fertilizer

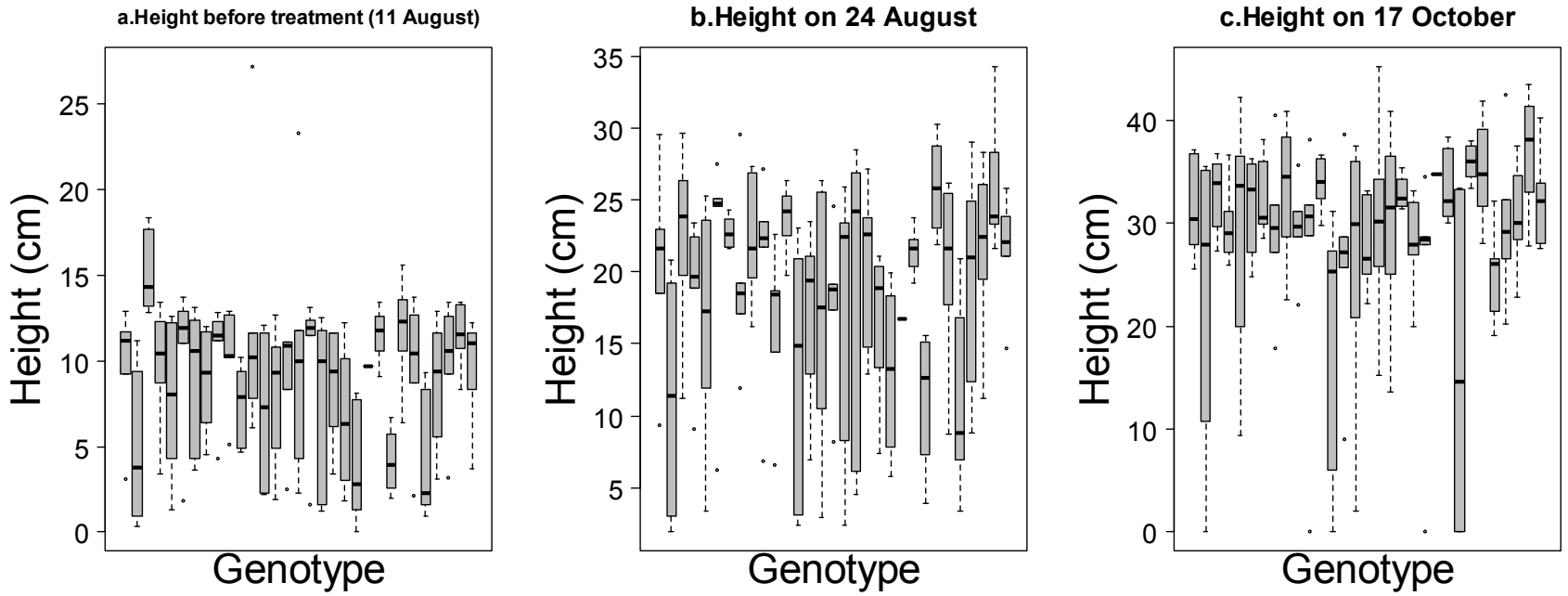

Figure 18. Genotypic differences in height of *Penthorum sedoides* in the first generation. Each bar represents an individual genotype (30 genotypes total).

Table XI. Maximum likelihood estimates and results of the likelihood ratio test for main stem, stolon and ramet mass for *Penthorum sedoides* in the first generation. "Fert" stands for the fertilizer treatment and "LR" stands for likelihood ratio.

Trait	Model	2*Negative log likelihood	Compared to	LR	P	Conclusion
Main stem mass						
	1. Full model	374.8				
	2. Minus fert*genotype	374.8	Model 1	0	1	Drop fert*genotype
	3. Minus genotype	374.8	Model 2	0	1	Drop genotype
	4. Minus fert	431.8	Model 3	57	<0.0001	Retain fert
Stolon mass						
	1. Full model	275.5				
	2. Minus non-stolon*fert*genotype	275.9	Model 1	0	1	Drop non-stolon*fert*genotype
	3. Minus fert*genotype	275.9	Model 2	0	1	Drop fert*genotype
	4. Minus non-stolon*genotype	277.4	Model 3	1.5	0.22	Drop non-stolon*genotype
	5. Minus non-stolon*fert	279.7	Model 4	2.3	0.13	Drop non-stolon*fert
	6. Minus genotype	279.7	Model 5	0	1	Drop genotype
	7. Minus fert	331.1	Model 6	51.4	<0.0001	Retain fert
	8. Minus non-stolon	372.8	Model 6	93.1	<0.0001	Retain non-stolon
Ramet mass						
	1. Full model	-3.3				
	2. Minus non-ramet*fert*genotype	-3.3	Model 1	0	1	Drop non-ramet*fert*genotype
	3. Minus fert*genotype	-3.3	Model 2	0	1	Drop fert*genotype
	4. Minus non-ramet*genotype	-3.3	Model 3	0	1	Drop non-ramet*genotype
	5. Minus non-ramet*fert	-2.9	Model 4	0.4	0.53	Drop Non-ramet*fert
	6. Minus genotype	-1.7	Model 5	1.2	0.27	Drop genotype
	7. Minus non-ramet	14	Model 6	15.7	<0.0001	Retain non-ramet
	8. Minus fert	36	Model 6	37.7	<0.0001	Retain fert

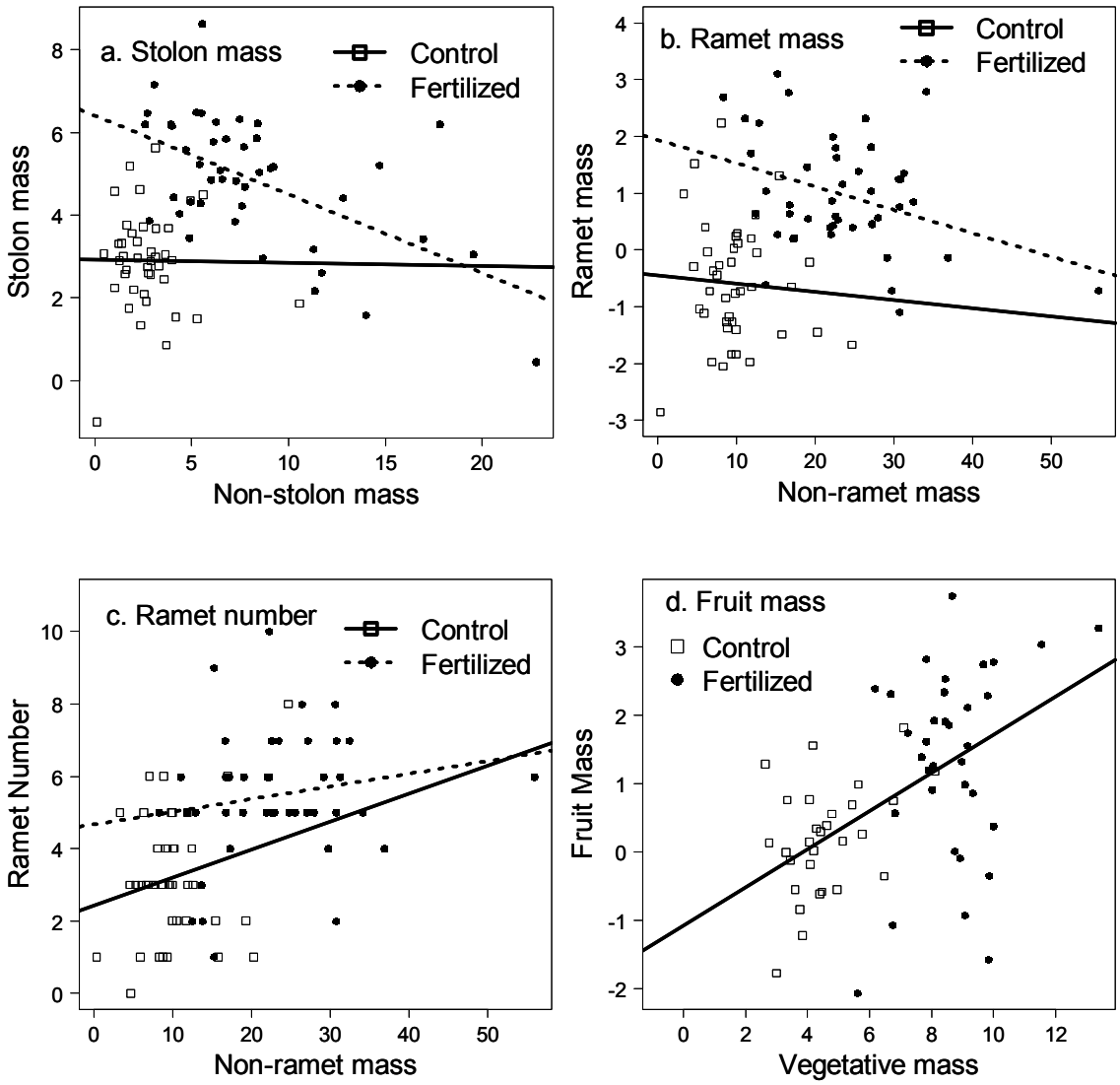

Figure 19. Effect of fertilizer treatment and size on a. stolon mass, b. ramet mass, c. ramet number and d. fruit mass in *Penthorum sedoides* in the first generation. Where treatments differed significantly in the likelihood ratio test, the dotted line represents the fertilizer treatment and the solid line is the control. One line indicates the two treatments did not differ significantly in slope or intercept. Data are Box-Cox transformed

Table XII. Maximum likelihood estimates and results of the likelihood ratio test for ramet number and fruit mass for *Penthorum sedoides* in the first generation . "Fert" stands for the fertilizer treatment, "veg" stands for vegetative mass and "LR" stands for likelihood ratio.

Trait	Model	2*Negative log likelihood	Compared to	LR	P	Conclusion
Ramet number						
	1. Full Model	328.2				
	2. Minus non-ramet*fert*genotype	329.6	Model 1	1.4	1	Drop non-ramet*fert*genotype
	3. Minus non-ramet*genotype	330.4	Model 2	0.8	0.37	Drop non-ramet*genotype
	4. Minus fert*genotype	331	Model 3	0.6	0.44	Drop fert*genotype
	5. Minus non-ramet*fert	331.3	Model 4	0.3	0.58	Drop Non-ramet*fert
	6. Minus genotype	332.1	Model 5	0.8	0.37	Drop genotype
	7. Minus non-ramet	790.6	Model 5	458.5	<0.0001	Retain non-ramet
	8. Minus fert	342.4	Model 5	10.3	0.0013	Retain fert
Fruit mass						
	1. Full model	183.1				
	2. Minus veg*fert*genotype	183.1	Model 1	0	1	Drop non-ramet*fert*genotype
	3. Minus fert*genotype	183.1	Model 2	0	1	Drop fert*genotype
	4. Minus veg*fert	183.3	Model 3	0.2	1	Drop veg*fert
	5. Minus veg*genotype	183.9	Model 4	0.6	0.37	Drop veg*genotype
	6. Minus genotype	190.7	Model 5	6.8	0.0091	Retain genotype
	7. Minus fert	186.1	Model 5	2.2	0.13	Drop fert
	8. Minus veg	366.9	Model 6	183	<0.0001	Retain veg

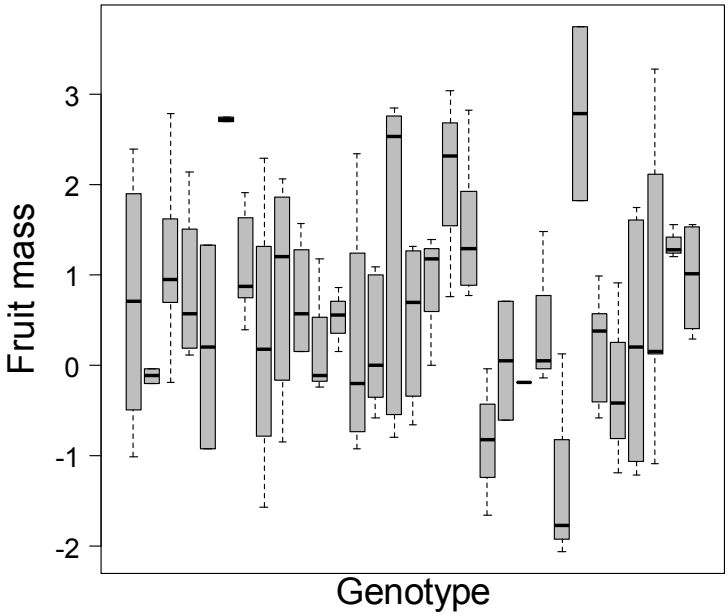

Figure 20. Genotypic differences in fruit mass in *Penthorum sedoides* in the first generation. Each bar indicates an individual genotype (30 genotypes total).

significant effect (Table XV; control mean was 4.5g ± 0.22 and fertilized was 9.45 g ± 0.32). Stolon mass was affected by fertilizer treatment (control mean was 11.53 g ± 0.70 and fertilized mean was 30.96 g ± 1.32), and main stem mass increased with plant size (Figure 22 a), and this trait was marginally influenced by genotype. Genotypes differed in the number of ramets they produced (Table XVI; Figure 23 b), and ramet number increased with size, although fertilizer had no significant effect (Figure 22 c). The only biomass component that varied by genotype was ramet mass, which was influenced by genotype nested in parent (Figure 23 a) and increased with non-ramet mass and fertilizer treatment (Figure 22 b). Only vegetative mass influenced fruit mass (Figure 22 d).

Table XIII. Maximum likelihood estimates and results of the likelihood ratio test for height on 11 August and 23 August for *Penthorum sedoides* in the second generation. "Fert" stands for the fertilizer treatment and "LR" stands for likelihood ratio.

Trait	Model	2*Negative log likelihood	Compared to	LR	P	Conclusions
Height before treatment						
11 August						
	1. Full model	784.3				
	2. Minus fert*parent	784.3	Model 1	0	1	Drop fert*parent
	3. Minus fert*parent(genotype)	784.4	Model 2	0.1	0.75	Drop fert*parent(genotype)
	4. Minus parent	784.4	Model 3	0	1	Drop parent
	5. Minus parent(genotype)	787.9	Model 4	3.5	0.06	Parent(genotype) marginal
	6. Minus fert	789.9	Model 5	2	0.16	Drop fert
Height after treatment						
23 August						
	1. Full model	863.6				
	2. Minus fert*parent(genotype)	863.6	Model 1	0	1	Drop fert*parent(genotype)
	3. Minus fert*parent	863.6	Model 2	0	1	Drop fert*parent
	4. Minus parent(genotype)	863.6	Model 3	0	1	Drop parent(genotype)
	5. Minus parent	863.6	Model 4	0	0	Drop parent
	6. Minus fert	867.1	Model 5	3.5	0.06	Fert Marginal

Table XIV. Maximum likelihood estimates and results of the likelihood ratio test for height on 7 September and 22 September for *Penthorum sedoides* in the second generation. "Fert" stands for the fertilizer treatment and "LR" stands for likelihood ratio.

Trait	Model	2*Negative log likelihood	Compared to	LR	P	Conclusions
Height on 7 September						
	1. Full Model	877.5				
	2. Minus fert*parent	877.5	Model 1	0	1	Drop fert*parent
	3. Minus fert*parent(genotype)	877.7	Model 2	0.2	0.65	Drop fert*parent(genotype)
	4. Minus parent	877.7	Model 3	0	1	Drop parent
	5. Minus parent(genotype)	880.2	Model 4	2.5	0.11	Drop Parent(genotype)
	6. Minus fert	894.7	Model 5	14.5	0.0001	Retain fert
Height on 22 September						
	1. Full Model	844.4				
	2. Minus fert*parent	844.4	Model 1	0	1	Drop fert*parent
	3. Minus fert*parent(genotype)	844.4	Model 2	0	1	Drop fert*parent(genotype)
	4. Minus parent	844.4	Model 3	0	1	Drop parent
	5. Minus parent(genotype)	846.0	Model 4	1.6	0.21	Drop parent(genotype)
	6. Minus fert	874.6	Model 5	28.6	<0.0001	Retain fert

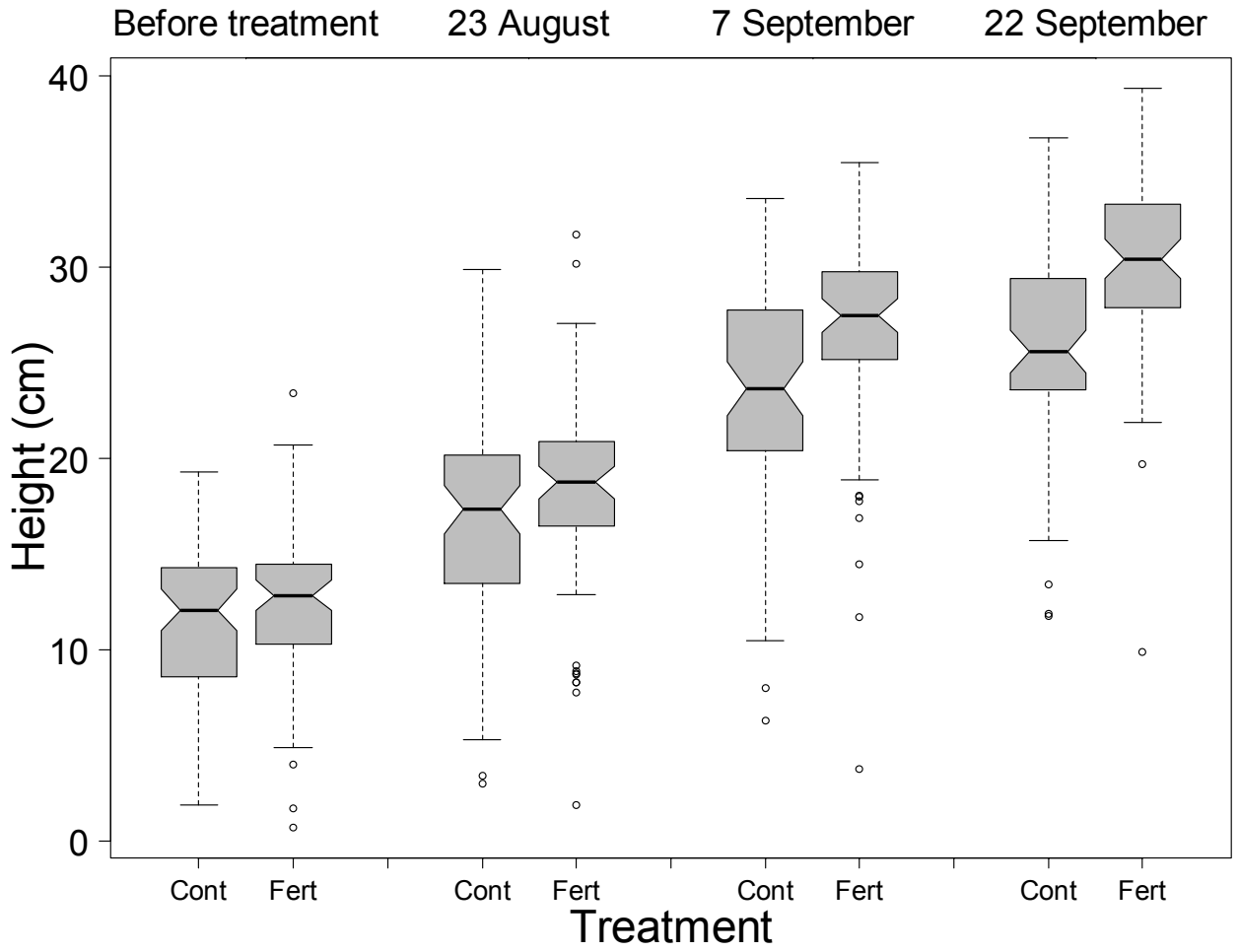

Figure 21. Effect of treatment on height of *Penthorum sedoides* in the second generation. "Cont" stands for control and "Fert" for the fertilized treatment.

Table XV. Maximum likelihood estimates and results of the likelihood ratio test for main stem and stolon mass for *Penthorum sedoides* in the second generation. "Fert" stands for the fertilizer treatment and "LR" stands for likelihood ratio.

Trait	Model	2*Negative log likelihood	compared to	LR	P	Conclusion
Main stem mass						
	1. Full model	449.9				
	2. Minus fert*parent	449.9	Model 1	0	1	Drop fert*parent
	3. Minus fert*parent(genotype)	449.9	Model 2	0	0.75	Drop fert*parent(genotype)
	4. Minus parent	449.9	Model 3	0	1	Drop parent
	5. Minus parent(genotype)	450.2	Model 4	0.58	0.48	Drop parent(genotype)
	6. Minus fert	554.9	Model 5	104.7	<0.0001	Retain fert
Stolon mass						
	1. Full model	182.6				
	2. Minus non-stolon*parent(genotype)*fert	182.6	Model 1	0	1	Drop 3-way interaction
	3. Minus Parent*fert	182.6	Model 2	0	1	Drop parent*fert
	4. Minus non-stolon*parent	182.6	Model 3	0	0	Drop non-stolon*parent
	5. Minus non-stolon*parent(genotype)	182.9	Model 4	0.3	0.58	Drop non-stolon*parent(genotype)
	6. Minus parent(gentoype)*fert	183.1	Model 5	0.2	0.65	Drop parent(gentoype)*fert
	7. Minus non-stolon*fert	183.7	Model 6	0.6	0.44	Drop non-stolon*fert
	8. Minus Parent	183.7	Model 7	0	1	Drop parent
	9. Minus Parent(Genotype)	186.7	Model 8	3	0.083	Parent(genotype) marginal
	10. Minus Fertilizer	236.0	Model 9	49.3	<0.0001	Retain fertilizer treatment
	11. Minus Non-stolon mass	256.3	Model 9	69.3	<0.0001	Retain non-stolon mass

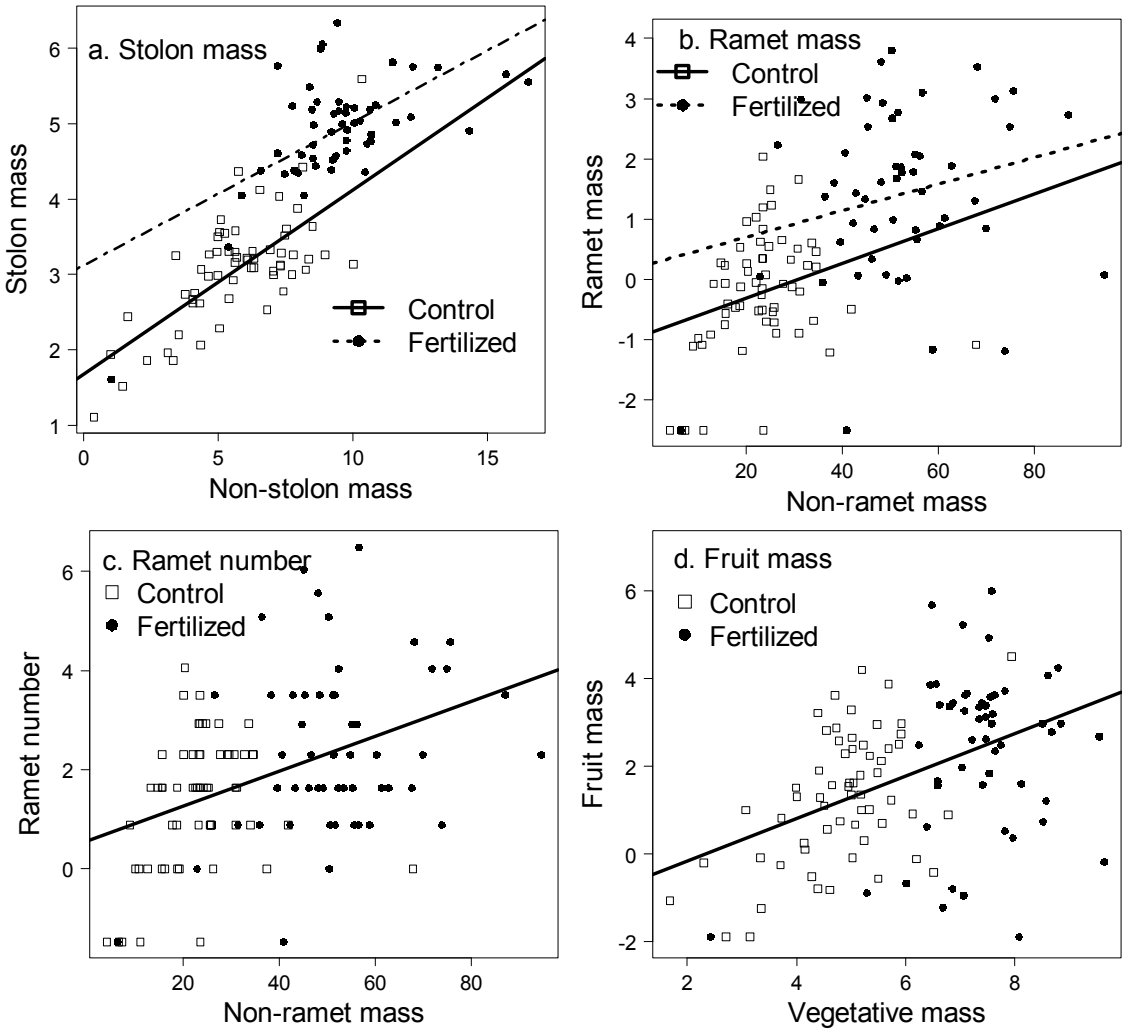

Figure 22. Effect of treatment and plant size on a. stolon mass, b. ramet mass, c. ramet number and d. fruit mass for *Penthorum sedoides* in the second generation. Where treatments differed significantly in the likelihood ratio test, the dotted line represents the fertilizer treatment and the solid line is the control. One line indicates the two treatments did not differ significantly. Data are Box-Cox transformed.

Table XVI. Maximum likelihood estimates and results of the likelihood ratio test for ramet mass and number for *Penthorum sedoides* in the second generation. "Fert" stands for the fertilizer treatment and "LR" stands for likelihood ratio.

Trait Model	2*Negative log likelihood	Compared to	LR	P	Conclusions
Ramet mass					
1. Full model	338.0				
2. Minus non-ramet mass*parent(genotype)*fert	338.0	Model 1	0	1	Drop 3-way interaction
3. Minus fert*parent	338.0	Model 2	0	1	Drop fert*parent
4. Minus fert*parent(genotype)	338.0	Model 3	0	1	Drop fert*parent(genotype)
5. Minus non-ramet*parent(genotype)	338.0	Model 4	0	1	Drop non-ramet*parent(genotype)
6. Minus non-ramet*parent	338.0	Model 5	0	1	Drop non-ramet*parent
7. Minus non-ramet*fert	338.3	Model 6	0.3	0.58	Drop non-ramet*fert
8. Minus parent	338.3	Model 7	0	1	Drop parent
9. Minus parent(genotype)	342.6	Model 8	4.3	0.038	Retain parent(genotype)
10. Minus fert	343.5	Model 8	5.2	0.023	Retain Fert
11. Minus non-ramet	458.8	Model 8	120.5	<0.0001	Retain non-ramet
Ramet number					
1. Full Model	390.8				
2. Minus non-ramet mass*parent(genotype)*fert	390.8	Model 1	0	1	Drop 3-way interaction
3. Minus fert*parent	390.8	Model 2	0	1	Drop fert*parent
4. Minus fert*parent(genotype)	390.8	Model 3	0	1	Drop fert*parent(genotype)
5. Minus non-ramet*parent	390.9	Model 4	0.1	0.75	Drop non-ramet*parent
6. Minus non-ramet*parent(genotype)	391.8	Model 5	0.9	0.34	Drop non-ramet*parent(genotype)
7. Minus non-ramet*fert	392.1	Model 6	0.3	0.58	Drop non-ramet*fert
8. Minus fert	392.2	Model 7	0.1	0.75	Drop fert
9. Minus parent	392.3	Model 8	0.1	0.75	Drop parent
10. Minus parent(genotype)	403.9	Model 9	11.6	0.0006	Retain parent(genotype)
11. Minus non-ramet	541.3	Model 9	149	<0.0001	Retain non-ramet

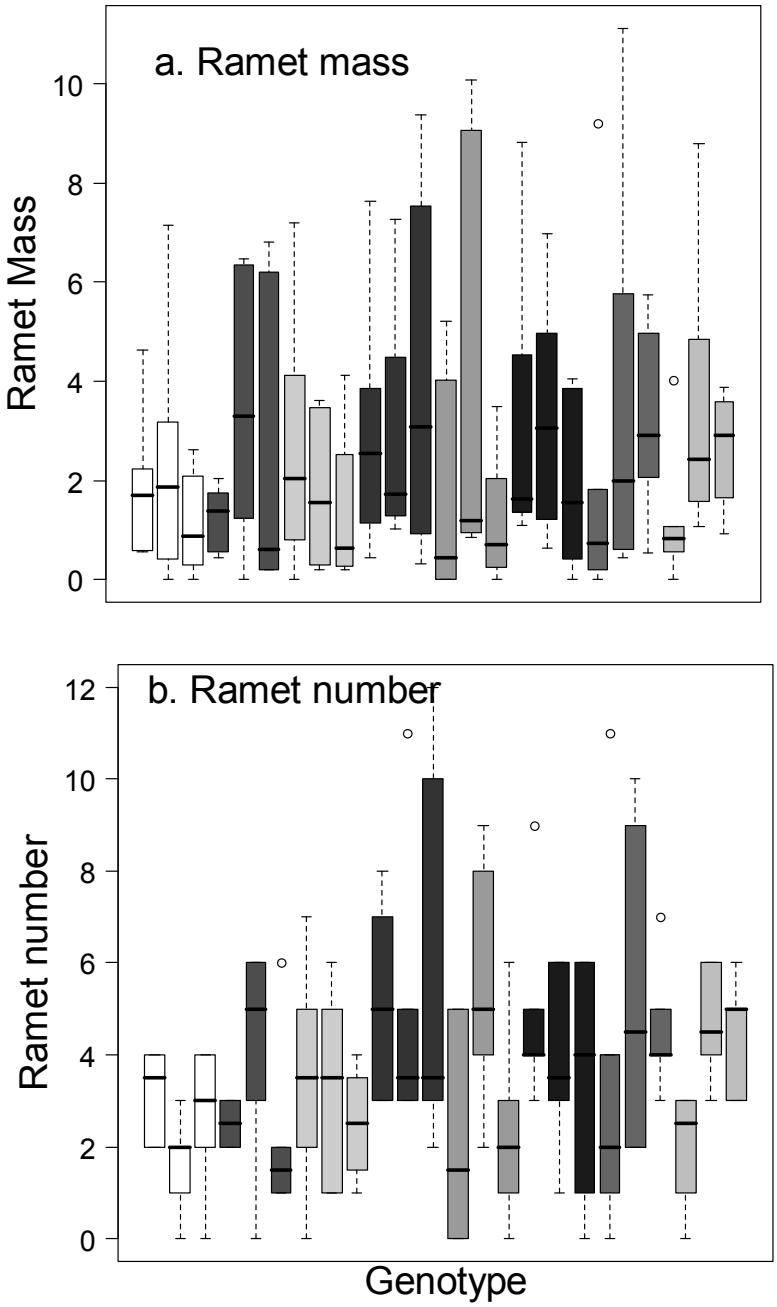

Figure 23. Genotypic differences in a. ramet mass and b. ramet number in *Penthorum sedoides* in the second generation. Each box indicates an individual genotype. Shading of adjacent genotypes indicates they are derived from the same maternal parent (8 parents and 24 genotypes total).

Table XVII. Maximum likelihood estimates and results of the likelihood ratio test for fruit mass for *Penthorum sedoides* in the second generation. "Fert" stands for the fertilizer treatment and "LR" stands for likelihood. "veg" stands for vegetative mass.

Trait	Model	2*Negative log likelihood	compared to	LR	P	Conclusion
Fruit mass						
	1. Full model	416.2				
	2. Minus vegetative mass*parent(genotype)*fert	416.2	Model 1	0	1	Drop 3-way interaction
	3. Minus fert*parent	416.2	Model 2	0	1	Drop fert*parent
	4. Minus fert*parent(genotype)	416.2	Model 3	0	1	Drop fert*parent(genotype)
	5. Minus veg*parent(genotype)	416.2	Model 4	0	1	Drop veg*parent(genotype)
	6. Minus veg*parent	416.4	Model 5	0.2	0.65	Drop veg*parent
	7. Minus veg*fert	417.9	Model 6	1.7	0.19	Drop veg*fert
	8. Minus parent(genotype)	417.9	Model 7	0	1	Drop parent(genotype)
	9. Minus fert	418.8	Model 8	0.9	0.34	Drop fert
	10. Minus parent	419.7	Model 9	0.9	0.34	Drop parent
	11. Minus veg	577.9	Model 10	158.2	<0.0001	Retain veg

Discussion

In both species and in both years, few traits showed genetic influence. It is clear that in these two species, plants bear relatively little resemblance to their genetically identical clones in the majority of the traits measured and, therefore plants of one genotype were not significantly different from plants of another genotype. This result is contrary to much of the genetic research in plants, which indicates that genotypes differ from each other in a variety of traits, both reproductive and vegetative. For example, Prati and Schmid (2000) found variation among clones for plant size (number of leaves and nodes, total biomass) and absolute and relative allocation to flowering, rooting and branching in *Ranunculus reptans*. Ronsheim and Bever (2000), also found significant broad sense heritability in the allocation of resources to seed production in *Allium vinale*. Even when maternal families (plants from seeds of the same maternal plant) rather than clones are considered, broad sense heritability is commonly detected (Cheplick 2001). For example, in *Mimulus guttatus*, maternal families within populations differed in time of flowering (phenology), plasticity in time to flowering when raised in contrasting moisture conditions, in height and number of upright branches, and both in number and length of stolons (van Kleunen 2007). In some cases, genetic variation influences the phenotype more than does environmental variation. Ceplitis (2001a) found this to be the case for number and proportion of seeds to bulbils**.** In contrast to these results, *L. salicaria* and *P. sedoides* showed minimal influence of genetics in the variation of traits, even though genetically identical clones were compared.

Nutrient addition showed a much greater influence over the growth and reproduction than did genotype. Most of the measured traits responded positively to

fertilizer addition, although root bud mass and ramet number in *L. salicaria*, fruit mass in the first generation of *P. sedoides* and the number of ramets and fruit mass in the second generation of *P. sedoides* did not. The overall large effect of environment and small effect of parental and/or genotypic identity indicate that *P. sedoides* and *L. salicaria* are highly plastic species and that many of their growth and reproductive aspects depend more on environmental conditions such as soil nutrient content than genotype. Such plasticity may aid these species in adapting to the variable environments experienced in wetland habitats (Mitsch and Gosselink 2000). However, since there were no detectable interactions between treatment and genotype effects, there was limited genetic variation in plasticity.

Lythrum salicaria

Both height and main stem mass in *L. salicaria* varied by genotype and increased with nutrient addition. The variation of the other traits, root bud mass, ramet mass and ramet number, were not influenced by genetics. Ramet mass increased under fertilizer treatment, but ramet number and root bud mass did not. Phenotypic variation in clonal growth in *L. salicaria* appeared to be under limited influence of either genetics or nutrient addition. These traits may have limited genetic variation, or may be responding to environmental factors other than nutrient levels**.** Relatively low environmental influence on root bud mass in a phalanx species emphasizes the rigidity of clonal expression of this growth form even under variable conditions.

Penthorum sedoides

The first and second generations of *Penthorum sedoides* showed similar responses to the tested variables for many of the traits studied. Stolon mass in both years was influenced by fertilizer treatment and non-stolon mass, while main stem mass was affected by fertilizer treatment. When there were discrepancies between the two years, the first generation tended to show more genotypic influences. Except for a marginal effect of genotype nested in parent for the pre-fertilization measurement, height measurements of the first generation depended on genotype while height of the second generation did not. Similarly, fruit mass varied with genotype only in the first generation. Clonal reproductive traits were the only cases where the second generation showed genetic variation while the first generation did not. There are several possible reasons for these between-year discrepancies. Environmental conditions in the second experimental year (2007) may have been more variable, causing increased plasticity that would mask the effect of genotype. Another possible explanation is that genotypes from the first generation were unrelated to each other and could be considered independent while genotypes in the second year of the experiment shared a maternal parent with some of the other genotypes, thus requiring a nested structure during the analysis. The fewer independent data points (30 genotypes in generation one vs. 24 genotypes nested in 8 parents in generation two) may have reduced the ability to detect genetic variability between the sets of clones in the second generation.

In the first generation, height measurements were consistently influenced by genotype and post-treatment heights increased with fertilizer treatment. Main stem mass was affected by fertilizer, with no genotypic influence. This implies that although genotype may dictate variation in plant height, it has little control over the amount of

tissue produced on the main stem. The stem thickness and the branching pattern perhaps depended more on environmental conditions than plant genotype. In the second generation, fertilizer again increased height and main stem mass, but there was only marginal variation due to genotype prior to fertilizer application and none on the later dates.

In both generations, there was an increase in mass to clonal organs in high nutrient conditions, which supports the hypothesis that clonal growth will increase under favorable conditions, helping plants retain a foothold and to produce more offspring in beneficial environments (Williams 1975, Chapter V of this dissertation). Size increase in clonal organs such as stolons and rhizomes have been found in response to nutrient increases (Lehmann and Rebele 2005, Liu et al. 2009a), although a lack of effect has also been reported (He et al. 2007, Bai et al. 2009). My results clearly showed an environmental effect on stolon mass and ramet mass in both years and in ramet number in the first generation. However, genetic effects on clonal traits differed between the two years; variation in stolon and ramet mass and ramet number was not significant in the first generation while broad sense heritability was seen in these traits in the second generation**.** Overall, the clonal mass data from *P.* sedoides suggest that environment has a large effect on clonal mass trait phenotypes while the genotypic effect is more limited. The large effect of environment on variation in stolon mass and ramet mass in the guerilla-like species may allow for strategic placement of clonal tissue in response to variable habitats.

For both generations of *P. sedoides*, fertilizer did not change the amount of sexually reproductive biomass produced. This result is contrary to much of the literature

(Biere 1995, Cheplick 1995, 2001, Thompson and Eckert 2004, Jongejans et al. 2006, Liu et al. 2009a). Sexual reproduction in *P. sedoides* is dependent on plant size and less on environmental factors such as nutrient availability. During the first set of experiments, genotype influenced fruit biomass, which reinforced the idea that fruit production has low plasticity and is determined more by intrinsic properties of the plant such as its size and genetic make-up than extrinsic environmental factors, meaning that sexual reproduction in *P. sedoides* has less plasticity than clonal growth.

Apart from height, the only measurements of *P. sedoides* to show genetic influence were related to sexual and clonal reproduction, including first year fruit mass and ramet number, and second year ramet mass and (marginally) stolon mass. These traits also tended to be less influenced by fertilizer treatment. This is surprising because life-history traits (such as reproduction) generally have lower heritabilities than morphological traits, such as mass of vegetative structures (Falconer 1981). One explanation for this low heritability is that life-history traits are closely tied to fitness and therefore under stronger selective pressure. The best alleles for the trait are more likely to be fixed while the deleterious alleles are removed, decreasing overall genetic variation in reproductive traits (Lynch and Walsh 1998). Life history traits are also more likely to experience high environmental variation because they depend on the variance of the traits that make them up (Lynch and Walsh 1998). Reviews of both the animal (Mousseau and Roff 1987) and plant (Geber and Griffen 2003) literature support the hypothesis that heritabilities are lower for life history traits. The discrepancies between these findings and mine may be due to their consideration of narrow sense heritability (additive genetic variation) while my study considers only broad sense heritability, which confounds

additive genetic variance with other effects, such as dominance and maternal effects (Falconer 1981).

Between species comparisons

For both *Lythrum salicaria* and the first generation of *Penthorum sedoides*, genotype consistently influenced height and post-treatment height increased with fertilizer treatment. However, unlike *P. sedoides*, main stem mass of *L. salicaria* was influenced by genotype. This implies that although genotype may dictate plant height in *P. sedoides*, it has little control over the amount of tissue produced on the main stem. In contrast, main stem mass of *L. salicaria* varied by genotype as was observed for height. This indicates that traits such as stem thickness and thickness and number of branches vary less with changes in the environment, or that main stem mass is more closely correlated with height in *L. salicaria* than *P. sedoides*.

Interactions between environmental variables and genotype are commonly reported and imply that the phenotypic plasticity of a species has a genetic component (Cheplick 1995, Ronsheim and Bever 2000, Pigliucci 2005). This was not the case for either *P. sedoides* or *L. salicaria* in this experiment. While these two species are highly plastic in their responses to fertilizer treatment, there appears to be no genetic component to this plasticity (Ronsheim and Bever 2000). Plants showed a similar response to the addition of nutrients regardless of their genotype or the identity of their maternal parent.

The variation in clonal structures due to genetics and treatment differed between the two study species in the first year of the experiment. For both *P. sedoides* and *L. salicaria*, ramet mass increased with fertilizer treatment but genetic variation in ramet mass was not significantly different from zero. Mass of root buds, *L. salicaria*'s

mechanism for clonal growth, was unaffected by genotype or fertilizer addition, while in the first generation of *P. sedoides*, stolon mass increased with fertilizer treatment. Another difference between the two species is that the fertilizer treatment did not affect ramet number in *Lythrum salicaria* while control and fertilized plants *P. sedoides* differed from each other. These differences relate to the nature of the two species' clonal growth. *Penthorum sedoides* has very extensive clonal growth, both in the amount of stolons and ramets produced and in how the ramets spread out spatially (personal observation). Clonal growth in *L. salicaria* is more limited. Root buds are much smaller than the stolons of *P. sedoides* and *L. salicaria* produces fewer of them. The growth form of *L. salicaria* is therefore more compact with fewer ramets (Mal et al. 1992). This growth form means that the ramets and root buds of individual genets are exposed to less environmental variation than the more wide-spread ramets and stolons of *P. sedoides*, which may explain the lack of plasticity in the ramet number and root bud mass of *Lythrum salicaria.* Environmental responsiveness of spacer size is more likely to be adaptive in *P. sedoides* since it's guerilla tendencies require flexibility to place ramets in the best microhabitats while ramet placement is more limited in the phalanx-like *L. salicaria*. It is also possible that root buds are too small to detect size variation in this trait.

Previous results are mixed about whether there is higher plasticity in wide-spread, "guerrilla"-like plants or compact, "phalanx"–like plants (as defined by Lovett Doust, 1981). For example, Schmid (1985) found higher plasticity in a phalanx species compared to a guerilla species and argues that unlike guerilla species, phalanx species require morphological plasticity because they cannot escape environmental change via

long spacers. However, Schmid (1985) did not specifically consider ramet or spacer mass. He et al.(2007) found more plasticity in ramet number of a phalanx species (*Stipa capitacea*) than a guerilla (*Carex monti-everestii*), although this was not true for other traits measured; however, spacer size between the two species was not compared. Similar to my results, Schmid and Bazzaz (1992) found less plasticity in clonal architecture (rhizome number and length) of a phalanx species (S*olidago canadensis*) and species intermediate in growth form (S*olidago altissima* and *Solidago gigantea*) relative to a guerilla species (*Aster lanceolatus*), which the authors attribute to the phalanx and intermediate species' more compact growth form. Since none of these studies investigated the genetic aspects of spacer size in relationship to phalanx and guerrilla growth strategies, the present research fills this gap and suggests that differences in clonal trait plasticity between phalanx and guerilla species is not due to varying levels of genotypic influence, but rather to differential responsiveness to environmental conditions.

Conclusions

Overall, in both species and both years for *P. sedoides*, variation due to genetic components was limited and no interactions between genotype and the fertilizer treatment were found. Traits that were influenced by genetic variation were restricted to height and clonal and sexual reproductive characteristics. This unexpected result may be due to a variety of factors. The two species studied may exhibit a low level of genetic control over their traits while being greatly affected by environmental conditions. It may also be that genetic effects were undetectable due to high variability in environmental conditions and their influence on phenotype of both species. Often, studies looking for genetic differences in traits use plants grown in greenhouses in very controlled environments,

which may overemphasize the importance of the genetic components on phenotype. On the other hand, almost all traits, with the notable exception of fruit mass of *P. sedoides*, increased under high nutrient treatment. The plastic response shown by these plants may assist in acclimatizing to variable environmental conditions. There were no interactions between genotype and fertilizer treatment, which was also contrary to many other studies, meaning all plants, regardless of genetic background, react in a similar way to environmental variation. Clonal growth characteristics responded more to environmental conditions than did sexual reproductive traits. However, whether clonal growth or sexual reproduction depended on genetic factors varied by the year. In the first year, fruit mass showed a genetic effect while clonal growth did not, but in the second generation, the opposite was true—variation in clonal reproduction had a genetic component while variation in sexual reproduction did not. Clonal characteristics, except ramet mass, did not exhibit environmental variation in the phalanx species *Lythrum salicaria*, but variation was also not significantly explained by genotypic differences. On the other hand, the guerilla species *Penthorum sedoides* exhibited fertilizer induced changes in its stolon and ramet mass, and in the second year, genotype was a significant source of variation.

CHAPTER V

SIZE-DEPENDENT ANALYSIS OF ALLOCATION TO SEXUAL AND CLONAL REPRODUCTION IN *PENTHORUM SEDOIDES* UNDER CONTRASTING NUTRIENT LEVELS.

Abstract

Reproductive output and reproductive allocation are important factors in the life history of any organism. In clonal plants, however, "reproductive" can refer to both sexual and asexual (clonal) replication. When investigating reproductive allocation, it is essential that the size of plants studied is taken into account and that the direct analysis of ratios (e.g. fruit mass/vegetative mass) is avoided for statistical reasons. The methods described by Klinkhamer et al. (1992) for investigating reproductive resource allocation are the most inclusive and versatile way of investigating allocation and its relationship to plant size. I investigated allocation of resources to both sexual (fruit mass) and clonal (stolon mass, ramet mass and ramet number) reproduction in *Penthorum sedoides* under two nutrient treatments; fertilized and control. Allocation to sexual reproduction was predicted to be higher in the control treatments while allocation to clonal reproduction

should be higher in the nutrient enriched treatment in an effort to produce the most fit offspring in a given environment. In many cases, such as stolon mass, ramet number, fruit mass in the second year and ramet mass in the first year, the relationship between size and allocation was negative, contrary to predictions, and results indicated that allocation to both sexual and clonal reproduction decreased as plants became larger. However, the hypothesis that allocation to clonal growth would increase in high nutrient environments was supported by the results on stolon and ramet mass, but not ramet number. The hypothesis that sexual allocation would increase in resource poor environments was only supported in the second year of the experiments.

Introduction

Since reproduction is an essential aspect of the life history of any organism, it is important to understand how individuals allocate resources to reproduction. The study of reproductive allocation (RA) is more complicated in clonal plants since they have two modes of reproduction, sexual and asexual (clonal growth) (Jackson et al. 1985). Plants in different environmental conditions are predicted to alter resource allocation between the two modes of reproduction in a way that will increase their genetic representation in subsequent years (Gardner and Mangel 1999). It has been hypothesized that sexual reproduction should increase in crowded or resource poor environments to allow escape for offspring and to generate new and potentially more fit genotypes (Nishitani et al. 1999, van Kleunen et al. 2002). In nutrient rich environment, clonal reproduction should be dominant to ensure offspring establishment in an environment conducive to growth

and to allow the genotype to retain a foothold in the beneficial environment (Williams 1975, Silander 1985, Gardner and Mangel 1999, van Kleunen et al. 2002).

Although reproductive allocation has commonly been studied in the past by comparing proportions (such as fruit biomass divided by total mass) between two populations or treatments using ANOVA (Weiner 2004), this method does not take into account that plant growth is allometric—some changes in allocation may simply be due to the nature of plant growth and development and not the plant's attempt to maximize success of reproduction and offspring fitness (Samson and Werk 1986, Weiner et al. 2009). If plants of different sizes vary in resource allocation, factors that influence plant size will also indirectly affect allocation patterns (Samson and Werk 1986, Weiner 2004).

The absolute amount of sexual reproductive biomass (e.g. total fruit or seed mass) produced by a plant is commonly called reproductive output (RO) (Bazzaz et al. 2000). Plant size and RO have been shown in most cases to be positively correlated (Klinkhamer et al. 1990, Aarssen and Taylor 1992, Mendez and Obeso 1993, Cain and Damman 1997, Sletvold 2002, Weiner 2004, Hawkins et al. 2005, Niu et al. 2009). RO increases with plant size because the total amount of resources and the number of meristems available for reproduction increases for larger plants (Clauss and Aarssen 1994, Weppler and Stocklin 2005). Clonal output (e.g. mass of stolons and ramets) is less frequently studied than sexual RO, but it also tends to increase with size and for similar reasons (Mendez and Obeso 1993, Schmid et al. 1995, Verburg et al. 1996, Brown and Eckert 2005, Hawkins et al. 2005, Wang et al. 2008).

While RO describes the absolute amount of seeds or fruits produced, sexual reproductive allocation (RA) describes the proportion of resources that are expended to

produce those seeds and fruits (Bazzaz et al. 2000). Since RA is an important trait in a plant's lifecycle, it has received a great deal of theoretical and experimental attention (Klinkhamer et al. 1992, Zhang and Jiang 2002, Niklas and Enquist 2003, Cheplick 2005, Wang et al. 2006, Niu et al. 2009), especially with regard to how to analyze the relationship between RA and plant size due to potential statistical problems. For example, a commonly used method for analyzing the size dependent relationship of RA is to perform a linear regression of RA (usually described as fruit mass/total mass or fruit mass/vegetative mass) on total mass or vegetative mass or determine the correlation coefficient between RA and total plant mass or vegetative plant mass (Klinkhamer et al. 1990, Mendez and Obeso 1993, Cheplick 2005). A problem arises because reproductive allocation and plant size are not independent; some measure of plant size is used as the denominator in the calculation of RA (Klinkhamer et al. 1990, Cheplick 2005), thereby violating an important assumption of linear regression and other methods of line fitting (Sokal and Rolf 1995, Cheplick 2005) and leading to spurious correlations between RA and plant size (Samson and Werk 1986, Klinkhamer et al. 1990). Similar methods have also been applied to clonal allocation studies, with similar risks of false correlation if the proportion of biomass to clonal growth is regressed on total biomass (Koivunen et al. 2004).

To solve these statistical problems, the best way to study allocation of resources to reproduction is to analyze allocation patterns (the relationship between reproduction and vegetative size) rather than using allocation ratios (Samson and Werk 1986, Klinkhamer et al. 1990, Klinkhamer et al. 1992, Weiner et al. 2009). Samson and Werk (1986) proposed the "graphical size-regression" approach, which looks at the relationship

between RA and plant size through linear regression of absolute sexual reproduction (R) on vegetative plant size (V). The coefficients derived from this regression are used to describe the relationship between RA and plant size (Samson and Werk 1986). However, the Samson and Werk (1986) model assumes that the relationship between R and V is linear, which is not always the case. It is possible that sexual reproductive mass may change disproportionately with vegetative size, giving rise to a non-linear relationship between reproductive output and plant size (Klinkhamer et al. 1990, Weiner et al. 2009). In these cases, the Samson and Werk (1986) model would not adequately describe the relationship (Klinkhamer et al. 1990, Klinkhamer et al. 1992). Klinkhamer et al. (1990) proposed a model to encompass potential nonlinearity, and later a general model that allows for the testing of both a minimum size for reproduction and a non-linear relationship between sexual reproductive mass and vegetative mass (Klinkhamer et al. 1990, Klinkhamer et al. 1992).

The relationship between size and sexual reproductive allocation (RA) has been analyzed using the methods of Samson and Werk (1986), and to a lesser extent, Klinkhamer et al. (1990, 1992), but few have used these approaches to investigate clonal reproductive allocation (Dong and Pierdominici 1995, Verburg and Grava 1998, van Zandt et al. 2003, Brown and Eckert 2005), and none has so far utilized the methods of Klinkhamer et al. (1992) to investigate potential non-linearity in the relationship between clonal mass and plant size. In this study, I examine sexual and clonal reproductive allocation patterns of *Penthorum sedoides* in response to nutrient conditions over two years. I use the methods of Klinkhamer et al. (1992) to test the importance of non-linearity in the reproduction-size relationship and the presence of a minimum size for

reproduction (positive x-intercept). The most common results of analysis using these methods is that RO increases with size while RA remains unchanged (reviewed by Cheplick, 2005 and Weiner et al., 2009), but I also predict that the allocation-size relationship will differ between the fertilizer and control group and that the fertilized group will allocate more biomass to clonal reproduction while the control group will allocate more biomass to sexual reproduction.

Methods

First generation

Seeds of *Penthorum sedoides* obtained from Ernst Conservation Seeds (Meadville, PA) were germinated on moist filter paper in Petri dishes in growth chambers on 24 January 2004. Seedlings were transplanted to small pots under grow lamps (16:8 light/dark cycle) on 23 February. Throughout the experiment, I watered the plants every other day as needed. Because these plants were also included in an experiment investigating genetic and environmental influences on phenotypic variation, I made six clones of each of 30 randomly chosen plants, assigning half of the clones to the treatment group while the other half served as controls (see methods of Chapter IV for details). My analyses of the results comparing clones, as described in Chapter IV, demonstrated little or no genotypic effect on traits in this species, so lack of independence between subjects is unlikely to distort the present analyses. Two weeks after cloning was completed (early July), I moved the plants to the outdoor ecological research area on the Cleveland State University campus (Cleveland OH) where they were transplanted into the larger pots. For the fertilizer group, I added commercial fertilizer (Miracle-Gro®) to

the pots following the manufacturer's instructions. The control group received water without fertilizer added. Treatments were applied four times at two week intervals. Plants were grown until the middle of October. Since this is close to the end of the growing season, all of the plants had produced fruits and very few were producing new flowers. When harvesting the plants, I counted the ramets and divided the plants into stem, leaves, stolons, ramets and fruits and placed them into individual paper bags. I then dried the harvested tissue, except the fruits, in an oven at 60 degrees C for at least 48 hours. These parts were weighed to the nearest tenth of a gram.

Second generation

In 2006, eight genotypes of *P. sedoides* from the 2004 experiment were randomly chosen to be the maternal parents of the second generation of plants. Seeds were taken from plants raised in the high nutrient treatment. I germinated the seeds on 15 December 2006 and grew the plants in a manner similar to the first generation When the plants were large enough, three young plants from each of the eight parents were cloned as described for the first generation experiment. Six clones were generated from each of the young plants (genotypes) with three being randomly assigned to the added nutrient treatment and three untreated. After the clones were established, they were transplanted to large pots in the outdoor experimental garden on the Cleveland State University campus in early July, 2007. Plants assigned to the fertilizer group were treated using commercial fertilizer following the company's instructions. Treatments were applied three times at 2 week intervals. Plants designated as control received only water at all times. Plants were again harvested at the end of the growing season (late September) when almost all plants had completed sexual reproduction. During harvest, I counted

ramets of each plant and divided the plants into main stem, fruits, ramets, stolons and roots and each of these parts were placed in separate paper bags and, except for the fruits, were dried in an oven at 60°C for at least 48 hr. These parts were weighed to the nearest tenth of a gram.

Statistical Analysis

Klinkhamer et al. (1992) devised a sequential approach to describe the relationship between RO and vegetative mass that allows for testing of non-linearity and an x-intercept (minimum size of reproduction) in the RO-size relationship. They contrast four models describing the relationship between reproductive biomass and vegetative biomass, the first three of which were already established. Model 0, where the equation is R=aV+E and R is the reproductive biomass, V is vegetative biomass, *a* is the slope of the regression and E is the error, predicts a linear relationship between R and V with no minimum size to initiate reproduction. Model 1 also describes a linear relationship, but it includes a minimum size requirement before a plant can reproduce— R=a(V-b) +E where *b* is the x-intercept. Klinkhamer et al. (1992) argue that for Model 1, *b* must be greater than zero since a negative value of *b* would imply that plants without vegetative tissue are capable of reproduction, which is unrealistic. Model 2 describes a nonlinear relationship between R and V with no minimum size for reproduction and is expressed as $R=aV^c$, with *c* being the allometric coefficient that indicates the degree of non-linearity. To explain relationships between R and V that are both nonlinear and have a minimum size for reproduction, Klinkhamer et al. (1992) developed Model 3—$R=a(V-b)^c$—which is an extension of models 2 and 3 and since it has parameters for both the X-intercept (*b*)

and the degree of nonlinearity (*c*) it can be used to test for both minimum size of reproduction and non-linearity of the relationship.

Each of the four models describing the relationship between sexual reproductive biomass and vegetative biomass have implications for the relationship between reproductive allocation and vegetative mass (Klinkhamer et al. 1992). For Model 0, in which there is no minimum size for reproduction and the relationship is linear, reproductive allocation does not change with plant size and mean RA is equal to *a*. In Model 1, RA increases with plant size above the minimum size threshold (assuming *b* is greater than 0), eventually approaching an asymptote. In situations where this is the case, RA increases with plant size at smaller sizes but remains relatively constant for larger plants. If the relationship can be described by Model 2, reproductive allocation increases with size if *c* is greater than 1 and decreases with size if *c* is less than 1.

In Model 3, the implications for the relationship between reproductive allocation and vegetative size are more complex. In cases where $b=0$ and $c=1$, no change in RA occurs with plant size (equivalent to Model 0). When $c=0$ and $b>0$ (model 1), RA increases with plant size (Klinkhamer et al. 1992). When $b=0$, and $c<1$, RA decreases with plant size and $c>1$, RA increases with plant size. If $b>0$ (increase in plant size) and where $c<1$ (decrease in plant size), this combination will give a humped relationship between RA and plant size.

Klinkhamer et al. (1992) suggest two mutually exclusive pathways to analyze the relationship between reproductive allocation and plant size, taking into account the possibility of both a minimum size for reproduction and a non-linear relationship between the two variables. The first route is to test first a minimum size for reproduction by

comparing Model 0 and Model 1 to determine if b=0. The non-linearity of the relationship can then be tested by comparing Model 1 and Model 3 to determine if c=1. The second route first tests for nonlinearity by comparing Models 0 and 2 to test if c=1, followed by a comparison between Models 2 and 3 to test if b=0, meaning the x-intercept does not differ significantly from zero. Klinkhamer et al. (1992) suggest the second path be utilized if estimates for b using the first path are unrealistic (i.e. less that zero).

Using the NLIN procedure in SAS (SAS Institute Inc 2010), I fit the four models described by Klinkhamer et al. (1992) to my data for fruit mass, stolon mass, ramet number and ramet mass, and then used the likelihood ratio test to determine which model best fit the data. The likelihood ratio (Λ) was estimated as $\Lambda = n*\log(SSE_{H0}/SSE_{H1})$ and follows a χ^2 distribution with 1 df (Niu et al. 2009).

Since b (x intercept) was frequently negative in my analyses using Model 1, which is biologically unrealistic, I first compared Model 2 to Model 0 and then Model 3 to Model 2 (Klinkhamer et al. 1992). I fit the models with fruit mass, stolon mass, ramet number and ramet mass as the dependent variable. Vegetative mass (total mass minus fruit mass; V), non-stolon mass (total mass minus stolon mass: NS) and non-ramet mass (total mass minus ramet mass: NR), respectively, were the independent variables. These independent variable are preferable over total biomass because they exclude the biomass value of the dependent variable thereby preventing artificial autocorrelation (Samson and Werk 1986). I ran the analyses with both treatments together and with the treatments considered separately to determine whether fertilizer addition alters the allometric patterns between reproduction and plant size. If results for the control and fertilizer groups were best described by the same model, the parameter values were compared

using 95% confidence intervals generated by the NLIN procedure (Sugiyama and Bazzaz 1998). If the value of *a* differed significantly between the treatments, fertilizer addition showed differences in the amount of resources allocated to reproduction. In addition to performing this analysis on fruit mass and vegetative mass to determine the relationship between RA and size, I also used these methods to examine the relationship between plant size and allocation to stolons (SA), ramet mass (RMA) and ramet number (RNA).

Transformation of the data was not done because this makes interpreting the relationships more difficult (Samson and Werk 1986). Klinkhamer et al. (1992) argue that the likelihood ratio test should be insensitive to deviations from normality that may arise when using untransformed data. In some cases, Model 2 was a significant improvement over Model 1, but Model 3 failed to converge when using NLIN in SAS. When this occurred, Model 2 was assumed to be the best model (SAS Institute Inc. 2010).

I produced figures representing the relationship between fruit mass and vegetative mass by plotting the results and drawing a line using the equation of the appropriate model and the parameters values for *a*, *b* and *c* estimated by the NLIN procedure. Graphs demonstrating the relationship between RA and vegetative mass were generated by calculating RA (fruit mass/vegetative mass) for each plant and plotting it against vegetative mass. To produce a line representing the relationship between RA and vegetative mass, I solved the model equation for RA (R/V) and used that equation with the parameter values for *a*, *b* and *c* estimated by the NLIN procedure. The RA equation for Model 0 was RA=*a* and for Model 2 it was $RA=a*V^{c-1}$ (These two models were the only ones required as *b* was always either negative or not different from zero). If the two

treatments did not differ in model or values of *a* and *c*, only the line representing all plants was drawn. Otherwise, two lines were drawn using the parameters and equations for each treatment. The same procedure was followed for stolon mass and non-stolon mass, for ramet number and non-ramet mass and for ramet mass and non-ramet mass. Graphics were generated in the statistics program R (R Development Core Team 2010).

Results

Summer 2004

Fruit mass

Model 0 best described the relationship between fruit mass and vegetative mass in the first year. RO increased linearly with size and RA remained constant (Figure 24). The likelihood ratios for the fruit data were Λ_{model2}=0.8, P>0.05 for all plants; Λ_{model2}=0, P>0.05 for the control; and Λ_{model2}=0.9, P>0.05 for the fertilized treatment (Table XVIII). The estimates of *a* (the slope of the regression line) were 0.14 ± 0.012 for total, 0.13 ± 0.014 for the control plants and 0.15 ± 0.017 for the fertilized plants (Table XIX). Estimates of the parameter *a* did not differ significantly between treatments.

Stolon mass

Model 2 gave the best fit for the relationship between stolon mass and non-stolon mass, and both stolon output and SA decreased with plant size (Table XVIII; Figure 25; Λ_{model2}=52.36, P<0.0001 and Λ_{model3}=0.33, P>0.05 for total; Λ_{model2}=57.37, P<0.0001

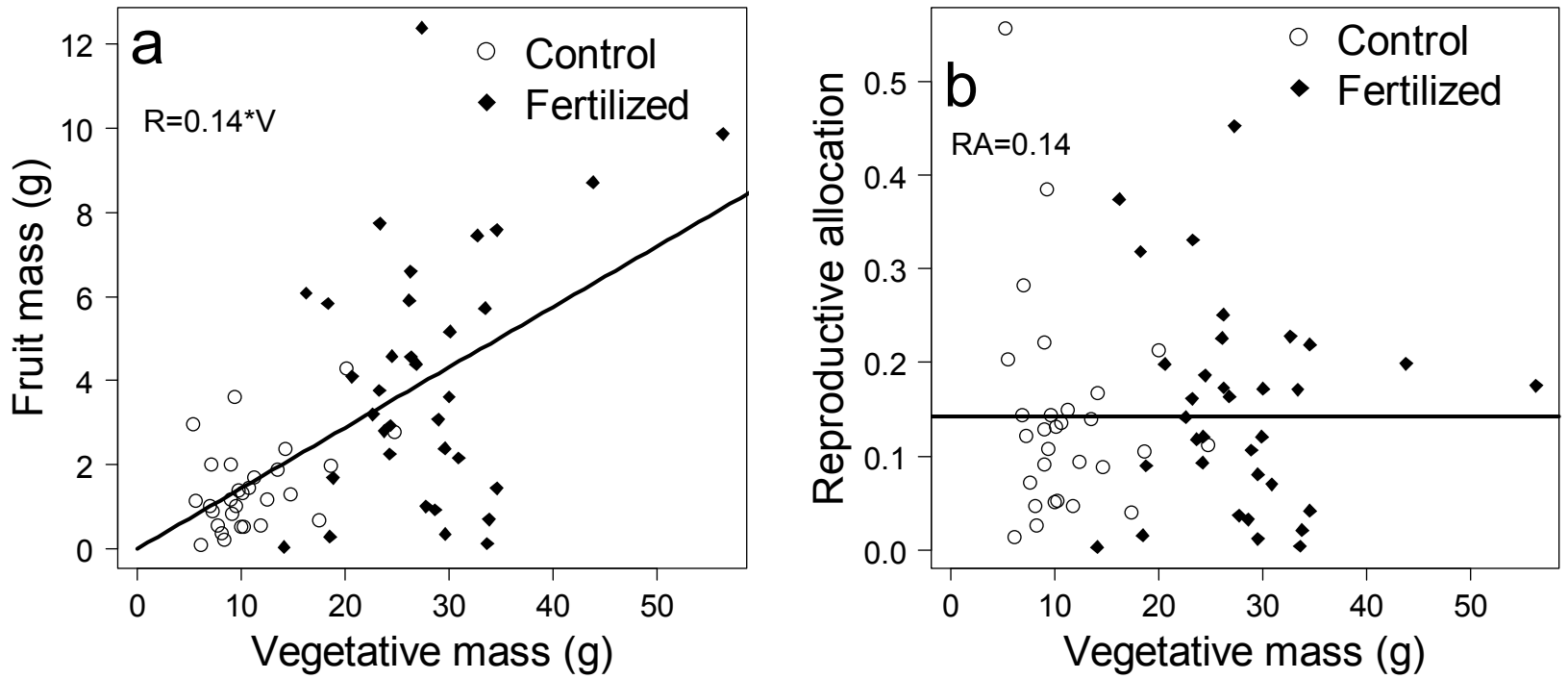

Figure 24. The relationship between vegetative mass (V) and a. fruit mass (R) and b. reproductive allocation (RA) in 2004. Open circles indicate control plants while filled diamonds represent fertilized plants. In Figure a the line describes the relationship between fruit mass and vegetative mass given by the equation $R=a*(V-b)^c$ using parameter values for *a*, *b*, and *c* that best fit the data. In Figure b, the parameters were inserted into the equation $RA=a*(V)^{c-1}$ to produce the line showing the relationship between RA and vegetative mass. The parameters values of the two treatments did not differ significantly from each other.

Table XVIII. Likelihood ratio test results comparing models describing the relationship between reproduction and plant size for the 2004 data. The residual sums of squares (SSE) for each of the three models are given. The likelihood ratios (Λ) comparing Model 0 and 2 and Model 2 and 3 are given, as are the P values for these comparisons. The symbol "--" in Model 3 columns means Model 2 was not found to give a sufficiently better fit then Model 1 and the test comparing Model 2 and Model 3 was therefore not performed. When convergence criteria were not met for Model 3, the symbol "nc" (not converged) appears.

Variable	n	SSE Model 0	SSE Model 2	SSE Model 3	Model 0 vs. Model 2		Model 2 vs. Model 3	
					λ	P	λ	P
Fruit								
Total	69	347.1	345.2	--	0.8	0.37	--	--
Fertilized	38	209.1	204.2	--	0.9	0.34	--	--
Control	31	137.3	137.3	--	0	1	--	--
Stolon mass								
Total	85	11791.5	6368.3	6310.9	52.36	<0.0001	0.77	0.38
Fertilized	47	10092.2	2978.36	nc	57.37	<0.0001	--	--
Control	38	1143	684.13	642.1	19.5	<0.0001	--	--
Ramet no.								
Total	85	421.4	268.8	268.8	38.21	<0.0001	0	1
Fertilized	47	246.85	130.99	nc	29.78	<0.0001	--	--
Control	37	156.025	112.71	nc	12.03	0.0005	--	--
Ramet mass								
Total	85	553.4	504.2	nc	7.91	0.0049	--	--
Fertilized	44	474.4	296.2	nc	20.72	<0.0001	--	--
Control	38	71.18	61.23	nc	6.02	0.014	--	--

Table XIX. Estimates for the parameters *a* and *c* ± standard error for the model $Y=a(X-b)^c$ for results from 2004. Values of *a* and *c* were compared using the 95% confidence intervals calculated by the NLIN procedure. Parameter *b* did not differ from 0 for any of the traits. If *c* did not differ significantly from one (i.e. Model 0), "--" appears in the *c* column.

Variable	Treatment	Model	*a*	*c*	Comparison of treatments
Fruit mass	Total	0	0.14 ± 0.012	--	
	Fertilizer	0	0.15 ± 0.017	--	
	Control	0	0.13 ± 0.014	--	Same model, parameters do not differ
Stolon mass	Total	2	10.49 ± 1.71	0.167 ± 0.091	
	Fertilizer	2	40.27 ± 9.93	-0.41 ± 0.14	
	Control	2	8.51 ± 1.34	-0.024 ± 0.16	Same model, *a* and *c* differ
Ramet No.	Total	2	1.48 ± 0.35	0.40 ± 0.08	
	Fertilizer	2	3.17 ± 1.33	0.18 ± 0.13	
	Control	2	2.02 ± 0.8	0.21 ± 0.17	Same model, parameters do not differ
Ramet mass	Total	2	0.84 ± 0.54	0.35 ± 0.22	
	Fertilizer	2	49.98 ± 37.04	-0.86 ± 0.30	
	Control	2	1.01 ± 0.69	-0.31 ± 0.31	Same model, *a* and *c* differ

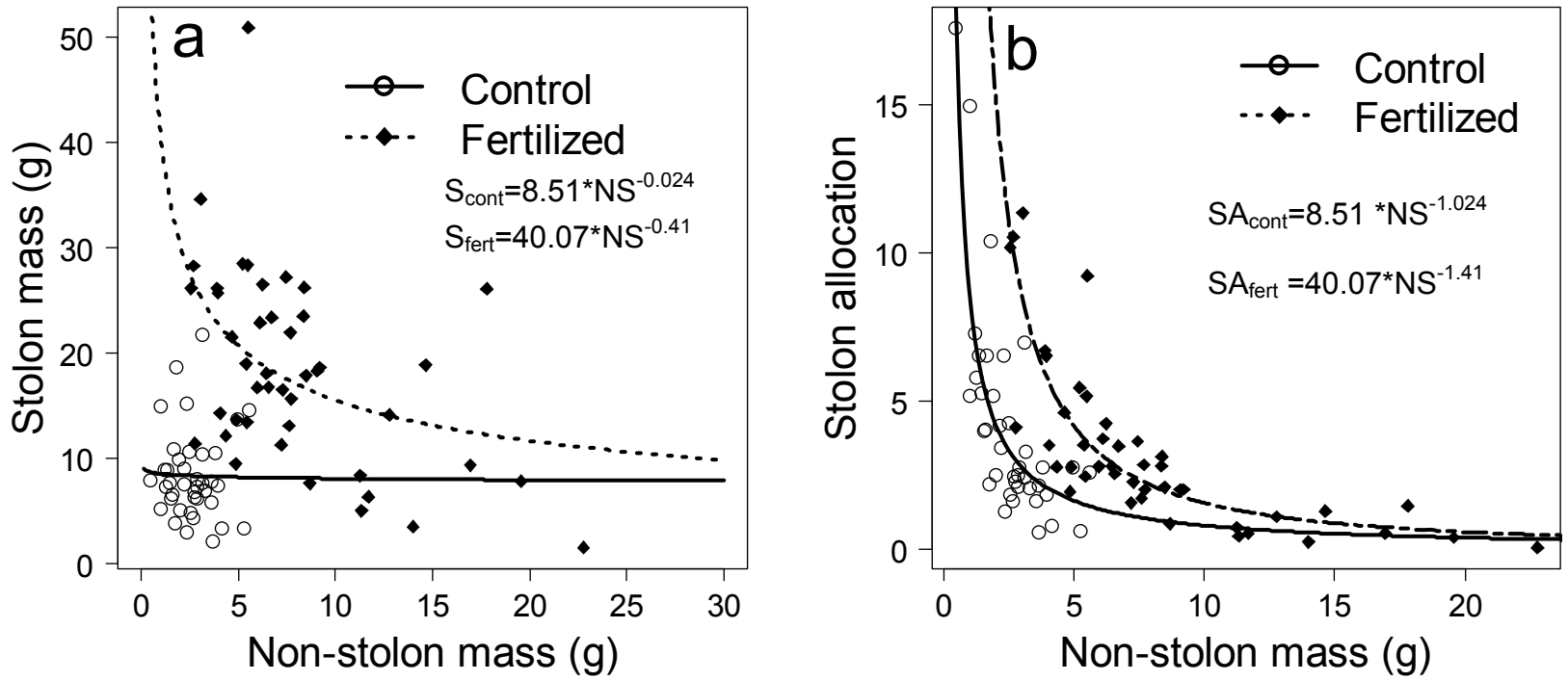

Figure 25. Effect of fertilizer treatment on the relationship between non-stolon mass (NS) and a. stolon mass (S) and b. stolon mass allocation (SA) in 2004. Open circles indicate control plants while filled diamonds represent fertilized plants. In Figure a the lines (solid for control and dotted for fertilizer) describe the relationship between stolon and non-stolon mass given by the equation $S=a*(NS-b)^c$ using parameter values for *a*, *b*, and *c* that best fit the data. In Figure b, the parameters were inserted into the equation $SA=a*(NS)^{c-1}$ to produce the line showing the relationship between stolon allocation and non-stolon mass.

and Model 3 failed to converge (FTC) for fertilized plants; and Λ_{model2}=19.5, P=0.0006 and Λ_{model3}=2.41, P>0.05 for control plants). The parameter estimates for total were *a*= 10.49 ± 1.7 and *c*= 0.0.17 ± 0.091; and for fertilized plants, *a*= 40.27 ± 9.93 and *c*= -0.41 ± 0.14. For stolon mass of the control, two outliers had a large influence on the relationship between stolon mass and plant size. With outliers removed, *a*= 8.51 ± 1.12 and *c*= -0.024 ± 0.16. The parameter estimates of both *a* and *c* differed significantly between the fertilized and the control groups; *a* was significantly larger in the fertilized group and *c* was larger in the control (Table XIX).

Ramet Number

As with stolon mass, Model 2 best described the relationship between ramet number and non-ramet mass. Ramet number increased with non-ramet mass and RNA decreased (Table XVIII; Figure 26; for total—Λ_{model2}=38.21, P<0.0001 and Λ_{model3}=0, p>0.05; for fertilized plants— Λ_{model2}=29.78, P=0.001, Model 3 FTC; and for control plants—Λ_{model2}=12.03, P=0.0005 and Model 3 FTC). The parameter estimates for total were *a*=1.48 ± 0.35 and *c*= 0.401 ± 0.08; for Fertilizer, *a*= 3.17 ± 1.33 and *c*= 0.18 ± 0.13; and for control, *a*= 2.02 ± 0.8 and *c*= 0.21 ± 0.17 (Table XIX). The parameter estimates did not differ significantly between the fertilizer and the control groups.

Ramet Mass

Model 2 again provide the best fit for the relationship between ramet and non-ramet mass; both ramet mass and RMA decreased with NR (Figure 27). The likelihood

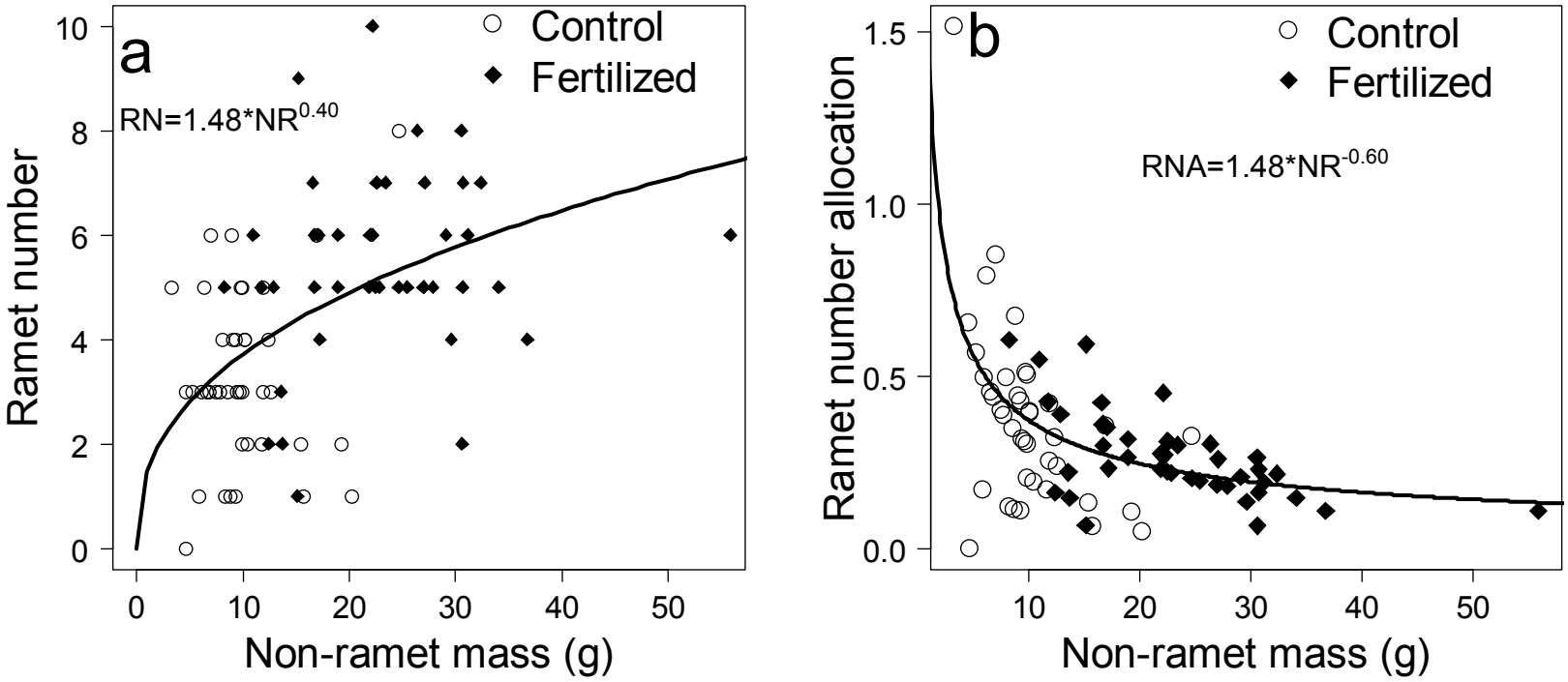

Figure 26. The relationship between non-ramet mass (NR) and a. ramet number (RN) and b. ramet number allocation (RNA) in 2004. Open circles indicate control plants while filled diamonds represent fertilized plants. In Figure a the line describes the relationship between ramet number and non-ramet mass given by the equation ramet $RN=a*(NR-b)^c$ using parameter values for *a, b*, and *c* that best fit the data. In Figure b, the parameters were inserted into the equation $RNA=a*(NR)^{c-1}$ to produce the line showing the relationship between ramet number allocation (RNA) and non-ramet mass. The two treatments did not differ significantly in parameter estimates**.**

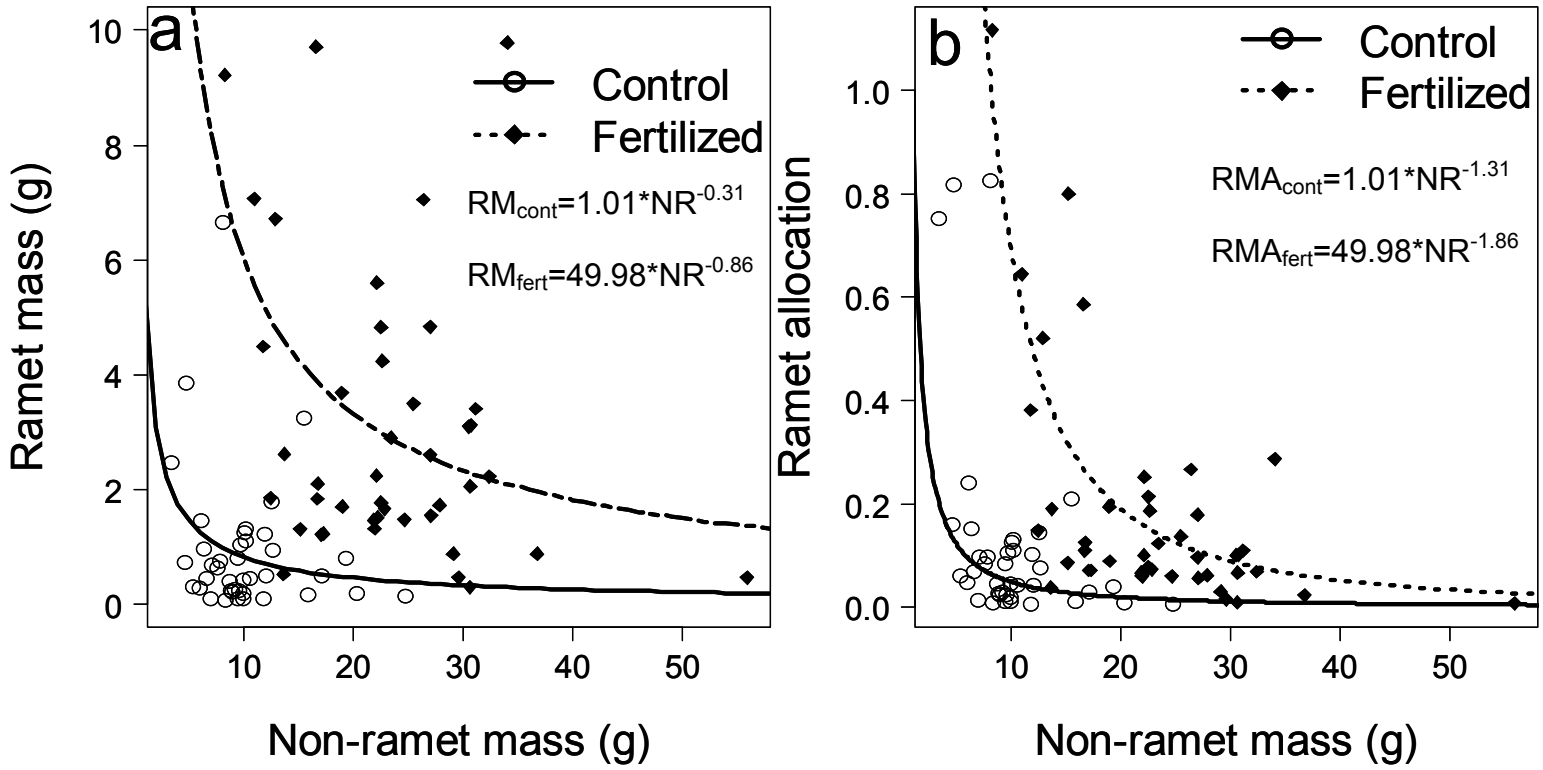

Figure 27. Effect of fertilizer treatment on the relationship between non-ramet mass (NR) and a. ramet mass (RM) and b. ramet mass allocation (RMA) in 2004. Open circles indicate control plants while filled diamonds represent fertilized plants. In Figure a the lines (solid for control and dotted for fertilizer) describe the relationship between ramet mass and non-ramet mass given by the equation $RM=a*(NR-b)^c$ using parameter values for *a*, *b*, and *c* that best fit the data. In Figure b, the parameters were inserted into the equation $RMA=a*(NR)^{c-1}$ to produce the line showing the relationship between ramet mass allocation and non-ramet mass.

ratios were Λ_{model2}=7.91, P=0.005 for all plants; Λ_{model2}=20.72, P<0.0001 for fertilized plants; and Λ_{model2}=6.02, P=0.01 for the control (Table XVIII). The parameter estimates for all plants were *a*=0.84 ± 0.54 and *c*=0.35 ± 0.22; for fertilizer only, *a*=49.98 ± 37.04 and *c*=-0.86 ± 0.3; and for control only, *a*=1.01 ± 0.69 and *c*=-0.0147 ± 0.31 (Table XIX). The parameters of the model differed significantly between the two treatments. As with stolon mass, *a* was higher in the fertilizer treatment while *c* was higher in the control.

Summer 2007

Fruit Mass

The model that fit the relationship between R and V differed between treatments in 2007 (Figure 28). For all plants, Model 2 best described the relationship and RO increased and RA decreased with vegetative mass (Table XX; Table XXI; Λ_{model2}=16.15, P<0.0001, Λ_{model3}=2.25, P>0.05; *a*=0.74 ± 0.33, *c*=0.50 ± 0.12). For the fertilized plants, Model 2 was also the best (Λ_{model2}=7.89, P=0.005, Model 3 FTC; *a*=2.14 ± 2.38, *c*=0.23 ± 0.28). However, for the control plants, Model 2 to was not a significant improvement on Model 0 and therefore RO increased linearly with size while RA remained constant (Λ_{model2}=1.06, P>0.05; *a*=0.15 ± 0.012), which also occurred in the 2004 results.

Because RA in the control was constant, it is possible to compare the means of the two treatments directly without risk of complications due to size dependence. When compared using the Wilcoxon two-sample test, RA for the fertilized plants was significantly less than RA for the control ($mean_{cont}$=0.14, $mean_{fert}$=0.11; W=1211, P=0.03).

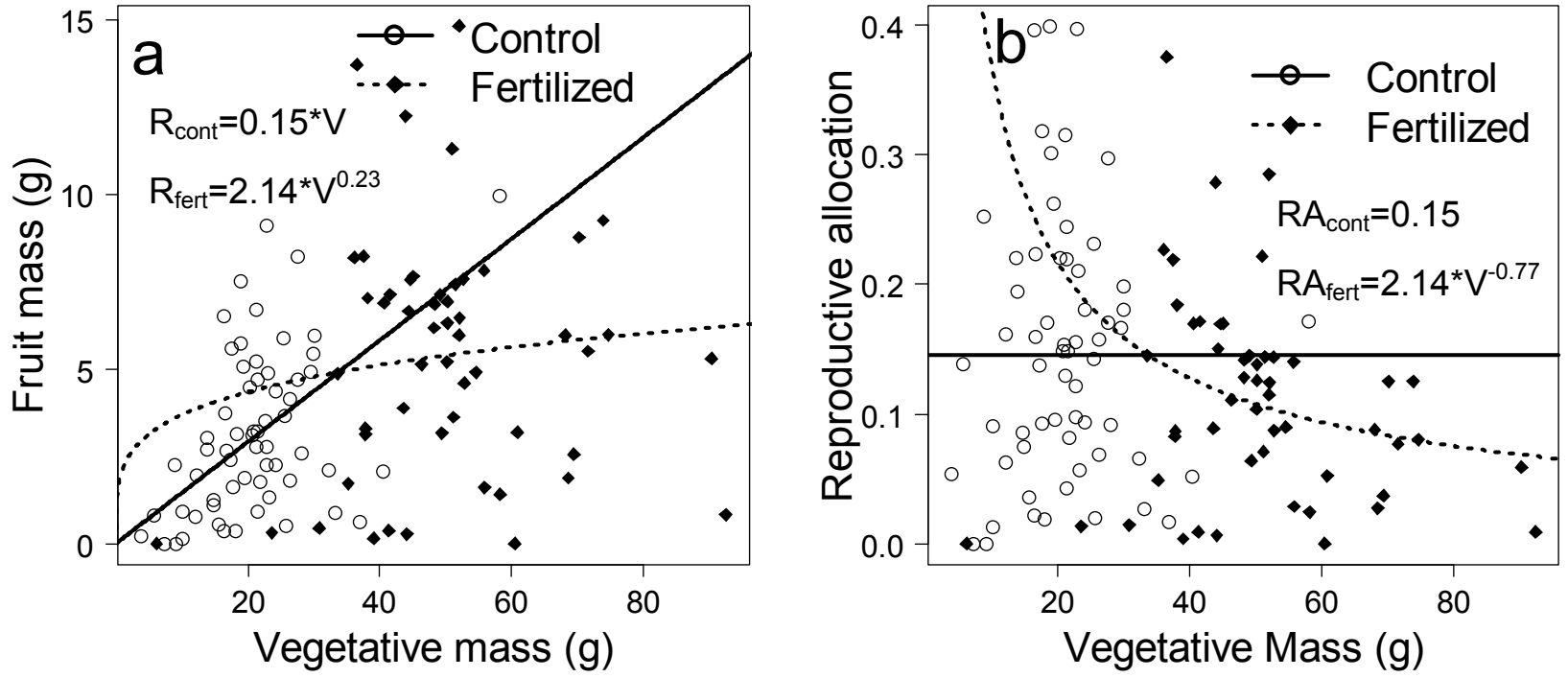

Figure 28. Effect of fertilizer treatment on the relationship between vegetative mass (V) and a. fruit mass (R) and b. reproductive allocation (RA) in 2007. Open circles indicate control plants while filled diamonds represent fertilized plants. In Figure a the lines (solid for control and dotted for fertilizer) describe the relationship between fruit mass and vegetative mass given by the equation $R=a*(V-b)^c$ using parameter values for *a*, *b*, and *c* that best fit the data. In Figure b, the parameters were inserted into the equation $RA=a*(V)^{c-1}$ to produce the lines showing the relationship between RA and vegetative mass.

Table XX. Likelihood ratio test results comparing models describing the relationship between reproduction and plant size for the 2007. The residual sums of squares (SSE) for each of the three models are given. The likelihood ratio (Λ) comparing Model 0 and 2 and Model 2 and 3 are given, as are the P values for these comparisons. The symbol "--" in Model 3 columns means Model 2 was not found to give a sufficiently better fit then Model 1 and the test comparing Model 2 and Model 3 was therefore not performed. When convergence criteria were not met for the model, the symbol "nc" (not converged) appears

Variable	n	SSE Model 1	SSE Model 2	SSE Model 3	Model 0 vs. Model2		Model 2 vs. Model 3	
					λ	P	λ	P
Fruit								
Total	113	1037.2	899.1	881.4	16.15	<0.0001	2.25	0.13
Fert	52	712.2	611.9	nc	7.89	0.005	--	--
Control	61	267.9	263.3	--	1.06	0.3	--	--
Stolon mass								
Total	113	7359.6	6954.5	6878	6.4	0.095	1.25	0.26
Fert	52	5396.1	3169.8	3092.6	27.4	<0.0001	1.28	0.26
Control	61	1269.6	1131.8	nc	7	0.008	--	--
Ramet no.								
Total	113	597.6	503.5	489.9	19.36	<0.0001	3.09	0.08
Fert	52	386.9	356.3	nc	4.28	0.04	--	--
Control	61	188.3	143.9	nc	16.4	<0.0001	--	--
Ramet mass								
Total	112	429.8	429	--	0.2	0.65	--	--
Fertilized	52	353	330.4	--	3.44	0.06	--	--
Control	61	53.35	47.08	46.14	7.63	0.006	1.23	0.27

Table XXI. Estimates for the parameters *a* and *c* ± standard error for the model $Y=a(X-b)^c$ for results from 2007. Values of *a* and *c* were compared using the 95% confidence intervals calculated by the NLIN procedure. Parameter *b* did not differ significantly from 0 from any of the traits. If *c* did not differ significantly from one (i.e. Model 0), "--" appears in the *c* column.

Variable	Treatment	Model	*a*	*c*	Comparison of treatments
Fruit mass	Total	2	0.74 ± 0.33	0.5 ± 0.12	
	Fertilizer	2	2.14 ± 2.38	0.23 ±. 28	
	Control	0	0.15 ± 0.012	--	Models differ
Stolon	Total	2	1.93 ± 0.46	0.82 ± 0.075	
	Fertilizer	2	8.33 ± 2.66	0.41 ± 0.10	
	Control	2	2.34 ± 0.77	0.64 ± 0.12	Same model, *a* and *c* differ
Ramet no.	Total	2	0.68 ± 0.28	0.47 ± 0.11	
	Fertilizer	2	0.91 ± 1.04	0.4 ± 0.28	
	Control	2	0.89 ± 0.49	0.37 ± 0.171	Same model, parameters do not differ
Ramet mass	Total	0	0.063 ± 0.0045	--	
	Fertilizer	0	0.069 ± 0.0068	--	
	Control	2	0.31 ± 0.26	0.38 ± 0.26	Models differ

Stolon Mass

Model 2 best fit the results for stolon mass in the second year of the experiment; stolon mass increased non-linearly with non-stolon mass (Tables XX and XXI; Figure 29; Λ_{Model2}=6.4, P=0.011; Λ_{Model3}=1.25, P>0.05, a=1.93 ± 0.46, c=0.82 ± 0.075 for total; Λ_{model2}= 7.0, P=0.081; a=8.33 ± 2.66, c=0.41 ± 0.10 for fertilized; and Λ_{model2}=27.4, P<0.0001 and Λ_{Model3}=1.28, P=0.26; a=2.34 ± 0.77, c=0.64 ± 0.12 for control). The value of *a* was significantly higher and *c* significantly smaller in the fertilized plants than the control.

Ramet Number

Model 2 also described the relationship between ramet number and non-ramet mass best, meaning that ramet number increased non-linearly with non-ramet mass while RNA decreased (Figure 30; Table XX; Table XXI; Λ_{model2}=19.36 , P<0.0001; Λ_{model3}=3.09, P>0.05; *a*=0.68 ± 0.28, *c*=0.47 ± 0.11 for total; and Λ_{model2}=16.4 P<0.0001, and Model 3 FTC; *a*=0.89 ± 0.49, *c*=0.37 ± 0.17 for the control; Λ_{model2}= 4.28, P=0.04; Model 3 FTC; *a*=0.91 ± 1.04 for fertilizer treatment. The parameters *a* and *c* did not differ between the treatment groups.

Ramet mass

The best model for the ramet mass results depended on treatment (Figure 31). For all plants together and the fertilized treatment alone, Model 0 adequately described the relationship, meaning that ramet mass increased linearly with non-ramet mass while

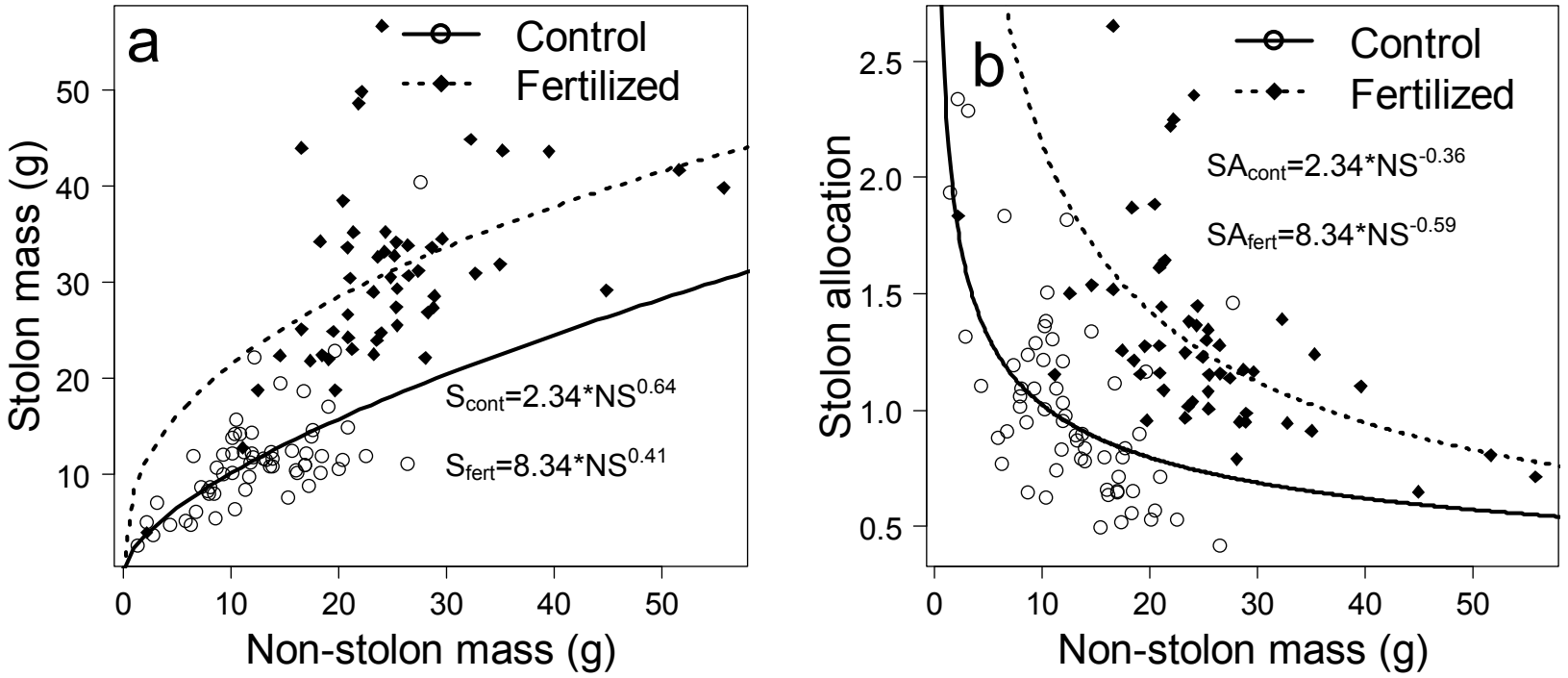

Figure 29. Effect of fertilizer treatment on the relationship between non-stolon mass (NS) and a. stolon mass (S) and b. stolon mass allocation (SMA) in 2007. Open circles indicate control plants while filled diamonds represent fertilized plants. In Figure a the lines (solid for control and dotted for fertilizer) describe the relationship between stolon and non-stolon mass given by the equation $S=a*(NS-b)^c$ using parameter values for a, b, and c that best fit the data. In Figure b, the parameters were inserted into the equation $SA=a*(NS)^{c-1}$ to produce the lines showing the relationship between stolon allocation and non-stolon mass.

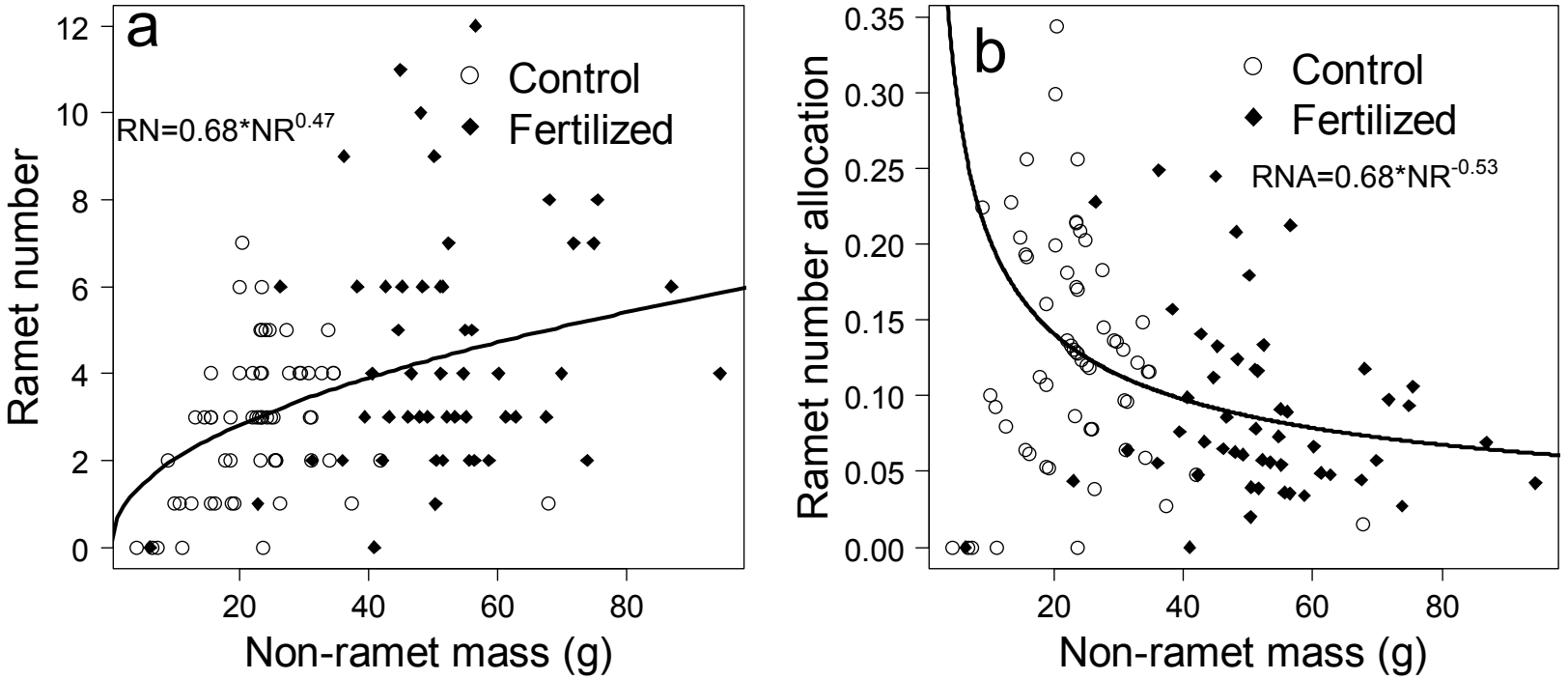

Figure 30. The relationship between non-ramet mass (NR) and a. ramet number (RN) and b. ramet number allocation (RNA) in 2007. Open circles indicate control plants while filled diamonds represent fertilized plants. In Figure a the line describes the relationship between ramet number and non-ramet mass given by the equation $RN=a*(NR-b)^c$ using parameter values for a, b, and c that best fit the data. In Figure b, the parameters were inserted into the equation $RNA=a*(NR)^{c-1}$ to produce the line showing the relationship between ramet number allocation and non-ramet mass. The two treatments did not differ in parameter estimates.

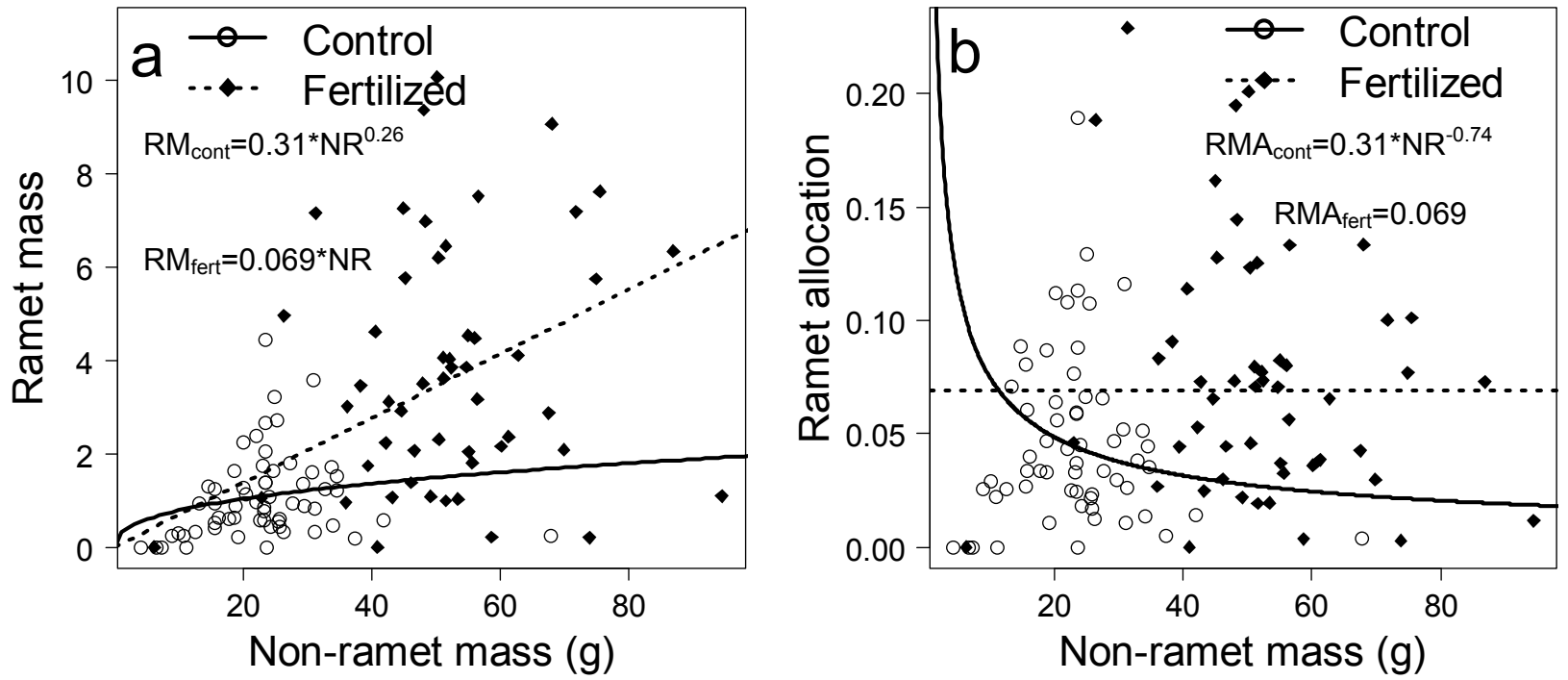

Figure 31. Effect of treatment on the relationship between non-ramet mass (NR) and a. ramet mass (RM) and b. ramet mass allocation (RMA) in 2007. Open circles indicate control plants while filled diamonds represent fertilized plants. In Figure a the lines (solid for control and dotted for fertilizer) describe the relationship between ramet mass and non-ramet mass given by the equation $RM=a*(NR-b)^c$ using parameter values for *a*, *b*, and *c* that best fit the data. In Figure b, the parameters were inserted into the equation $RMA=a*(NR)^{c-1}$ to produce the line showing the relationship between ramet mass allocation and non-ramet mass.

RMA remained constant (Table XX; Table XXI; Λ_{model2}=3.44. P=0.06, a=0.069 ± 0.007 for fertilized treatment; Λ_{model2}=0.2, P>0.05, a=0.063 ± 0.0045 for all plants), but for the control plants, Model 2 provided a significantly better fit; ramet mass increased non-linearly with NR and RMA decreased with NR (Λ_{model2}=7.63, P=0.006 and Λ_{model3}=1.23, a=0.31 ± 0.26, c=0.38 ± 0.26).

Since RMA in the fertilized treatment was constant, I compared RMA in the control with RMA in the fertilized treatment using the Wilcoxon two-sample test and found that RMA in the fertilizer treatment was greater than in the control ($mean_{cont}$=0.05, $mean_{fert}$=0.07; W=1211, P=0.003).

Discussion

Sexual and clonal output

Sexual reproductive output (RO), measured as fruit mass, increased with plant size for both years, which was unsurprising since large plants have more resources to support reproduction and increase in RO with size is frequently reported (Bazzaz et al. 2000, Weiner 2004, Weppler and Stocklin 2005, Niu et al. 2009). Although RO is usually discussed as sexual reproductive output, increases in clonal output with size are also common (Brown and Eckert 2005, Hawkins et al. 2005, Wang et al. 2008). Although clonal output increased with size in the second generation, a surprising result from the first generation of *P. sedoides* is that stolon and ramet mass decreased as plant size increased. The negative relationship between stolons and plant size may have been due to increased ramet number with size. Plants may produce many stolons when small as a way of establishing themselves in an area and as they mature, these same stolons become

ramets as they grow vertically and expand their leaves. Once a stolon began to grow upright to become a ramet, I no longer considered it a stolon and the horizontal portion of the ramet was not included with the stolon mass. Therefore, larger plants that produced many ramets may appear to have less stolon mass even though they initially produced many stolons. In an extreme case, a plant that produced a ramet at the end of each of its stolons would appear to have no stolon mass. However, this explanation seems unlikely since the same procedure was used in the second generation, where stolon mass increased with size.

A decrease in stolon and ramet mass with plant size can also imply a cost to asexual reproduction, meaning plants that produced large ramet and stolon masses were less likely to grow larger. Few studies have looked for a cost to clonal reproduction. A decrease in survival and future reproduction when a plant reproduced asexually has been reported in some studies (Eriksson 1988, Wijesinghe and Whigham 1997). Koivunen et al. (2004) found a negative relationship between clonal and total biomass at the ramet level (not for the whole genet) and only in flowering ramets. More research is required to investigate the costs of clonal reproduction, especially using manipulation of the amount of clonal growth rather than purely correlational analyses (Hartemink et al. 2004).

Effect of fertilizer treatment on allocation

Support for the hypothesis that clonal growth will increase under beneficial conditions was provided by the increase in allocation to stolon (both years) and ramet mass (the first year) observed in the nutrient enriched treatments. However, treatment did

not influence the nature of relationship between size and allocation in these cases; clonal reproductive allocation decreased with size regardless of nutrient addition.

Also as predicted, sexual reproductive allocation decreased while ramet mass allocation increased under fertilizer treatment in the second generation. However, unlike stolon allocation, nutrient addition altered the nature of the relationship between allocation and size, with allocation to a trait decreasing with size in one treatment but remaining unchanged in the other. The differing nature of the relationship between these traits and plant size demonstrates how the developmental and resource needs of differently sized plants can change in response to nutrient supply. If allocation to a trait decreases with size, the trait is important while a plant is small, but it receives a smaller proportion of resources as the plant grows larger and allocates more to other aspects of growth and reproduction (Cheplick 2005). However, if allocation to a trait stays the same regardless of size, the trait likely remains important throughout the plant's life and is therefore maintained, perhaps at the expense of other plant functions. Overall, my results indicate that sexual reproduction is more important in the low nutrient condition (and therefore maintained at a constant level across plant sizes), while clonal growth (ramet mass) was more important in the fertilized treatments.

Sexual reproductive allocation

In a review of the relationship between sexual reproductive allocation and plant size, Weiner et al. (2009) found that the most common relationship is linear, passing through the origin [analogous to model 0 of Klinkhamer et al. (1992)] which means that sexual reproductive allocation does not change with size. Most of my results for fruit mass also fell into this category (all of summer 2004 and the control treatment of summer

2007). However, there was a negative relationship between RA and vegetative size for all plants together and fertilizer treated plants in summer 2007, which was the pattern least frequently observed by Weiner et al. (2009). A decrease in RA as plant size increases can be explained by costs of supporting structures and transport increasing as the plant grows larger (Klinkhamer et al. 1992, Obeso 2002, Weiner et al. 2009). Another explanation is that larger plants tend to allocate more resources to growth, clonal reproduction and/or storage for future growing seasons while for smaller plants, sexual reproduction is a priority. Small plants may allocate more to flowering and fruiting than larger plants if there is a high probability that they may die (Cheplick 2005, Aarssen 2008). This allows plants to initiate sexual reproduction early in case they do not survive long into the growing season.

In the second year of this experiment, environmental conditions altered the nature of the relationship between sexual reproduction and plant size, which has also been reported by others. For example**,** Wang et al. 2006 found that RA increased with size across density treatments and decreased in size across sowing dates in *Amaranthus retroflexus*. Likewise**,** RA was either positively or negatively correlated with plant size depending on species identity, grazing and nutrient addition (Niu et al. 2009). In the present study**,** RA decreased with plant size under fertilizer treatment but remained constant in the control, meaning fruit production is more important, and therefore maintained at a constant level for all plant sizes, under nutrient poor conditions. Large plants in the control group may maintain RA at the expense of growth and clonal reproduction. Overall, fertilized plants allocated less biomass to sexual reproduction than did controls. Sexual reproduction may be more important in the control than in the

fertilized group because seeds may serve as both a potential escape mechanisms for offspring and to produce genetically diverse offspring that may better able to cope with lower nutrient conditions (van Kleunen et al. 2002).

Clonal allocation

My assessment of stolon and ramet allocation provides one of the few studies that takes size into account when investigating clonal allocation and is the first to consider non-linearity of the clonal mass-plant size relationship. Clonal biomass and plant size had a non-linear relationship and allocation declined with size for almost all the clonal traits (with the exception of ramet mass in 2007). This non-linearity would have not be detectable with the more commonly used methods of Samson and Werk (1986). The decrease in clonal allocation with plant size could be a result of smaller plants requiring more clonal growth than larger plants or of plants with a large amount of clonal growth producing less non-clonal tissue, such as the main stem, indicating a potential cost to stolon and ramet production.

Theoretically, allocation to clonal growth characteristics should increase in high quality environment (Gardner and Mangel 1999). This allows the plant to place its genetically identical offspring in an environment in which the parent plant has reproduced successfully (Silander 1985). This prediction was supported by my results for stolon mass and ramet mass, both of which showed increased allocation under nutrient addition. However, unlike ramet and stolon mass, allocation to ramet number did not support this hypothesis In both years, plants in the two treatments did not differ in resource allocation to ramet formation. Since the number of offspring (ramets) produced is perhaps the most accurate measure of clonal reproduction, this result weakens the

support for the hypothesis provided by stolon and ramet mass. Variation in ramet number was more genetically controlled than other traits (Chapter IV of this dissertation), which may explains its lack of responsiveness to fertilizer treatment. The increased RMA and SA in fertilized plants relative to control may be due to their other functions, such as storage, nutrient uptake and overwintering (for stolons), increased photosynthesis (for ramets) and genotype persistence (Hutchings and Mogie 1990, Pluess and Stocklin 2005). Larger ramet size may also increase the likelihood of ramet survival and more and larger stolons may give the genet a good "head start" and increase ramet number in the following growing season.

Conclusions

Resource allocation to clonal growth was investigated for the first time using the methods proposed by Klinkhamer et al. (1992), which concurrently tests for both a minimum size for reproduction and a non-linear relationship between allocation and size. Contrary to my prediction that the relationships between reproductive output and size would be linear, clonal characteristics tended to have a nonlinear relationship and allocation to these traits decreased with size. A non-linear relationship between size and fruit mass was also seen in the fertilizer treatment plants in the second year. My hypothesis that treatments would influence the relationship between reproduction and plant size was partially supported; in the first year, plants showed the same relationship regardless of treatment. It is therefore important to look at different environmental variables and growing seasons when studying allocation patterns.

Support for the hypothesis that plants will allocate more to sexual reproduction in nutrient poor conditions while clonal reproductive allocation is higher in fertilized conditions was mixed. In the first year, the two treatments did not differ in RA. However, the second year, RA in the control remained constant while RA in the fertilized group decreased with size and tended to fall below the mean value of RA for control. Although plants of the fertilizer group allocated more to ramet mass and stolon mass than the control, the number of new ramets produced did not differ between treatments, providing limited support that clonal reproductive allocation should be higher in high resource environments.

CHAPTER VI

CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions

Overall, the hypothesis that plants would alter sexual and clonal output and allocation based on environmental conditions was not consistently supported by my results. From Optimal Partitioning Theory, I predicted clonal growth allocation would increase with nutrients while sexual reproduction would decrease with nutrient addition. When there was support for this hypothesis, it was mixed, only during one year or only under certain conditions. For example, sexual reproductive allocation (RA) decreased with nutrients but only in the second generation of *Penthorum sedoides*. Likewise, although allocation to ramet and stolon mass was greater in the high nutrient treatment, supporting my hypothesis, ramet number did not differ between treatments. Ramet number, which describes the number of clonal offspring produced by a plant, is perhaps the best measure of clonal reproduction and therefore the support provided by stolon and ramet mass allocation is weakened by the lack of treatment effect on ramet number.

Using Optimal Partitioning Theory, I further hypothesized that sexual reproduction would decrease and clonal reproduction increase under simulated herbivory treatments, both as methods to increase resources put towards survival and to repair tissue. However, this hypothesis was also only partly supported, depending on species. In *Lythrum salicaria*, clonal mass was unaffected by treatment. In *Eupatorium perfoliatum*, both clonal growth and fruit mass were increased in the presence of root herbivory. In fact, most non-reproductive biomass factors also increased in the root damage treatments, which was unexpected. *Penthorum sedoides* showed a decrease in fruit mass with root damage as predicted. Most other traits in *P. sedoides* were unaffected by herbivory, implying that reduced sexual reproduction may be the cost for maintenance of other functions, in support of my hypothesis. Although stolon mass in *P. sedoides* was unaffected by treatment, branches, which can act as clonal organs if they come into contact with the soil, increased in the root damaged treatments. My herbivory experiment revealed previously undescribed effects of herbivory—in particular, the increased branching exhibited by all three species following root damage. The ecological reasons behind this response, and whether it occurs in other species and with actual herbivore damage, will require future study.

In both *Lythrum salicaria* and *Penthorum sedoides*, few of the traits showed significant amounts of variation due to genetics, even though I was comparing groups of genetically identical clones. The addition of fertilizer had a much stronger effect on variation, indicating that environmental differences had more influence on the phenotype than did genotype. My results suggest that *L. salicaria* and *P. sedoides* are highly plastic species, able to acclimatize to variable environmental conditions and utilize available

resources. For clonal characteristics, the effect of nutrient addition was stronger in the wide-spread, guerilla-like *P. sedoides* than the more compact *L. salicaria*. This may allow *P. sedoides* more flexibility in stolon and ramet placement to better utilize variable environments.

Many of the reproductive traits in both the herbivory and nutrient experiments increased with size. This is to be expected since larger plants have more resources available to support sexual and clonal reproduction. However, in the nutrient experiment, stolon and ramet mass in the first generation of *P. sedoides* decreased with increasing plant size. While this unusual response may be an artifact of my methodology in defining and collecting stolons and ramets, it may reflect a cost of clonal reproduction; plants that produce many stolons or ramets may have fewer resources available for other structures. The pattern of stolon and ramet decrease with size occurred only in the first generation but since these experiments were not designed to test for reproductive costs, they may not have been detectable if present in the second generation. Stolon mass did decrease slightly with size in the herbivory experiment, but only in the root damage treatments and the relationship was not significant.

Using methods proposed by Klinkhamer et al. (1992), I have shown for the first time that clonal reproduction can have a non-linear relationship with size while allocation to clonal growth can decrease with size. This knowledge has many implications for the study of clonal plants. Studies of clonal allocation may need to be reconsidered in the light of these findings, since most studies that consider size-dependence assume a linear relationship between size and clonal output. These results also have implications for studies of clonal reproductive costs. On a more practical note, since clonal growth is a

major component of many plant's life-cycles, including endangered and invasive species (particularly *Lythrum salicaria* and *Phragmetes*), increased understanding of clonal growth may assist in the management of these species.

Future directions

Improvement of plant size range in size dependent allocation experiment.

As seen in Chapter V, there were few plants representing smaller size ranges, especially for the fertilizer treatments. This may have reduced my ability to detect a minimum size for reproduction if one existed. Therefore, my further research would include an experiment to measure size dependence of clonal growth and sexual output and allocation, but include multiple sampling points throughout the growing season to better represent smaller plant sizes. Measurements from smaller plants should improve my ability to detect a minimum size of reproduction and refine the estimates of the relationship between plant size and clonal and sexual allocation. This will also provide data for young, and therefore small, high nutrient plants, creating a better overlap in size between treatments and more accurate comparisons between fertilized and control plants.

To further increase the size range of plants, I would carry out the experiment over several years, allowing the plants to overwinter outdoors. This will allow *P. sedoides* to reach larger sizes more similar to those seen in the field. Multi-year research will also allow me to investigate why ramet number does not increase with fertilizer while stolon mass does. I can test whether the disproportionate increase in stolon mass with fertilizer

treatments leads to increased survival over the winter, higher ramet number, and overall better health of the plant in subsequent years.

Cost of asexual reproduction and trade-offs between clonal and sexual reproduction.

In the first year of the nutrient experiment, there was a decrease in both ramet and stolon mass with plant size. This trend was also seen in *P. sedoides* in the herbivory experiment, although the relationship was not significant. This could be caused by a cost of clonal reproduction, meaning that plants with large amounts of clonal and ramet mass may not have resources available for other aspects of growth. The cost of sexual reproduction is determined experimentally by removing flower buds or in other ways preventing flower formation on some plants and comparing these to plants that were allowed to flower naturally. However, research using similar methods to determine the costs of stolon or ramet production has not been reported. My further research plans include comparing plants prevented from reproducing clonally to controls to determine whether stolon or ramet production decreases overall plant growth and to detect a trade-off between clonal and sexual reproduction.

Effect of herbivory on allocation to reproduction

As mentioned in Chapter III, the effect of herbivore damage on plant reproduction is not often studied with size dependence taken into account. I would like to further investigate the reaction of *P. sedoides* to herbivory and determine how it affects allocation to reproduction using the methods described in Chapter V. For this experiment, I would increase the levels of damage induced by repeating the damage

treatments to better imitate herbivore attack or by manipulating the exposure of plants to insect herbivores. I would also include multiple harvest dates so that small plant sizes would be included in the analysis.

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