PLANT COMPETITIVE RESPONSES TO NEIGHBOURS

COMPETITIVE BEHAVIOURS IN RESPONSE TO NEIGHBOURS OF TWO WOODLAND PLANT SPECIES

BY

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ABSTRACT

Plants often grow in communities closely surrounded by neighbouring plants. Plants can actively and intensely compete for resources and also anticipate competition by sensing environmental cues from the presence and identity of neighbours. Moreover, it’s been proposed that the evolution of both increased and decreased competitive ability may serve as a mechanism for invasiveness. However, still little is known about how plants integrate competitive responses when sensing multiples cues of competition and which individual competitive traits respond to the identity of competitors. In addition, whether and why the evolution of competitive traits may contribute to the ability of introduced species to become invasive is also poorly understood.

Here I present a body of work that examined the competitive responses of a native and an invasive plant species to cues of competition and the identity of neighbours. I also examined how experimental manipulation of pot volume, to control belowground resources, affects plant growth and allocation. In one study I tested the competitive responses of the North American native, *Impatiens pallida*, to cues signalling the presence of neighbours above and belowground simultaneously in competitive environments composed of either siblings or strangers. I demonstrate that *I. pallida* can recognize siblings and shows more aggressive competitive behaviours towards strangers than kin.

In two other studies, I compared the competitive responses of the invasive and native ecotypes of *Alliaria petiolata* to changes in density, as well as to the presence and identity of neighbours. I found that invasive ecotypes produced less competitive phenotypes especially under high density. Moreover, I found that invasive ecotypes performed better when sharing rooting space with neighbours that were siblings.

Taken together, these results demonstrate the ability of these plant species to respond to the identity of neighbours and provide strong evidence in support of the evolution of reduced competitive ability hypothesis in invasive plant species potentially mediated by the action of kin selection in invasive ecotypes.

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THESIS ORGANIZATION AND FORMAT

This thesis is organized in a sandwich format approved by McMaster University and with the recommendation of the supervisory committee. The thesis consists of six chapters. Chapter one provides a general introduction and objectives of the thesis. Chapters 2-5 are manuscripts that have been either already published (Chapter 2), submitted for review (Chapter 3), or are soon to be submitted for review (Chapters 4 and 5). Chapter 6 summarizes the results, discusses limitations and suggests directions for future research.

CHAPTER 1: **General Introduction**

CHAPTER 2: **Kin Recognition: Competition and Cooperation in Impatiens (Balsaminaceae)**

Authors: Guillermo P. Murphy and Susan A. Dudley

Publication: *American Journal of Botany*, 96(11): 1990-96 (2009)

Comments: This study was conducted by G.P.M. under the supervision of S.A.D.

CHAPTER 3: **Differentiating the effects of pot size and nutrient availability on plant biomass and allocation**

Authors: Guillermo P. Murphy, Amanda L. File, and Susan A. Dudley

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Comments: The idea for the study was proposed by G.P.M., the study was conducted by G.P.M. and A.L.F. and supervised by S.A.D.

CHAPTER 4: **Comparison of competitive behaviours between native and invasive ecotypes of Garlic Mustard under high, medium, and low density conditions**

Authors: Guillermo P. Murphy and Susan A. Dudley

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CHAPTER 5: **Comparison of competitive behaviours between native and invasive ecotypes of Garlic Mustard in response to density, presence, and identity of neighbours**

Authors: Guillermo P. Murphy

Publication: Manuscript will be submitted to *Proceedings of the Royal Society B*

Comments: This study was conducted by G.P.M. under the supervision of S.A.D.

CHAPTER 6: **General conclusion**

Table of Contents

List of Tables

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

List of Figures

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 1:

GENERAL INTRODUCTION

In nature, plants constantly interact with neighbouring plants and one of the most important interactions is competition for limited resources (Novoplansky, 2009). As sessile organisms that disperse stochastically as seeds, plants cannot choose their neighbours but must cope with the competitive environment (Novoplansky, 2009). It is not surprising then, that in the past, plants were thought of as victims of their environments that could only passively respond to the conditions they encountered. Recently however, plants have been shown to not be passive at all. Instead, they are active organisms capable of gathering information about their environments and responding to neighbours and resource availability independently through phenotypic plasticity (Aphalo & Ballaré, 1995; Callaway, 2002), which can be considered analogous to behaviour.

How plants respond to active and impending competition aboveground and belowground has been well documented (Casper & Jackson, 1997; Novoplansky, 2009; Schenk, 2006; H. Smith, 1995). However, the recent demonstration that plants are capable of recognizing their siblings (Dudley & File, 2007) has opened a new range of questions regarding whether and how plants behave in social situations and how kin selection may influence the evolution of competitive traits. Although the fitness outcomes of growing in groups of siblings or groups of strangers have been measured before, the limitations of those studies have led researchers to call for approaches that measure specific traits instead of fitness to elucidate how plants interact competitively with sibling neighbours (File, Murphy, & Dudley, 2011).

Species that form genetically structured populations due to limited seed dispersal, inbreeding, and self-fertilization are good candidate species to be subjected to kin selection and to evolve kin recognition mechanisms (File et al., 2011; Kelly, 1996). Two such species are the North American native Jewelweed (*Impatiens pallida*) and the North American invasive Garlic Mustard (*Alliaria petiolata*). Invasives in general, because they form high-density monospecific stands (Williamson, 1996) likely composed of related individuals in their new habitats, create conditions favourable for kin selection. Thus, it is possible that the observed reduction of competitive ability in invasive ecotypes of some invasive plants like Garlic Mustard (Bossdorf, Prati, Auge, & Schmid, 2004) may be due to kin selection.

In the following section I provide an overview of the role of individual competitive behaviours and the importance of kin recognition for plants. I examine individual competitive behaviours in the context of plant competition and plant invasion as well as how competitive behaviours may be influenced by kin selection.

COMPETITIVE BEHAVIOURS AND THEIR ROLE IN COMPETITION

Aboveground, plants compete strongly for access to light (H. Smith, 1982, 1995). For plants, light serves as both a resource and a cue (H. Smith, 1995;

Stuefer & Huber, 1998). As a resource light is essential for plants to be able to photosynthesize. As a cue, light signals the presence and proximity of competitors aboveground (H. Smith, 1995; S. Smith, Casal, & Jackson, 1990; Stuefer & Huber, 1998). Plants respond to the availability of light as a resource by producing phenotypes (i.e. phenotypic plasticity) that will maximize light acquisition (Franklin & Whitelam, 2005; Schlichting & Smith, 2002; Schmitt, Stinchcombe, Heschel, & Huber, 2003; H. Smith, 1995; Sultan, 2003). Plant responses to light as a cue involve sensing competitors and thus anticipating competition (Ballaré, Sánchez, Scopel, Casal, & Ghersa, 1987; Ballaré, Scopel, & Sánchez, 1990; Franklin & Whitelam, 2005; S. Smith et al., 1990).

Plants perceive light as a cue through phytochromes, a family of photoreceptors (Ballaré, 1999; Franklin & Whitelam, 2005; H. Smith, 1995; H. Smith & Whitelam, 1997). Phytochromes are molecules that serve plants as light sensors and can exist in two photoconvertible isomeric forms: the Pr form which absorbs red light (600-700 nm) and the Pfr form which absorbs far-red light (700-800 nm) (H. Smith, 1982, 1995). When light passes through or is reflected from green vegetation, chlorophyll absorbs red light and reflects far-red light reducing the ratio of red to far-red (R:FR) (Taiz & Zeiger, 1998). This lower ratio of R:FR causes phytocrhomes in the Pr state to switch into the Pfr state triggering a suite of developmental responses (H. Smith, 1982, 1995).

Responses to low R:FR include changes in morphology, such as elongation of vertical spacers (stem internodes and petioles) (Ballaré et al., 1990;

Huber, Fijan, & During, 1998; Weijschede, Berentsen, de Kroon, & Huber, 2008) and increases in leaf area (Stuefer & Huber, 1998; vanHinsberg & vanTienderen, 1997; Weijschede, Martinkova, de Kroon, & Huber, 2006), as well as changes in allocation of resources among plant organs (Cipollini & Schultz, 1999; Huber et al., 1998; Maliakal, McDonnell, Dudley, & Schmitt, 1999; Stuefer & Huber, 1998; vanHinsberg & vanTienderen, 1997). Elongation of vertical spacers is a competitive response (Huber et al., 1998; Stuefer & Huber, 1998; vanHinsberg & vanTienderen, 1997; Weijschede et al., 2008) that allows plants to position leaves closer to the light resource while at the same time shading competitors (H. Smith, 1995). This better positioning of leaves along with increases in leaf area help plants maximize light acquisition in competitive environments. However, these adaptive responses (Dudley & Schmitt, 1996) can be costly involving allocation of resources into shoots at the expense of other plant organs (Cipollini & Schultz, 1999; Schmitt & Wulff, 1993).

Belowground, plants compete for water and mineral nutrients (Casper & Jackson, 1997). Competition occurs when the presence of competitors reduces resource availability (Casper & Jackson, 1997). However, other forms of belowground competition also exist among plants, like allelopathy, the production of harmful chemical compounds that inhibit the growth of neighbouring plants (Mahall & Callaway, 1992). Moreover, belowground, plants are not restricted to competition with other plants but may also experience competition for resources with other organisms such as soil microbes (Kaye & Hart, 1997).

Plants can respond to competition belowground by avoiding competition, tolerating competition, or engaging in competition by producing traits that maximize the occupation of soil space and that increase resource uptake (Novoplasnky, 2009). These traits include root depth, fine root biomass, density, and surface area (Casper & Jackson, 1997). In addition, plants show spatial and temporal partitioning of the soil as well as morphological and physiological plasticity to belowground competition (Cahill & McNickle, 2011; Casper & Jackson, 1997). This allows plants to choose among different strategies for competing belowground like tolerating competition, avoiding competition, or responding aggressively to competition (Cahill & McNickle, 2011; Novoplansky, 2009).

An important aspect of plant responses to competition belowground is the ability of plants to proliferate roots in resource rich patches (Cahill & McNickle, 2011; Casper & Jackson, 1997; Hodge, 2009; Marschner, 1995). However, the root competition responses are not limited to active competition for resources (Schenk, 2006). In addition to active competition plants can also sense and respond to impending competition belowground (Callaway, 2002). In the presence of neighbouring roots, even when resources are controlled experimentally, plants increase root proliferation by increasing root biomass and allocation. These responses to the presence of neighbours were argued to be a pre-emptive competitive response (Gersani, Brown, O'Brien, Maina, & Abramsky, 2001; Maina, Brown, & Gersani, 2002; O'Brien, Gersani, & Brown, 2005) although see

(J.F. Cahill, 2003; Kembel & Cahill, 2005). However, increased allocation of resources to roots may come at a cost of aboveground traits and ultimately reproductive yield (Gersani et al., 2001; Maina et al., 2002; O'Brien et al., 2005) but see (Murphy & Dudley, 2007). Therefore, because in nature both above and belowground competition are likely to occur simultaneously, it is important for studies that examine responses to either active competition or responses to the presence of competitors, to consider possible synergistic and antagonistic interactions between responses to above and belowground competition.

THE POT SIZE ARGUMENT

Studies that have examined plant responses to the presence of competitors instead of active competition for resources belowground and some studies that have examined identity recognition in plants have used a methodology that has been challenged theoretically. Plants have been shown to respond to impending competition belowground by changing allocation of resources between plant organs in the presence of neighbouring roots before resources are depleted (Cahill et al., 2010; Gersani et al., 2001; Maina et al., 2002; Murphy & Dudley, 2007; O'Brien et al., 2005). Similarly, plants have been shown to respond differently to the presence of self and non-self roots also before resource depletion occurs (Falik, Reides, Gersani, & Novoplansky, 2003; Gruntman & Novoplansky, 2004; Holzapfel & Alpert, 2003). The methodology used in the studies that demonstrate these responses involves controlling the availability of resources experimentally by increasing pot size proportionally with number of plants per pot. This methodology has been challenged and argued back and forth theoretically as some consider it to be flawed (Hess & de Kroon, 2007; Schenk, 2006; Semchenko, Hutchings, & John, 2007) and others do not (O'Brien & Brown, 2008). Hess and de Kroon (2007) provide a set of formal hypotheses as an alternative explanation for the results found by previous studies that used this methodology. These hypotheses propose that root mass should be a function of pot size only, while plant growth should be limited by nutrient availability. Then, as a result of a trade-off between root and shoot biomass, root allocation should increase with pot size. In chapter 3, I provide the first empirical tests of these hypotheses.

THE ROLE OF COMPETITIVE BEHAVIOURS IN PLANT INVASION

Invasive plant species are introduced species that displace local species by often forming dense, monospecific stands in their new habitats (Williamson, 1996). Invasive plant species disrupt ecosystem stability and reduce biodiversity by altering both the biotic and abiotic components of ecosystems (Vitousek, Dantonio, Loope, & Westbrooks, 1996). Invasive plants impact community composition, soil characteristics, and nutrient cycling (Wilcove, Rothstein, Dubow, Phillips, & Losos, 1998). Invasive species are considered to be the second most important factor contributing to biodiversity loss after habitat destruction and have been reported to be a contributing factor in more than half the cases of threatened plant species (Vitousek et al., 1996; Wilcove et al., 1998).

When introduced into new habitats, invasive species are released from the stresses imposed by herbivores, pathogens, and competitors that have coevolved with them in their native habitats (Callaway, Ridenour, Laboski, Weir, & Vivanco, 2005). However, not all introduced species become invasive and many invasive species are fairly minor components of their home communities (Callaway & Maron, 2006; Williamson, 1996). In addition, those species that ultimately invade often do so after a considerable lag time (Mack et al., 2000; Sakai, 2001) indicating that an adaptation period might be necessary before they can successfully invade the new habitat.

Invasive species are subjected to novel selection pressures in their new habitats (Mooney & Cleland, 2001). Thus, the observed lag time between introduction and invasion may be the time that is required for rapid evolutionary changes to occur that will allow introduced species to adapt and invade their new habitat (Bossdorf et al., 2004). In fact, rapid evolution in invasive plants has been shown to be a widespread occurrence (Buswell, Moles, & Hartley, 2011). Rapid evolution in invasive plants has been reported for effect of allelotoxins (Prati & Bossdorf, 2004), dispersal ability (Cheptou, Carrue, Rouifed, & Cantarel, 2008), phenotypic plasticity (Richards, Bossdorf, Muth, Gurevitch, & Pigliucci, 2006), mycorrhizal dependence (Seifert, Bever, & Maron, 2009), morphological and physiological responses to climate (Maron, Elmendorf, & Vila, 2007), vegetative reproduction (Lavergne & Molofsky, 2007) and fitness (seed and fruit production) (Ridley & Ellstrand, 2009). However, it has been the evolution of competitive ability in invasive species that has received a great deal of attention because of two hypotheses that predict opposing outcomes: the “evolution of increased competitive ability” hypothesis (EICA) (Blossey & Notzold, 1995), and the “evolution of reduced competitive ability” hypothesis (ERCA) (Bossdorf et al., 2004).

The evolution of increased competitive ability hypothesis (Blossey & Notzold, 1995) was proposed as an explanation for the observation of plants being larger and more vigorous in their introduced habitat compared to conspecifics in their native habitat (Crawley, 1987). EICA argues that because invasive species are released from predators and diseases in their new habitats, natural selection will act against costly defense traits and instead favour traits that increase competitive ability (Blossey & Notzold, 1995). Several studies have tested the EICA hypothesis by examining performance and fitness outcomes of native and invasive populations in common environments and have found only mixed support for it (summarized by (Bossdorf et al., 2005)). As a result an alternative explanation was proposed that predicted the opposite outcome of evolutionary forces on competitive ability: the “evolution of reduced competitive ability” hypothesis (ERCA) (Bossdorf et al., 2004).

The ERCA hypothesis was proposed based on a prediction derived from the EICA hypothesis. Bossdorf et al. (2004) predicted that, if invasive ecotypes evolved to be more competitive in their new habitats, then they should outcompete ecotypes from the native habitat. They tested this prediction in an invasive North American species, Garlic Mustard (*Alliaria petiolata*) and found that in this species invasive ecotypes were less competitive than native ecotypes (Bossdorf et al., 2004). Based on these results they proposed the alternative hypothesis that natural selection may select against competitive traits and lead to the evolution of reduced competitive ability (Bossdorf et al., 2004). They argue that this may occur because competitive traits are costly and invasive species may encounter fewer or weaker competitors in the new habitat. Therefore, natural selection will favour the production of less competitive phenotypes. Another possible explanation however, is the introduction by invasive species of “novel weapons”. (Callaway & Ridenour, 2004). Invasives may gain a competitive advantage by producing allelopathic compounds that may negatively affect competitors that have not co-evolved to tolerate them (Callaway & Ridenour, 2004). Thus, the advantage of producing other costly competitive traits may be reduced and natural selection may select against them.

Although many studies have looked at the evolution of competitive ability in plants, most have focused on performance and fitness outcomes. These studies often compare invasive and native ecotypes in common gardens but overlook important confounding factors that can affect those outcomes, such as latitudinal clines (Colautti, Maron, & Barrett, 2009). A more appropriate approach has been proposed that involves measuring differences in individual putative competitive traits that respond to competition cues instead of performance or fitness outcomes that can be more susceptible to confounding environmental conditions (File et al., 2011). Here, I utilize this approach to examine responses of Garlic Mustard to density, presence and identity of neighbours in chapters 4 and 5.

In principle, the EICA and ERCA hypothesis are not mutually exclusive and both outcomes are possible depending on the environmental conditions and selection pressures that each species encounters in the new habitat. However, why the evolution of reduced competitive ability will favour invasiveness is still unclear. A possible explanation involves the action of kin selection on competitive traits of invasive ecotypes.

COMPETITIVE BEHAVIOURS AND KIN SELECTION IN PLANTS

Kin selection proposes that a particular behaviour that does not convey direct benefits to the individual may still be selected for if it provides a sufficiently large benefit to a relative (Hamilton, 1964). This idea has been formalized in what is known as Hamilton's rule: assuming heritability of the trait/behaviour, kin selection will favour a trait/behaviour if $C < B*r$, where C is the cost to the organism performing the behaviour, B is the benefit to the relative, and r is the relatedness between the two (Hamilton, 1964). Kin selection theory has often been used to explain the evolution of altruism. In a context of plant competition altruism is analogous to reduced competitive behaviour, thus the evolution of reduced competitive ability in invasive individuals can be considered equivalent to evolution of altruistic traits.

Two possible situations may occur that can allow for kin selection to act on a plant population. One situation involves plants having a high probability of interacting with siblings making sibling interactions predictable (File et al., 2011; Kelly, 1996). Although the likelihood of predictable interactions with siblings is low for plants, mainly because seed dispersal is stochastic (File et al., 2011), invasive species provide a unique case where the likelihood of interacting with siblings is increased. In their new habitat, many invasive plants like Garlic Mustard, the model species used to develop the ERCA hypothesis, form monospecific stands of closely related individuals due to limited seed dispersal and high levels of selfing (Anderson, Dhillion, & Kelley, 1996; Williamson, 1996). This way, they create genetically structured populations that meet the requirements for kin selection (Kelly, 1996). A more reliable mechanism that favours kin selection is if plants can recognize siblings from strangers (Dudley & File, 2007; Waldman, 1988).

KIN RECOGNITION IN PLANTS

The ability to recognize siblings from strangers has been shown in a variety of plant species like *Cakile edentula* (Bhatt, Khandelwal, & Dudley, 2010; Dudley & File, 2007) *Impatiens pallida* (Murphy & Dudley, 2009), *Arabidopsis thaliana* (Biedrzycki, Jilany, Dudley, & Bais, 2010), and *Chenopodium album* (unpublished data). It has been proposed that this ability is mediated by root interactions, more precisely by responses to root exudates (Biedrzycki et al., 2010). In all of these species differential responses to siblings compared to strangers have involved changes in competitive behaviours that are consistent with kin selection. In *C. edentula* and *A. thaliana* plants allocated resources to fine roots and increased the number of lateral roots respectively as a competitive response to strangers but not to siblings (Biedrzycki et al., 2010; Dudley & File, 2007). In *I. pallida*, a plant more limited by the light resource, plants allocated more resources to leaves when competing with strangers and showed changes in canopy morphology that reduced interference with siblings (Murphy & Dudley, 2009). In *C. album*, plants increased competitive responses to strangers but not to siblings under good resource availability conditions while showing no differential behaviour under low resource availability (unplublished). Therefore, kin recognition allows for altruistic traits to be expressed only in those cases when plants compete with siblings. If invasive plants can recognize siblings, then kin selection may favour the evolution of less competitive phenotypes, as proposed by the ERCA hypothesis, by providing a benefit in indirect fitness from reduced competition with relatives. Moreover, if competitive traits are costly, the benefits will be twofold because plants will also avoid the cost of producing competitive traits. Here, I provide evidence for kin recognition in a native plant (*I. pallida*) in chapter 2 and in an invasive plant (*A. petiolata*) in chapters 4 and 5.

OBJECTIVES

The general objective of this work is to better understand the competitive behaviour of plants in response to interactions with their neighbours. Competitive behaviours are important because they will partially, but substantially, determine how well an organism will perform through its life cycle. This is particularly true for plants, as they have often no means of escaping their competitive environment. The competitive behaviours of plants in response to their neighbours are complex. They depend on whether plants perceive cues of above or belowground competition, the proximity of neighbours, and the identity of neighbours. To better understand plant competitive behaviour in response to neighbours, I performed a series of experiments that asked the following questions: Chapter 2) How does the North American native species *Impatiens pallida* respond to cues of above and belowground competition as well as the identity of neighbours? Chapter 3) Can the manipulation of rooting volume affect how plants perceive and respond to neighbours? Chapter 4) How do invasive and native ecotypes of the invasive species *Alliaria petiolata* respond to competition with conspecifics under high, medium, and low density conditions? Chapter 5) How do invasive and native ecotypes of *Alliaria petiolata* respond to the presence and identity of neighbours?

To answer these questions I performed a series of experiments. In chapter 2, I examined the responses of plants of the native species *I. pallida* to cues of competition aboveground and belowground simultaneously and how those responses differed depending on the relatedness of neighbours. In chapter 3, I tested how experimental manipulations of rooting volume affected traits involved in competitive behaviour of *C. edentula*, a species previously showed to demonstrate differential competitive behaviour towards siblings and strangers. Finally, in chapters 4 and 5, I compared the responses of native and invasive ecotypes of *A. petiolata* to density conditions, the presence of neighbours belowground, and how those responses differed between siblings and strangers.

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CHAPTER 2:

KIN RECOGNITION: COMPETITION AND COOPERATION IN *IMPATIENS* (BALSAMINACEAE)

ABSTRACT

The ability to recognize kin is an important element in social behaviour and can lead to the evolution of altruism. Recently it has been shown that plants are capable of kin recognition through root interactions. Here we tested for kin recognition in a North American species of *Impatiens* that has a high opportunity of growing with kin and demonstrates strong responses to aboveground competition. We measured how the plants responded to the aboveground light quality cues of competition and to the presence of the roots neighbours, and determined whether the responses depended on whether the neighbours were siblings or strangers. The study families were identified by DNA sequencing as members of the same species, provisionally identified as *Impatiens pallida* (hereafter *I.* cf. *pallida*). We found that *I.* cf. *pallida* plants were capable of kin recognition, but only in the presence of another plant's roots. Several traits responded to relatedness in shared pots, including increased leaf to root allocation with strangers and increased stem elongation and branchiness in response to kin, potentially indicating both increased competition towards strangers and reduced interference (cooperation) towards kin. *I.* cf. *pallida* responded to both competition cues simultaneously, with the responses to the aboveground competition cue dependent on the presence of the belowground competition cue.

INTRODUCTION

In nature, plants often grow in close proximity to other plants and they actively acquire information from their environments about competitive situations (Callaway, 2002). Phenotypic plasticity to that information allows plants to adapt to resource availability and competition (Ballare, Scopel, and Sanchez, 1990; Dudley and Schmitt, 1995; Schlichting and Smith, 2002; Sultan, 2003). Plants interact and respond to the presence of competitors both aboveground (Smith, 1982, 1995; Schmitt and Wulff, 1993) and belowground (Casper and Jackson, 1997; Gersani et al., 2001; Maina, Brown, and Gersani, 2002; Falik et al., 2003; Holzapfel and Alpert, 2003; Gruntman and Novoplansky, 2004; O'Brien, Gersani, and Brown, 2005; Murphy and Dudley, 2007). Recently it was shown in the annual species *Cakile edentula* (Dudley and File, 2007) that responses to belowground neigbours depended on whether those neighbours were siblings or strangers. *C. edentula* had greater root allocation when sharing a pot with strangers than when sharing a pot with kin, indicating that they recognized kin, and consistent with the hypothesis that kin competed less than strangers. However, it is not yet known whether the ability to discriminate kin from strangers is widespread in plants.

The ability to discriminate kin from non-kin is an important element in social behaviour. Kin recognition is especially advantageous under competitive situations if it prevents costly competitive behaviour towards kin or promotes cooperation among siblings (Axelrod and Hamilton, 1981; Waldman, 1988). Plant life histories often result in highly related groups of neighbours, creating conditions in which kin recognition is most likely to evolve (Waldman, 1988). The ability of plants to sense and respond to other plants is analogous to animal behaviour. Thus, like animal behaviour, phenotypic plasticity of plants in response to other plants could be subject not only to natural selection but to kin selection as well (Hamilton, 1964; Waldman, 1988; Stevens, Goodnight, and Kalisz, 1995; Kelly, 1996). Traits that demonstrate phenotypic plasticity in response to other plants are candidate competitive traits likely to affect the fitness of neighbouring plants, and so therefore more likely to exhibit kin discrimination. These traits include changes in allocation to roots, stems and leaves, changes in the number of branches, plant height, and stem elongation.

North American *Impatiens* species offer an ideal system for testing for kin recognition. *Impatiens pallida* Nutt. and the closely related species *Impatiens capensis* Meerb. (Balsaminaceae) are herbaceous annual plants that are found in moist, shady, woody areas of eastern North America (Newmaster, Harris, and Kershaw, 1997). *Impatiens capensis* are well known for their adaptive responses to low red to far-red ratio (R:FR), a cue of aboveground competition (Dudley and Schmitt, 1995, 1996; Maliakal et al., 1999). These adaptive responses (Dudley and Schmitt, 1996), which are phytochrome mediated (Smith, 1995), include increased stem elongation and shifts in allocation towards stems and away from roots and leaves (Maliakal et al., 1999). Seed dispersal occurs by explosive dehiscence, which allows seeds to land in close proximity to the mother plant therefore creating dense stands of closely related individuals (Stevens, Goodnight, and Kalisz, 1995). *I. capensis* meets criteria established for the action of kin selection: 1) heritable variation in potentially competitive traits, 2) structured populations, 3) phenotype of neighbours, particularly aboveground plant architecture, affects the focal plant's fitness (Kelly, 1996). The ecology of these *Impatiens* species contrasts with that of *C. edentula,* the species found to show kin recognition. *C. edentula* grows in sandy beaches where light is plentiful and belowground resources are limiting (Dudley and File, 2007), while North American *Impatiens* spp. grow as understory species that often experience light limitation (Schmitt et al., 2003). Therefore, it can be expected that if *Impatiens* species are capable of kin recognition, their responses to kin and strangers may differ from those of *C. edentula.* While *C. edentula* has been shown to respond to strangers by increasing allocation to belowground competitive traits, we predict that *Impatiens* should respond to strangers by increasing allocation to aboveground competitive traits.

In this experiment we tested whether kin recognition is found in *Impatiens* cf. *pallida.* We grew plants in a factorial design that included the presence and absence of root neighbours, the identity of the neighbour (kin or strangers), and high or low R:FR light. We measured internode length and biomass components. Density, irradiance, and average soil volume per plant were maintained constant. We tested the hypotheses that (i) *I.* cf. *pallida* plants are capable of kin recognition through root interactions, (ii) *I.* cf. *pallida* respond to root neighbours as well as R:FR; and (iii), responses to the aboveground competition cue are conditional on the presence of root neighbours (the belowground competition cue).

MATERIALS AND METHODS

Sample materials- *Impatiens* seedlings were collected from two different sites at the Royal Botanical Gardens in Hamilton, Ontario during the summer of 2005. These seedlings were grown in a growth room and their seeds collected to obtain maternal sibships (hereafter families). All seeds resulted from cleistogamous flowers, which are inconspicuous and obligately self-pollinated. Both *I. capensis* Meerb. and *I. pallida* Nutt. are often found in the sampled sites. These species can only be distinguished morphologically by their chasmogamous flower characteristics. However, because the *Impatiens* plants produced only cleistogamous flowers in the growth room, it was not possible to identify them to species through morphology. Therefore, DNA was sequenced to test whether more than one species had been used in the study. Two samples from each family were analysed, as well as reference samples of *I. capensis* (Rhode Island, USA) and *I. pallida* (Dundas, Ontario, Canada), which were identified from their distinct floral morphology. Total genomic DNA was extracted using QIAGEN DNeasy plant mini kit (QIAGEN Inc., 2800 Argentia Rd., Mississauga, Ontario, Canada). The designs for specific primer sequences for the *atpB-rbcL* spacer in Balsaminaceae were obtained from Janssens et.al. (2006) as well as amplification and purification methods. Sequences were added to Genbank (accession numbers: FJ490153-71). We found that all individuals in the study were identical for the analyzed sequence. This result confirmed that all families used belonged to the same species. This sequence was also identical for the reference sample of *I. pallida* but differed from the reference sample of *I. capensis*. This suggests that the species used was *I. pallida.* However, when we compared our sequences with those provided by Janssens et al. (2006), we found that all of our sample and reference sequences were more similar to the *I. capensis* Meerb. (Ohio) sequence provided by Janssens et.al. (2006), than to the sequence identified as from *I. aurea* Muhl. (Ohio). *I. aurea* Muhl is sometimes identified as a synonym for *I. pallida* Nutt. (e.g. Lunell, 1916), though only *I. pallida* Nutt. is accepted by the Integrated Taxonomic Information System (ITIS Taxonomic Serial No.: 29189). Because it is most probable that the species Janssens et al. (2006) used was *I. pallida* Nutt., we cannot conclusively establish that the species we used was *I. pallida* and we will thus refer to the study species as *Impatiens* cf. *pallida* (cf. is used to indicate that a species is “comparable” to another, but by definition is not confirmed to be the same).

Experimental design-.

The experiment was a complete factorial design (2^3 combinations) with treatments that consisted of: root neighbours present (root neighbours) or absent (solitary), siblings (kin) or non-siblings (strangers), and high R:FR (high) or low

R:FR (low) light. The experiment consisted of 8 trays, each containing 64 plants. Within each tray seeds of 4 families from the same population were used to create 4 groups of 4 sibling plants and 4 groups of 4 stranger plants, with 2 solitary and 2 root neighbour treatments for each relatedness treatments, arranged randomly. Half the trays were placed into high R:FR treatments, and half into low R:FR treatments. Total sample size was 512 plants.

The seeds collected from field seedlings reared in a growth room were stored in moist conditions at 2°C until they germinated in mid January 2006. Once the radicle had appeared, sprouted seeds were planted directly into their pots in a mix of 3:1 sand and turface (Profile Products LLC, Buffalo, Grove, IL, USA) and grown for 42 days in a growth room at 22°C and a 15-h photoperiod. Plants were watered daily and fertilized biweekly with 20-20-20 NPK (Plant-Prod fertilizer, Plant Production Co. Ltd., 314 Orenda Road, Brampton, Ontario, Canada). Pots, trays and paper towels used to line the bottom of the trays were soaked with fungicide (Maestro 80DF, Active ingredient: Captan 80%, Arysta Life Sciences North America Corporation, Cary,NC 27573 USA. Plant Products Company Limited, Bramalea, Ontario Canada L6T 1G1) prior to planting to prevent fungus growth. Fungicide was later applied weekly during the study by spraying over the plants. This method proved only partially effective since no fungal contamination was seen on the roots, though the pots still showed some contamination.

Treatments- *Root neighbours- I.* cf. *pallida* seeds were planted in cuboid-shaped board pots (Zipset Plant Bands, bleached board, light weight, from Stuewe & Sons, Inc., Corvallis, Oregon, USA) with constant cross-sectional area. The solitary treatment consisted of one plant per pot (3.8x3.8x35.5 cm) while the root neighbour treatment consisted of four plants per pot of four times the cross-sectional area (7.6x7.6x35.5 cm). Each pot in the root neighbour treatment was matched with four pots in the solitary treatment, so that sample size was equal for each treatment. The pots were arranged in 8 square trays (30x30 cm) in a checker-board pattern that held 64 plants per tray for a total of 512 plants. Utilizing these dimensions ensured that a solitary plant had the same soil depth and average soil volume as a plant with root neighbours. It also allowed us to maintain a constant aboveground density of 689 plants per square meter, and so create equivalent aboveground competition conditions for plants in solitary and root neighbour treatments (Fig. 1).

Kin vs. stranger- Groups of four plants were either kin (selfed siblings) or strangers (from four different families from the same field population). A total of 8 families were utilized. Each tray had a subset of four of those families with every family being equally represented in both the root neighbour and kin treatments within each tray.

Light- In the light treatments the ratio of red to far-red light (R:FR) was manipulated through lateral shades while overhead irradiance was kept constant

(Ballaré, Scopel, and Sanchez, 1991; Dudley and Schmitt, 1995, 1996; Maliakal et al., 1999). Each tray of 64 plants was placed into one of the light treatments, either high R:FR or low R:FR (four trays per light treatment). Light was provided by a mix of fluorescent lights (fluorescent cool white Philips lamps), which provided light rich in red relative to far-red, and incandescent lights that provided further far-red light. To create the low R:FR treatment, each tray was fenced with hardware cloth covered with banana leaves that provided reflected light low in red compared to far-red (R:FR *c*. 0.2) (Ballare, Scopel, and Sanchez, 1990; Schmitt and Wulff, 1993). The hardware cloth was initially placed 15 cm above the canopy level and was periodically raised to compensate for canopy growth. The high R:FR treatment was created in a similar fashion with a neutral shade attached to the hardware cloth instead of the banana leaves (R:FR *c*. 1.9). This created different R:FR conditions while keeping similar transmittance levels in both treatments.

Data collection- Aboveground harvesting was done at an early stage in reproduction (six weeks) so that allocation during vegetative growth could be determined. Measurements of node height, total plant height, and branching were taken and aboveground biomass was partitioned into leaves, stems, and reproductive structures, dried at 50°C and weighed. Belowground harvesting was done a week after aboveground harvesting. Roots were collected by washing, dried at 50°C, weighed, and then roots larger than 1 mm diameter were isolated and weighed. Fine root mass (roots less than 1 mm diameter) was calculated from the difference of total root mass and larger roots. During that time, trays containing the roots were stored in a cold room at 5°C. For plants in the root neighbour treatments, all roots in a single pot were weighed together because of the difficulty of assigning roots to individual plants. Appendix 1 contains tables with means, standard errors, and significance values for root, stem and leaf biomass.

Data analysis- The data were analyzed utilizing SAS statistical software (version 8.02). We used PROC GLM to carry out analyses of variance and covariance. We used analysis of covariance to test for differences in allocation and elongation (Coleman, McConnaughay, and Ackerly, 1994; McConnaughay and Coleman, 1998). Elongation was measured as the least square mean (lsmean) from an analysis of covariance with plant total height as the dependent variable and stem weight as the covariate (LSMEANS option, PROC GLM). Because trays were assigned to different light treatments, F-ratios for light effects were tested over the mean square for trays, nested within the light effect, in the denominator. Kin and root neighbour main effects and interactions, because these treatments were applied within trays, were tested over the mean square error. Because of the difficulty of separating the roots of plants in the root neighbours present treatment, the experimental unit for belowground traits and total biomass were groups of four plants, either the four in a large pot, or four adjacent plants in single pots. For aboveground traits the experimental unit was the individual. The data for belowground analysis was log-transformed to ensure that the residual variance was homoscedastic and the distribution of the residuals did not differ significantly from normality. Parameters are presented untransformed for clarity. Following Coleman, McConnaughay, and Ackerly, 1994; Cahill, 2003 we used differences in the estimated relation between components of plant mass rather than proportional biomass ratios to test for differences in allocation. Allocation to roots was measured as the least square mean from an analysis of covariance with fine root mass as the dependent variable and leaf mass as the covariate (Dudley and File, 2007). These traits were selected because they function directly in aboveground and belowground resource acquisition respectively (Givnish, 1986). Root:shoot ratios are sensitive to increases in stem mass resulting from increased stem elongation. For allocation to leaves, leaf mass was the dependent variable and stem mass the covariate, with a second-order polynomial term included. Branchiness was determined by using branch number as the dependent variable and leaf mass as the covariate.

RESULTS

No direct effects of root neighbours were found for morphological or allocation traits (Tables 1 and 2). There were significant differences between kin and stranger treatments for several traits. Plant height, number of branches, branchiness (number of branches corrected for leaf mass), and elongation were increased in response to kin (Tables 1 and 2, Fig. 2). More alternate branches were found for plants in the kin treatment, but opposite branching did not differ between kin and strangers (Table 1). Allocation to leaves relative to stem and allocation to leaves relative to roots were increased in response to strangers (Table 2, Fig. 2). For each trait, there was a significant kin by root interaction (Fig. 2), where the differences between kin and strangers occurred only in the presence of root neighbours. Solitary plants showed no difference between kin and strangers for any trait.

Surprisingly, in the root neighbours treatment the hypocotyl ($F_{1,477}$=7.48**) and internode 1 ($F_{1,477}$=5.50*) were longer than in the solitary treatment. Significant kin and root × kin effects in internode 1 (solitary: kin= 4.5cm, strangers= 4.6cm; root neighbours: kin= 5.2 cm, strangers= 4.5cm; $F_{1,477}$ (kin)= 5.63* , $F_{1,477}$ (kin×root)=9.15**) and internode 2 (solitary: kin= 4.1cm, strangers= 4.2cm; root neighbours: kin= 5.0 cm, strangers= 3.9cm; $F_{1,474}$ (kin)= 5.61* , $F_{1,474}$ (kin×root)=10.02**) could be attributed to the increased internode lengths in plants growing with kin in the root neighbours treatments compared to all others.

The light treatment directly affected plant height, elongation and leaf allocation (Tables 1 and 2). Under low R:FR, *I.* cf. *pallida* became taller (height:high R:FR = 16.4 cm, low R:FR = 21.4 cm; SE = 0.58), more elongated (height corrected for stem mass: high R:FR = 15.9 cm, low R:FR = 21.8 cm; SE = 0.24), and allocated more to leaves compared to stem (leaf mass corrected for stem mass: high R:FR = 0.14, low R:FR = 0.10; SE = 0.001). The expected increase in internode length in lowered R:FR was found for the hypocotyl ($F_{1,6}$=6.08*) , internode 1($F_{1,6}$=12.1*), and internode 2 ($F_{1,6}$=12.02). Low R:FR also decreased root allocation, although only in the presence of root neighbours (Fig. 3).

Total biomass and leaf area were not affected by any treatment (results not shown, all P > 0.1).

DISCUSSION

In this study we examined the responses in *I.* cf. *pallida* (Yellow Jewelweed) to the aboveground cue of competition, R:FR, and to the presence of belowground neighbours, testing for kin recognition by determining if the responses depended on whether the neighbours were siblings or strangers. As found in previous studies performed in legumes and *C. edentula* (Great Lakes Sea Rocket) (Gersani et al., 2001; Maina, Brown, and Gersani, 2002; O'Brien, Gersani, and Brown, 2005; Murphy and Dudley, 2007), *I.* cf. *pallida* showed responses to the presence of root neighbours. However, these responses differed between plants interacting with kin and those interacting with strangers. These findings provide strong evidence in support of the hypothesis that *I.* cf. *pallida* are capable of kin recognition. They also indicate that root interactions are required for kin recognition since, as also found by Dudley and File (2007), kin effects were only observed in the presence of root neighbours. In contrast to *C. edentula,* however, *I.* cf. *pallida* increased allocation towards competitive traits aboveground instead of belowground in response to strangers.

We had predicted that groups of kin would demonstrate cooperation, defined as the absence or reduction of a competitive response, while groups of strangers would demonstrate a competitive response. The results for the trait leaf:root allocation met this expectation. In root neighbour groups of kin, root allocation did not differ from that of solitary plants. However, when root neighbour groups were strangers, *I.* cf. *pallida* showed shifts in allocation that would allow the plant to be more competitive by increasing its carbon acquiring capabilities. These shifts in allocation included increased allocation to leaves relative to stems and roots. These results contrast with the previous kin recognition study (Dudley and File, 2007) which found that under the presence of strangers *C. edentula* plants had increased root allocation as a competitive trait. Taken together, these results show that differential responses to kin and strangers are likely to be species-specific. While *C. edentula* grows in a beach environment where belowground resources are the limiting factors (Dudley and File, 2007), *I.* cf. *pallida* grows in woody areas where light acquisition is essential (Schmitt et al., 2003). Therefore, the responses of each species are consistent with their ecology, with *C. edentula* showing increased allocation towards competitive traits belowground and *I.* cf. *pallida* showing increased allocation towards competitive traits aboveground in response to strangers.

In animals, kin selection often results in positive behaviours towards kin but not strangers, such as alarm calls, grooming and foodsharing (Waldman, 1988). In this study, *I.* cf. *pallida* showed changes in aboveground morphology in the presence of kin that differed from the phenotype demonstrated by solitary plants and strangers sharing pots. We observed an increase in elongation as well as branchiness in plants sharing a pot with kin. Increased elongation was seen in height relative to stem mass and in increased length of the hypocotyl and the first two internodes, similar to elongation induced by R:FR. Are these potentially altruistic responses? Although elongation is usually considered a competitive response when associated with low R:FR (Smith, 1982; Dudley and Schmitt, 1995, 1996), the degree of elongation showed by *I.* cf. *pallida* in response to kin was not as pronounced as that elicited by low R:FR, suggesting the role of elongation here to be different. Further evidence that the observed elongation was not a competitive response was the lack of the shift in allocation of biomass towards stem that is often observed accompanying the competitive elongation response (Cipollini and Schultz, 1999). Moreover, branchiness is normally reduced when plants respond to low R:FR, increasing apical dominance and vertical growth to allow the plant to gain access to light and shade neighbouring competitors in the process (Smith, 1995). Here however, in response to kin, we observed increased branchiness, a trait argued to reduce self-shading (Pearcy, Muraoka, and Valladares, 2005). Kelly (I996) found that in *I. capensis,* target plants performed better when plants surrounding them had a more bushy phenotype. Several studies show that small changes in aboveground traits can increase a stand's competitive ability (Sakai, 1991; Schieving and Poorter, 1999; Falster and Westoby, 2003). Therefore, we hypothesize that the increases in elongation and branchiness observed as responses to kin, are changes in morphology with the objective of reducing leaf overlap in the stand and thus neighbouring shading (reduced interference). Reduced mutual shading in a stand of siblings is consistent with kin selection theory.

This study, because young plants were measured, doesn't address the question of whether differential behaviour towards kin and strangers in *I.* cf. *pallida* conveys significant fitness benefits. In the case of reduced competition towards siblings the expected benefits are twofold: plants increase their inclusive fitness by making valuable resources more available to their neighbouring kin, and avoid paying the cost of competition. Several studies have followed groups of plants through to measure final fitness (Donohue, 2003). Some have found that groups of kin outperform groups of strangers in competitive environments. However, other found that strangers outperform kin, which is more consistent with the resource partitioning hypothesis (Maynard Smith, 1978; Price and Waser, 1982; Barton and Post, 1986; Cheplick and Kane, 2004). The resource partitioning hypothesis suggests that relatives are more phenotypically similar, and so will compete more intensely for the same share of resources than would more dissimilar plants. Relatedness may both elicit phenotypic plasticity and affect the competitive environment. These processes are not mutually exclusive, so both could co-occur.

Although these results indicate that *I.* cf. *pallida* is capable of kin recognition, the mechanism by which plants recognize kin is still unknown. In their experiment with *C. edentula,* Dudley and File suggested that plants were able to recognize kin through some form of root communication, since *C. edentula* only showed responses to kin in the presence of root neighbours. In this experiment we provide evidence in support of that hypothesis. In *I.* cf. *pallida*, like in *C. edentula,* all traits that showed a differential behaviour towards either kin or strangers did so only in the presence of root neighbours (Tables 1 and 2, Fig. 2).

I. cf. *pallida* also responded to low R:FR, the aboveground cue of competition. These findings are in accordance with previous studies performed in *I. capensis* (Dudley and Schmitt, 1995, 1996; Maliakal et al., 1999). In contrast to a previous study performed in soybean however (Murphy and Dudley, 2007), *I.* cf. *pallida* showed a reduction in root allocation under low R:FR that occurred only in the presence of root neighbours. This result shows for the first time the existence of an interaction between the above and belowground competition cues, where a response to one cue is triggered only in the presence of the other. This result suggests that in *I.* cf. *pallida*, future studies that look at responses to cues that signal competition above and belowground should examine these responses simultaneously.

Some recent studies have suggested that the experimental design used here to determine the effects of belowground neighbours may be flawed because of confounding effects of pot size (Schenk, 2006; Hess and de Kroon, 2007). Others however, argue differently (O'Brien and Brown, 2008). While the possibility of pot size being perceived as a resource itself by plants is an interesting hypothesis, Schenk (2006) and Hess and de Kroon (2007) only argue in its favour from a theoretical standpoint by demonstrating that much of the published data is consistent with this hypothesis. In a very large empirical study however, McConnaughay and Bazzaz (1991) showed that while pot size does affect size traits, it had no effect on allocation, which is the main focus of this study. We also performed a parallel study where we compared the effects of the different pot sizes used in this study on *C. edentula* and also found no effects on allocation (unpublished data). Moreover, predictions by Schenk (2006) and Hess and de Kroon (2007) consider previous results that only found an increase in root allocation with root neighbours present to confirm their hypotheses, since they suggest that root allocation increases with greater pot size. However, we found the opposite result, decreased root allocation with root neighbours, to occur in this study, which suggests that the changes in allocation found here were not an artifact of inadvertently manipulating pot size but a response to the presence of neighbours belowground.

This study provides evidence in support of the hypothesis that plants are capable of kin recognition. It demonstrates that kin recognition does occur in *I.* cf.

pallida and through a mechanism that involves root interactions. Moreover, it shows for the first time that responses to above and belowground competition cues can be dependent on one another, and demonstrates that a considerable degree of complexity is possible in response to kin or strangers. Future studies should be performed in other species to determine how widespread kin recognition is in plants, and how diverse is the spectrum of plant responses to growing with siblings. Past studies measured the fitness consequences of growing with kin versus growing with strangers and identified kin selection and niche partitioning as potential driving forces. However, we don’t know yet to what extent these forces can co-occur. The challenge for future studies is to be able to identify how plants recognize kin so we can manipulate their responses to kin. Only then will we be able to elucidate the fitness consequences of kin selection and niche partitioning.

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Table 1. Analyses of variance for above and belowground traits for growth-room grown *I.* cf. *pallida.*

		Height		Branch #		Opposite Branches		Alternate Branches	
Source	Df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Light*	1	8.12	**0.0292**	1.71	0.2393	0.01	0.9231	2.50	0.1650
Neighbours	1	3.46	0.0634	2.35	0.1256	0.91	0.3397	1.70	0.1929
Kin	1	1.30	0.2557	0.31	0.5797	0.10	0.7487	1.40	0.2377
LightxNeighbours	1	0.73	0.3949	0.01	0.9159	1.74	0.1881	1.13	0.2891
LightxKin	1	1.60	0.2060	0.59	0.4438	0.34	0.5589	2.67	0.1028
NeighboursxKin	1	8.49	**0.0037**	8.05	**0.0047**	0.67	0.4139	9.30	**0.0024**
LightxNeighboursxKin	1	0.58	0.4448	0.32	0.5707	0.01	0.9126	0.77	0.3804
Tray(Light)	6	4.58	**0.0002**	6.02	**<.0001**	5.49	**<.0001**	7.90	**<.0001**

Notes:

* Treatment tested over tray nested with light interaction.

Degrees of freedom for the error terms were: height 476, branch number 475, opposite branches 477, alternate branches 477.

Bold numbers indicate significant values

Table 2. Analyses of covariance for above and belowground traits for growth-room grown *I.* cf. *pallida.*

		Elongation (plant height/stem mass)		Leaf Allocation (leaf mass/stem mass)		Branchiness (# of branches/leaf mass)		Root Allocation (fine root mass/leaf mass)	
Source	Df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Light*	1	32.33	**0.0013**	22.62	**0.0032**	0.27	0.6210	1.62	0.2497
Neighbours	1	1.97	0.1610	0.78	0.3789	0.51	0.4742	0.28	0.5952
Kin	1	8.29	**0.0042**	7.57	**0.0062**	1.52	0.2186	1.46	0.2294
LightxNeighbours	1	2.41	0.1212	0.74	0.3906	2.37	0.1245	4.04	**0.0467**
LightxKin	1	0.35	0.5550	1.73	0.1896	0.01	0.9191	1.26	0.2645
NeighboursxKin	1	6.14	**0.0136**	5.99	**0.0147**	5.18	**0.0233**	6.51	**0.0120**
LightxNeighboursxKin	1	0.26	0.6127	1.48	0.2250	0.10	0.7502	2.78	0.0983
Tray(Light)	6	8.70	**<.0001**	7.09	**<.0001**	14.86	**<.0001**	2.97	**0.0099**
Covariate	1	1126.21	**<.0001**	920.05	**<.0001**	414.24	**<.0001**	239.35	**<.0001**

Notes:

* Treatment tested over tray nested with light interaction.

Degrees of freedom for the error terms were: elongation 474, leaf allocation 475, branchiness 471, root allocation 113.
Stem mass and leaf mass were used as covariates for elongation, leaf allocation and branchiness, root allocation respectively.

Bold numbers indicate significant values

APPENDIX 1

Table 1. Part A: Means and standard errors (in brackets) for root, stem, and leaf biomass for growth-room grown *I.* cf. *pallida.*

Part A	Treatment combination							
Variable	NHK	NHS	NLK	NLS	YHK	YHS	YLK	YLS
Total root biomass (g)	0.2555	0.3752	0.3015	0.2826	0.4989	0.3256	0.2908	0.2548
	(0.0222)	(0.0485)	(0.0390)	(0.0215)	(0.0756)	(0.0320)	(0.0284)	(0.0252)
Stem biomass (g)	0.0483	0.0784	0.0905	0.0827	0.1120	0.0785	0.0963	0.0762
	(0.0060)	(0.0086)	(0.0134)	(0.0096)	(0.0157)	(0.0112)	(0.0122)	(0.0111)
Leaf biomass (g)	0.0876	0.1320	0.1214	0.1073	0.1825	0.1502	0.1239	0.1009
	(0.0101)	(0.0144)	(0.0205)	(0.0143)	(0.0254)	(0.0215)	(0.0192)	(0.0161)

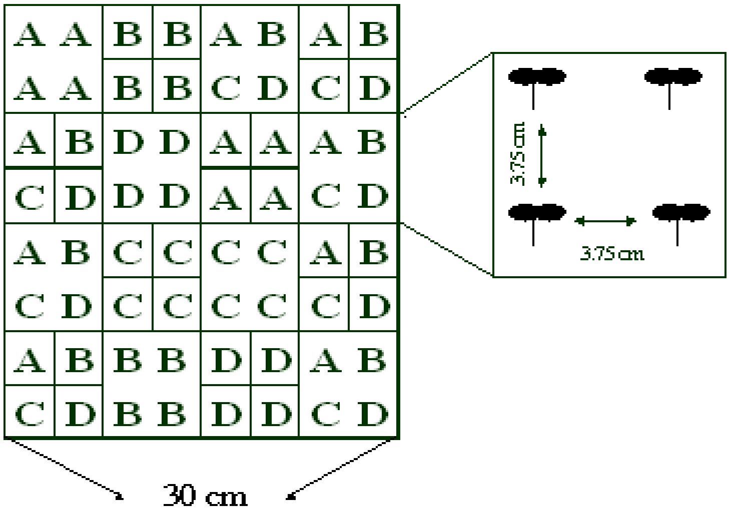
N: neighbours absent
Y: neighbours present
H: high R:FR
L: low R:FR
K: kin
S: stranger

Part B: *P* values for root, stem, and leaf biomass for growth-room grown *I.* cf. *pallida*.

Part B	Source						
Variable	Light	Neighbours	Kin	LxN	LxK	NxK	LxNxK
Total root biomass	0.1012	0.2595	0.8272	0.1054	0.6573	**0.0105**	0.1312
Stem biomass	0.5746	0.2792	0.4196	0.5778	0.2980	**0.0099**	0.4846
Leaf biomass	0.1113	0.3184	0.8007	0.2508	0.3030	0.1131	0.7909

Bold numbers indicate significant values from ANOVA

Fig. 1) Overtop two-dimensional representation of a typical tray used in the experiment containing 8 big pots and 32 small pots. Letters represent plants of a particular family. Groups of four plants of the same family form the “kin” treatment while groups of four plants of different families form the “stranger” treatment. Pots that contain 4 plants represent the “root neighbours” treatment while pots containing only one plant represent the “solitary” treatment. The distance between plants is 3.75 cm in all instances, thus maintaining aboveground density constant. Thickness of the pots is negligible (<1 mm).



A	A	B	B	A	B	A	B
A	A	B	B	C	D	C	D
A	B	D	D	A	A	A	B
C	D	D	D	A	A	C	D
A	B	C	C	C	C	A	B
C	D	C	C	C	C	C	D
A	B	B	B	D	D	A	B
C	D	B	B	D	D	C	D

Fig. 2) Effect of root neighbours and kin on root allocation, leaf allocation, branch number, branchiness, elongation, and plant height for growth room grown *I.* cf. *pallida*. Letters indicate significant statistical differences between treatments at the 0.05 level. Bars indicate 1 SE. Data averaged over both light treatments.

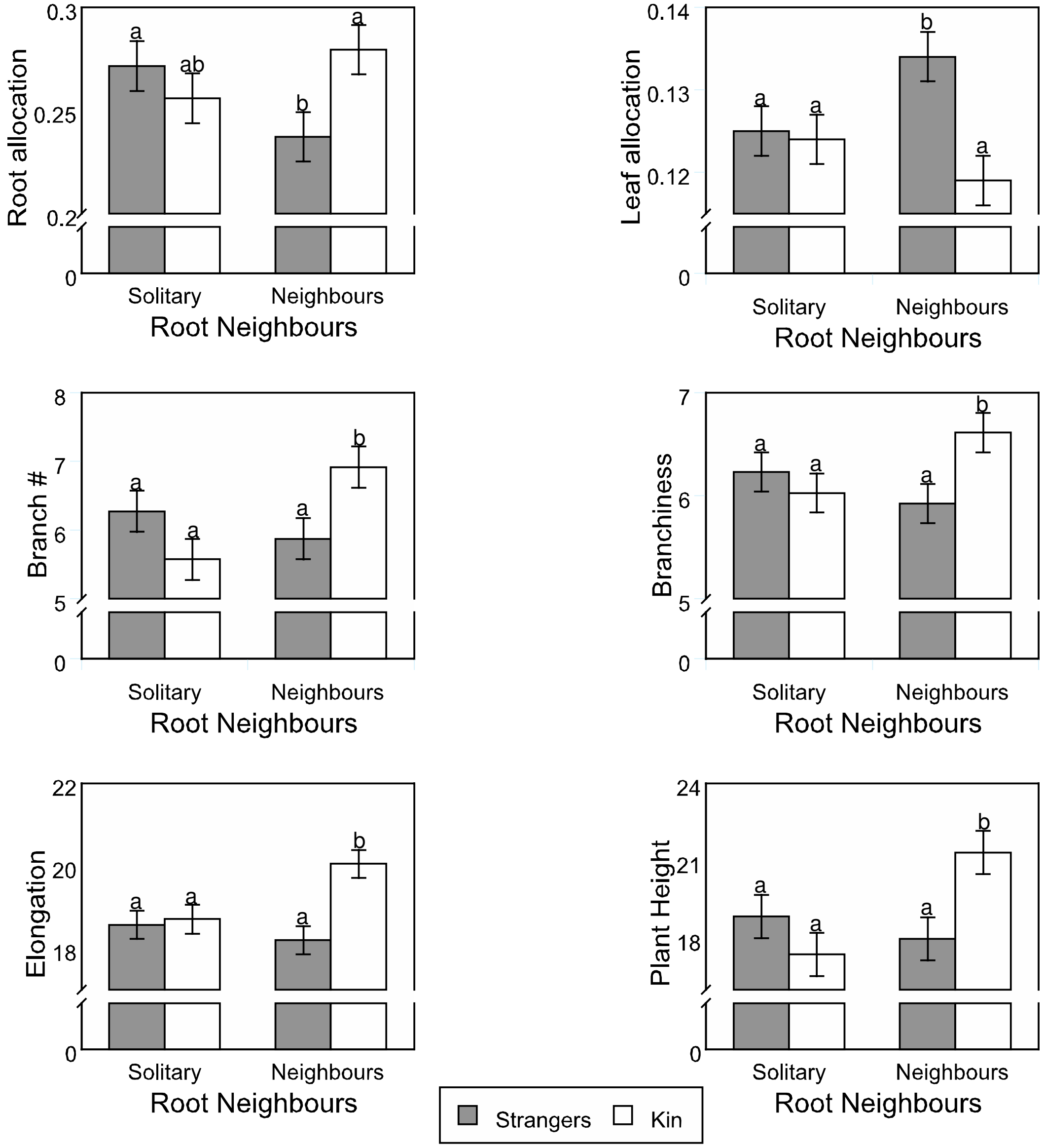
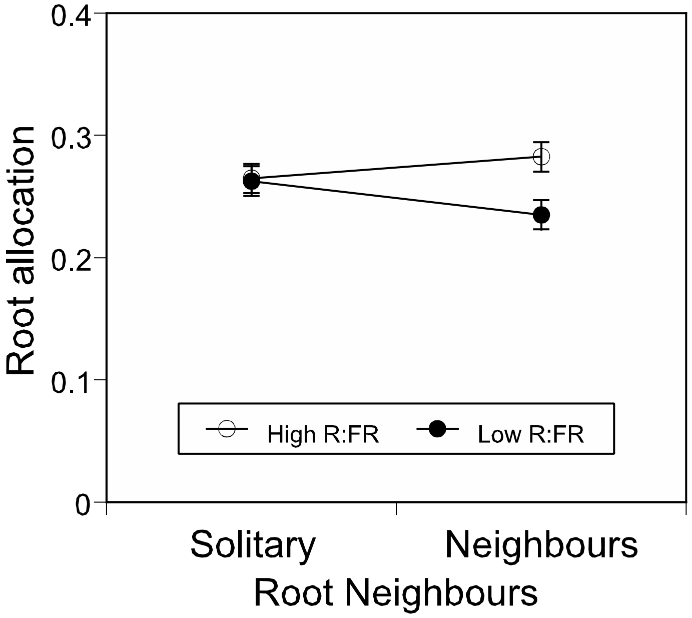

Root allocation
0.3
0.25
0.2
0
a
ab
b
a
Solitary
Neighbours
Root Neighbours
Leaf allocation
0.14
0.13
0.12
0
a
a
b
a
Solitary
Neighbours
Root Neighbours
Branch #
8
7
6
5
0
a
a
a
b
Solitary
Neighbours
Root Neighbours
Branchiness
7
6
5
0
a
a
a
b
Solitary
Neighbours
Root Neighbours
Elongation
22
20
18
0
a
a
a
b
Solitary
Neighbours
Root Neighbours
Plant Height
24
21
18
0
a
a
a
b
Solitary
Neighbours
Root Neighbours
▨ Strangers
☐ Kin

Fig. 3) Interaction plot for root allocation (least square means of fine root biomass using leaf biomass as a covariate) in response to root neighbours and light for growth room grown *I.* cf. *pallida*. Bars indicate 1 SE.


0.4
0.3
0.2
0.1
0
Root allocation
High R:FR
Low R:FR
Solitary
Neighbours
Root Neighbours

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CHAPTER 3:

DIFFERENTIATING THE EFFECTS OF POT SIZE AND NUTRIENT AVAILABILITY ON PLANT BIOMASS AND ALLOCATION

ABSTRACT

An alternative proposed explanation to apparent plant responses to neighbours is pot size effects caused by experimental manipulation. The idea is differences in pot size alone can elicit increases in root production and allocation, so that plant responses attributed to the presence and identity of neighbours, could instead result from plants growing in bigger pots. We tested the effects of pot size on growth and allocation while controlling for nutrient amount, concentration, plant density, and life stage. We found that total biomass and allocation to roots either decreased or remained unaffected in response to increases in pot size for all treatment combinations except for plants in high water-soluble fertilizer, which showed increased total biomass and allocation to roots in response to pot size. Therefore, pot size itself was not a good predictor of changes in growth or allocation. Instead, the effects of pot size were dependent on nutrient amount, nutrient concentration and life stage, which were better predictors. These results do not support the hypothesis that plant responses attributed to the presence of neighbouring plants are an artefact of pot size manipulation.

INTRODUCTION

An increasing body of research demonstrates that plants obtain information about the presence and identity of neighbours through root interactions (Novoplansky 2009). Several studies have shown that plants are capable of identifying whether roots from neighbours are present nearby even when controlling for resource depletion (Cahill et al. 2010; Gersani et al. 2001; Maina et al. 2002; Murphy and Dudley 2007; O'Brien et al. 2005) and that plants can distinguish self from non-self roots (Falik et al. 2003; Gruntman and Novoplansky 2004; Holzapfel and Alpert 2003), siblings from strangers (Bhatt et al. 2010; Biedrzycki et al. 2010; Biernaskie 2011; Dudley and File 2007; Murphy and Dudley 2009), and same from different species (Maestre et al. 2009; Mommer et al. 2010; Semchenko et al. 2007b). Presence/absence recognition studies have shown variation in how plants respond to the presence of neighbouring roots from a different individual. *Impatiens pallida* has been shown to decrease root production (Murphy and Dudley 2009), as did *Chenopodium album* (unpublished data). However, studies done in legumes found increased root production, even when average soil resources were kept constant. Root overproduction may allow plants to pre-empt competition and could result in a tragedy of the commons because competing plants overproduced roots at the cost of reproductive biomass (Gersani et al. 2001; Maina et al. 2002; O'Brien et al. 2005). Using root growth as an indicator, studies that looked at self/non-self recognition have demonstrated that plants recognized a physiologically connected plant as self, while separated clones were recognized as non-self (Falik et al. 2003; Gruntman and Novoplansky 2004; Holzapfel and Alpert 2003). These presence/absence and self/non-self studies, in particular, have been controversial.

The issue with many presence/absence and identity recognition studies is whether there are confounding effects caused by the method through which resource availability is controlled. Many studies maintained average resources per plant constant by increasing pot size (absolute rooting volume) proportionately with number of plants to uncouple the effects of resource availability from the responses specific to the presence or identity of the competitor (Gersani et al. 2001; Maina et al. 2002; Murphy and Dudley 2007; O'Brien et al. 2005). If pot size can affect plant growth, then apparent responses to the presence or identity of neighbours could instead be attributed to pot size effects (Hess and de Kroon 2007; Schenk 2006; Semchenko et al. 2007a).

Hess and de Kroon (2007) provided a set of formal hypotheses that, coupled with the root foraging response in which root proliferation increases in a high nutrient patch (Kembel and Cahill 2005), although not every species shows this proliferation response, provide an alternative explanation for most of the results of the presence/absence and self/non-self studies where pot size was manipulated. Their hypotheses are:

I. "Root mass is a function of available rooting volume independent of nutrients, i.e. a plant will fill up the available volume with its roots."

II. “The growth of a plant is limited by the total amount of nutrients available to the plant.”

III. “If a plant produces more root biomass but similar total biomass, it will produce less reproductive and/or shoot biomass.”

According to their hypotheses, root mass should be a function of pot size alone, while plant growth should be limited by nutrient availability. Then, as a result of a trade-off between root and shoot biomass, root allocation should increase in larger pot volumes. Although elegantly argued, these controversial hypotheses by Hess and de Kroon (2007) have not been empirically tested. These arguments have been considered plausible (Hodge 2009) but have also been disputed (O'Brien and Brown 2008).

Indirect evidence against Hess and De Kroon hypotheses already exists from a study that has shown plants to respond to the presence of neighbours by reducing root allocation, which contradicts the predictions made by the pot size hypotheses (Murphy and Dudley 2009 (Chapter 2)). New evidence has also arisen for plant identity recognition that does not involve pot size manipulation (Bhatt et al. 2010; Biedrzycki et al. 2010; Biernaskie 2011; Mommer et al. 2010) or compares responses to identity within the same pot size (Dudley and File 2007; Murphy and Dudley 2009). However, although researchers have investigated the role of pot size before, no one has yet empirically tested whether pot size affects root allocation as predicted by the pot size hypotheses proposed by Hess and de Kroon (2007).

In previous pot size studies, the control of confounding variables has depended on the question of the study. For example, studies that investigated the effect of pot size on plants with the objective of propagation for production often confounded the effects of increased aboveground density because plants were simply grown in smaller plugs (e.g. Di Benedetto and Klasman 2004). Similarly, when asking whether plants should be initially grown in smaller pots and then progressively transplanted into larger pots, Beeson (1993) applied fertilization proportional to pot size, thus confounding the effects of nutrient availability (Beeson 1993). Because of the confounded nature of pot size and nutrient availability, understanding the relative roles of pot size versus nutrient amount on growth and allocation while controlling for density is crucial. Two studies have tested rigorously for the effects of volume and nutrients across multiple species (Gurevitch et al. 1990; McConnaughay and Bazzaz 1991). McConnaughay and Bazzaz (1991) manipulated volume and nutrient concentration of water-soluble fertilizer (WSF), but found no significant effect of pot volume on root:shoot ratio. Gurevitch et al. (1990) manipulated pot volume and added controlled release fertilizer (CRF) proportionately to volume, and found decreased root: shoot ratio with increased pot volume. Neither of these studies supported the prediction that increased root:shoot allocation found by presence/absence and identity recognition studies are a result of pot size. However, these studies were not designed to test Hess and De Kroon predictions and make no mention on controlling for density effects.

Here we conducted two experiments specifically designed to determine whether pot size itself affects plant growth and allocation. We kept aboveground density constant and tested the effects of rooting volume and rooting depth on juveniles and early-reproductive single plants of the annual plant *Cakile edentula*. We applied nutrients in two ways in order to experimentally separate nutrient concentration and nutrient amount. A water-soluble fertilizer (WSF) treatment resulted in constant nutrient concentrations as pot size changed while a controlled release fertilizer (CRF) treatment resulted in a constant amount of nutrients as pot size changed. The first experiment was harvested when plants were juveniles while the second was harvested with most plants at an early-reproductive stage. We asked the following questions: 1) Does pot size affect plant growth and allocation? 2) Are the effects of pot size conditional on nutrient type, nutrient availability and life stage? 3) Are these pot size effects and interactions those predicted by Hess and de Kroon (2007)?

MATERIALS & METHODS

Experimental Design

Great Lake Sea Rocket (*Cakile edentula* (Bigelow) Hook. ssp. *edentula* var. *lacustris*) was used for these experiments because it has been shown to respond to the presence and identity of neighbouring plants (Bhatt et al. 2010;

Dudley and File 2007). *C. edentula* also germinates quickly and naturally grows in sand, which facilitated the harvesting of roots.

Two experiments were conducted using the same experimental design. In the first experiment, plants were harvested at 7 weeks (at a juvenile stage) while harvesting of the second experiment begun at week 11 (to also look at responses at an early-reproductive stage). Both experiments were composed of the same treatments in a factorial design: 4 pot widths, 3 pot depths, 2 nutrient types, and 2 nutrient levels. However, we analyzed the data using volume (with and depth combined) and depth by itself as treatments, rather than width and depth, to provide a better representation of the effects of pot size. We also considered life stage as treatments (for an average representation of size differences between life stages see supplementary figure 1).

Seeds from a single population of *C. edentula* were collected from Confederation Park, Hamilton, Ontario in September 2008 and used in both experiments. The juvenile experiment was conducted in November-January, 2008 and the early-reproductive experiment in September-November, 2009. For each experiment, a total of 360 seeds were surface sterilized using a 10% bleach solution for 15 minutes. Seed coats were scarified, seeds were soaked in distilled water overnight and then planted the following day. Seeds were planted singly in a pot with 3:1 sand and turface (Profile Products LC, Buffalo Grove, IL, USA) mix. For the first experiment, seeds that did not germinate within 7 days of planting were replaced. For the second experiment, seeds that did not germinate within 19 days of initial planting were replaced. All plants were grown in a greenhouse and received natural and supplemental light.

Treatments

Pot volume

Six different volume treatment classes were created by using open-ended cuboid cellulose pots (Plant Bands; Monarch manufacturing, Colorado) of four widths (3.81 cm, 5.08 cm, 7.62 cm, and 10.16 cm) and three depths (8.89 cm, 17.78 cm, or 35.56 cm) (Table 1). Trays contained nine pots of randomly selected volumes. The distance between the centers of the pots was kept equal in order to maintain a constant density within and across trays. All pots were raised to an equal height at 35.56 cm above bench level by placing shorter pots on Styrofoam so that they would not be shaded by taller pots (Fig.1). Trays were arranged randomly across three adjacent greenhouse benches. In the early-reproductive experiment, these benches were considered blocks in the statistical analysis because of large differences in performance among greenhouse benches.

In the juvenile life stage experiment, each pot width and depth combination was replicated 30 times. Thus, the extreme volume treatment classes consisted of 30 replicates while the intermediate volume treatment classes consisted of 60 and 90 (Table 1). In the early-reproductive stage experiment, we increased the replication of the extreme volume treatment classes (0.13L and 3.67L) from 30 to 40 and reduced the replication of the intermediate volume classes (0.50L and 0.95L) from 90 to 79 pots for the 0.50L treatment and from 90 to 81 pots for the 0.95L treatment.

Nutrients

We created two nutrient type treatments by fertilizing with either a controlled release fertilizer (CRF) (SmartCote, Plant Product Co Ltd, Brampton, ON; 14-14-14 NPK) or a water soluble fertilizer (WSF) (PlantProd, Plant Products Co Ltd, Brampton, ON; 20-20-20 NPK). CRF fertilizer was applied to soil surface one week after germination and gradually dissolved each time the plants were watered. By applying a CRF fertilizer, the total amount of nutrients per plant was kept constant but the concentration of nutrients decreased as pot volume increased. Such CRF fertilizers create a gradient of decreasing nutrient concentration with depth that does not differ between a leaching column and the field (Hanafi et al. 2002). WSF fertilizer was applied weekly by watering the plants until the soil was saturated with the nutrient solution. This kept the concentration of nutrients per unit of soil volume constant, while the total amount of nutrients available increased with increasing pot volume.

Two nutrient level treatments were created: high or low (one tenth of the high level). In both experiments, we used a high level of WSF (680 PPM) to compensate for the high leaching of nutrients from the sand-turface mix. For another fast-growing annual in sand-turface substrates, it was shown that growth and root allocation responses to nutrient availability showed thresholds at this level over which increases in nutrients create no further effects, neither positive nor deleterious (Sultan and Bazzaz 1993). In the juvenile life stage experiment, plants given CRF fertilizer received 3g for high nutrients, the supplier's recommended dosage and 0.3g for low nutrients. In the early-reproductive stage experiment we increased the CRF fertilizer to 6g per plant in the high nutrient treatment and 0.6g per plant in the low nutrient treatment because of the large differences seen between CRF and WSF nutrient treatments in the first experiment.

Data Collection

Aboveground biomass was separated into stems, leaves, and fruits (fruits were only present in the early-reproductive experiment), and dried in an oven at 65^{o}C for 72h, then weighed to obtain dry weights. During aboveground harvest, completed trays with only roots remaining were stored in a cold room at 5^{o}C. After aboveground harvest was finished, roots were washed of substrate, oven dried, separated into coarse (>1mm diameter) and fine roots (<1mm diameter), then weighed to obtain dry weights.

Data Analysis

The data was analyzed using SAS statistical software (version 10.1; SAD, Cary, NC, USA). We used PROC GLM to conduct analysis of variance and covariance. All variables were log transformed to satisfy the assumptions of GLM. Analysis of covariance (ANCOVA) was used to test for differences in allocation (Coleman et al. 1994; McConnaughay and Coleman 1998). Volume was considered a discrete treatment with six classes. We used PROC GLM with predetermined contrast statements to distinguish between the effects of each volume category. The data from both experiments were analyzed as a single data set with plant life stage at time of harvest (juvenile or early-reproductive) as a treatment. Root allocation was measured as the least square mean (lsmean) from an analysis of covariance with root mass as the dependent variable and shoot mass (the sum of leaves and stems) as the covariate (LSMEANS option, PROC GLM). Partial correlation analysis was used (PROC CORR) to look for evidence of trade-offs between allocation to root biomass and number of fruits, controlling for aboveground mass.

RESULTS

Total plant biomass

Plants at the early-reproductive stage showed increased total biomass compared to juveniles (early-reproductive=0.18g, juveniles =0.09g; SE=0.01) as did plants that were supplied with high nutrients (high=0.18g, low=0.09g; SE=0.009) and WSF (WSF=0.20g, CRF=0.09g; SE=0.009) (Table 2). However, interactions among these treatments influenced the degree of increased growth with, for example, early reproductive plants in high-WSF showing the highest biomass compared to juveniles while plants in low-CRF showed almost no difference between life stages (supplementary tables 1 and 2).

Pot volume had complex effects on total biomass that varied with nutrient type, nutrient level, life stage, and their interactions (Table 2). Overall, increasing volume reduced total biomass (linear effect of volume F=4.12, P<0.0429), with a significant intermediate optimum at 0.24L and 0.50L (quadratic effect of volume F=4.71, P<0.0303). However, this effect occurred in juveniles but not in early-reproductive plants, which were unaffected by changes in volume (volume × life stage: linear F=4.83, P<0.0284; quadratic F=8.21, P<0.0043) (supplementary table 3), indicating that big pot sizes were deleterious at the juvenile stage but had no effect at the early-reproductive stage. The effects of pot volume however, also depended on nutrient type (volume × nutrient type: linear F=22.41, P<0.0001), and on the interaction between nutrient type and nutrient level (volume × nutrient type × nutrient level; linear F=5.17, P<0.0233). Though pot volume did not affect total biomass in WSF overall, it increased total biomass in high WSF (supplementary table 4). In CRF, pot volume decreased total biomass regardless of nutrient level (supplementary tables 3 and 4). However, these interactions between volume and nutrients differed between early-reproductive and juvenile plants (volume × nutrient type × nutrient level × life stage: linear F=7.86, P<0.0052). Compared to juveniles, early-reproductive plants showed greater biomass increases with volume in high- and low WSF (Fig. 2). Though volume still decreased biomass in high CRF, the effect was smaller in early-reproductive plants. However in low CRF, volume decreased biomass even more strongly for early-reproductive plants than juveniles (Fig. 2). Therefore, increases in pot size had either negative or no effects for both juvenile and early-reproductive plants with the exception of early-reproductive plants in high WSF, which showed a positive effect.

Root biomass

The direct effects and interactions of life stage, nutrient type, and nutrient level on root biomass were similar to those of total biomass (Table 2). Plants at the early-reproductive stage showed increased root biomass (early-reproductive=0.05g, juveniles =0.02g; SE=0.002) as did plants that were supplied with high nutrients (high=0.04g, low=0.02g; SE=0.002) and WSF (WSF=0.04g, CRF=0.02g; SE=0.002). Significant interactions among these treatments influenced the degree of increased root growth with each of these treatments (supplementary tables 5 and 6).

Although volume had no direct effect on root biomass (Table 2), there were effects of volume that depended on plant life stage, nutrient type and the interaction between nutrient type and nutrient level (Table 2). Root biomass decreased with volume in juvenile plants but increased with volume in early-reproductive plants (volume× life stage: linear F=24.54, P<0.0001) (Fig. 3), indicating that plants may only benefit from increases in pot volume at a later life stage. Volume had no effect on root biomass for plants in either low WSF or low CRF (Fig. 4). However, root biomass increased with volume in high WSF and decreased with volume in high CRF (volume × ntype × nlevel: linear F=8.13, P<0.0045) (Fig. 4). These results indicate that only in one particular nutrient combination (high WSF) did increases in pot volume elicit increases in root biomass.

Root allocation

Plants at the early-reproductive stage had higher root allocation than juveniles (juveniles=0.02, early-reproductive=0.04; SE=0.001), as did plants in high nutrients compared to low nutrients (high=0.034, low=0.029; SE=0.001). However, increased root allocation in high nutrients occurred only in CRF but not in WSF, which did not differ significantly from low CRF and low WSF (high CRF=0.037, high WSF=0.031, low CRF=0.028, low WSF=0.029; SE=0.001) (Table 3).

Pot volume increased root allocation (linear volume F=17.81, P<0.0001). However, this effect also depended on life stage, nutrient type, nutrient level, and the interaction between nutrient type and nutrient level (Table 3). Volume increased root allocation in early-reproductive plants but had no effect in juvenile plants (volume× life stage: linear F=34.93, P<0.0001) (supplementary table 7). In WSF, volume increased root allocation but in CRF volume had no effect (volume× ntype: linear F=9.59, P<0.0021) (supplementary table 7). Volume increased root allocation in high nutrients (volume× nlevel: linear F=12.07, P<0.0006) while in low nutrients there was an intermediate optimum (volume× nlevel: quadratic F=8.19, P<0.0044) (supplementary table 7). The interaction between volume, nutrient type and nutrient level was significant (Table 3); however, neither linear nor quadratic effects were significant. Root allocation remained constant across volumes except at the highest volume (3.67L), where there was a marked increase in root allocation for plants in high WSF and a marked decrease in root allocation for plants in low CRF (Fig. 5).

Survival

Survival was lower in the highest pot volume compared to all other volumes for all nutrient combinations (volume=3.67L, survival= 73%, all other volumes, survival= 88%; P< 0.0189, from a generalized linear model of both experiments with a binomial link function).

Reproduction

The only treatment that affected fruit number was nutrient level (Table 4). Plants grown in low nutrients had more fruits than plants grown in high nutrients (low nutrients=1.58, high nutrients=1.55, SE=0.008, Table 4) possibly indicating delayed reproduction in high nutrient conditions. A partial correlation between fruit number and total root biomass was positive, even controlling for aboveground biomass (correlation coefficient=0.14324, P=0.0192) demonstrating that there was no evident tradeoff between root production and fruit production.

DISCUSSION

Pot size manipulation is a part of many plant studies, particularly those looking at the effects of presence and identity of neighbours. Here we ask whether pot size, measured as pot volume, affected plant growth and allocation, and whether those effects depended on nutrient type, nutrient availability and life stage. Notably, in this study density was controlled to a constant level, so neither differences in light availability nor cues of competition could have caused the pot size effects. Pot size did affect plant growth and allocation, but those effects strongly depended on nutrient type, nutrient availability, life stage, and interactions among these treatments. To interpret these complex effects, we will compare these results to predictions derived from Hess and de Kroon (2007).

The hypotheses proposed by Hess and de Kroon (2007) suggest four predictions for the present study. The first two predictions arise directly from the Hess and de Kroon (2007) hypotheses. Prediction 1) plants grown in larger pots will have greater root mass, regardless of nutrient type and nutrient level (hypothesis I). Prediction 2) plants given higher nutrient levels will have greater total mass, compared within the same volume, nutrient type, and life stage (Hypothesis II). The last two predictions are more complex, because they depend on nutrient type. Prediction 3 for WSF) plants grown in larger pots will have greater total mass because nutrient amount is the product of WSF concentration and pot volume (Hypothesis II, and see Table 3(b) from Hess and de Kroon (2007)). Prediction 3 for CRF) plant mass will be unaffected by pot size because the same amount of CRF was applied to all volumes (Hypothesis II). These effects of nutrient-type on the relationship between pot size and total mass are then carried through to predict root allocation across pot sizes (Hypothesis III). Prediction 4 for WSF) root allocation will remain constant across volumes because pot size simultaneously increases root mass and, from prediction 3 for WSF, total mass. Prediction 4 for CRF) root allocation will increase with volume because pot size increases root mass but, from prediction 3 for CRF, not total mass (See table 5 for a summary of these predictions).

Hess and de Kroon (2007) do not make predictions about change over time. However, life stage clearly affected the nature of the responses. Pot volume had negative effects on plant mass in juvenile plants, but not in early-reproductive plants. Root allocation was not affected by pot volume in juveniles, but did show an increase with pot volume in early-reproductive plants. Consequently, we will take life stage differences in consideration in interpreting how these results compare with the predictions.

A key prediction from Hess and de Kroon (2007) is that roots should fill up the available volume. Consequently, plants in bigger pots should have more roots at any given time and under any given nutrient conditions. However, in our study, root mass as well as the relation between root mass and pot volume were affected by nutrient treatments and life stage. Early-reproductive plants had more roots than juveniles. In early-reproductive plants, root mass did increase with volume, but root mass decreased with volume in juveniles. In one nutrient combination, high WSF, root mass increased with volume, but in two others root mass remained constant, and in high CRF, root mass decreased with volume. These results do not support the postulate that plant roots respond to pot size independently from other factors.

Increased nutrient availability resulted in greater plant size when the nutrient level was increased across all treatment combinations. Unsurprisingly, plants grown in high WSF and high CRF were bigger than plants grown in low WSF and low CRF. This finding supported prediction 2 that if the growth of a plant is limited by the total amount of nutrients available to the plant then for any given pot size, total plant biomass will increase with more nutrients, whether WSF or CRF.

Prediction 3 suggests that giving plants more nutrients by applying a constant level of WSF, but to larger pots, should also increase plant growth. This was partially supported by our results. Early-reproductive plants grown in larger volumes were bigger when given high and low WSF. In juveniles, though, increasing pot volume did not result in bigger plants in high WSF, and resulted in smaller plants in low WSF. Prediction 3 suggested that pot volume would be neutral for plants in the CRF treatment, since volume would not affect nutrient availability. However, we found that plants in CRF were smaller in greater pot sizes at both life stages. In juvenile plants, the effect of pot size was negative, and the highest biomass at intermediate pot sizes suggested an optimum size for growth. Thus total plant biomass was affected by volume, but not as predicted by nutrient availability at different pot sizes.

From these results, it is clear that pot size had mostly negative or neutral effects on growth, particularly in juvenile plants, while positive effects were only observed in early-reproductive plants supplied with WSF. Further evidence of negative effects is the low survivorship in larger pot sizes. These results were consistent with the common horticultural advice to match pot volume to plant size, as small pots may dry too quickly, while big pots may stay too wet. Soil that is more wet than necessary for the plant can be detrimental as it can create anoxic conditions (Agrios 1997). When more nutrients are present in the soil than can be used by the plant it may increase the presence of microbes.

The most important prediction for interpreting studies of recognition however, is how volume affects root allocation. It is possible that even with negative effects of volume on overall growth, volume may still affect root allocation as predicted by Hess and De Kroon (1997). In these results, the trends for root allocation with volume conflicted with the predictions by nutrient type. Prediction 4 for WSF indicated that root allocation would remain constant with volume, but in this study, root allocation tended to increase with pot volume in WSF. Prediction 4 for CRF indicated that root allocation would increase with volume, but in this study, root allocation was not affected by volume in CRF. In juveniles, volume had no effect on root allocation. This is important because some of the studies that showed responses to neighbours measured juvenile plants (Dudley and File 2007; Murphy and Dudley 2007; 2009). Therefore our findings do not support the alternative hypothesis that those results were caused by pot size manipulations.

In the largest volume, the differences among the nutrient combination effects were most variable, which may indicate deleterious effects of volume. Such deleterious effects were most important in juveniles, while early-reproductive plants showed increasing root allocation with pot volume. This trend was driven by plants grown in WSF, though the interaction was not significant. We speculate that the differences between early-reproductive plants and juvenile plants may occur because when plants are older and larger, they are able to forage for the more abundant resources in larger pots supplied with WSF by producing more roots, potentially to gain in higher aboveground mass later on.

Reproduction in the early-reproductive plants was not affected by pot size but was affected by nutrient level (Table 4). Plants that had fewer available resources produced more fruits at an early-reproductive stage, suggesting that plants will use different reproductive strategies depending on availability of resources. It has been suggested that there is a trade-off between root production and fitness (Hess and de Kroon, 2007). Here, however, root and fruit biomass were positively correlated. Therefore, these results suggest that no trade-off exists, at least in an early-reproductive stage.

Taken together, these results do not support the hypothesis that pot volume alone drives root mass and therefore root allocation. Instead, they show that pot volume can have negative effects on growth and survivorship, particularly at an early stage. While the decline in size with increasing volume for plants in CRF nutrients could result from dilution of those nutrients, it still supports the alternative hypothesis that excess volume can have deleterious effects. Therefore, we argue that by matching number of plants to pot size, researchers can avoid the deleterious effects of too small or too large pot size. The response seen here in older plants to forage for highly available nutrients would not be expected when pot size is matched to number of plants. Moreover, these results support the methodology of earlier harvesting during juvenile growth in annuals, when plants only grow vegetatively, and root allocation is not affected by pot volume.

In conclusion, these results resolve a current dilemma regarding methodological issues. They indicate that the alternative hypotheses suggested by Hess and de Kroon do not explain effects of pot volume in plants grown in individual pots. Taken together with the increasing body of work showing effects of identity in the same size of pot, this study indicates that the results of presence/absence and identity recognition studies cannot be dismissed as artifacts of pot size manipulation.

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Table 1 Pot widths and depths used to create six volume treatment classes. The twelve pot sizes derived from different widths and depths were used to create six volumes (in parenthesis) and correspond to the following volume treatment classes: 1=0.13L, 2=0.24L, 3=0.50L, 4=0.95L, 5=1.95L, 6=3.67L. Values in the table are rooting volume liters.

Width Depth	3.81 cm	5.08 cm	7.62 cm	10.16 cm
8.89 cm	0.129 (1)	0.229 (2)	0.516 (3)	0.918 (4)
17.78 cm	0.258 (2)	0.459 (3)	1.03 (4)	1.84 (5)
35.56 cm	0.516 (3)	0.918 (4)	2.06 (5)	3.67 (6)

Table 2 Analysis of variance for solitary plants of *C. edentula.* Log root mass and log total plant biomass are the dependent variables. Volume, life stage, nutrient type and nutrient level are the independent variables. Bold numbers indicate significant values.

		Log root biomass (g)		Log total plant biomass (g)	
Source	df	F	P	F	P
Volume	5	2.16	0.0574	3.53	**0.0038**
Life stage	1	171.65	**<.0001**	77.12	**<.0001**
Vol X life stage	5	9.05	**<.0001**	3.29	**0.0062**
Nutrient type	1	61.85	**<.0001**	121.8	**<.0001**
Vol X nutrient type	5	6.16	**<.0001**	5.31	**<.0001**
Nutrient type X life stage	1	0.23	0.6324	0.02	0.8878
Vol X nutrient type X life stage	5	0.72	0.6098	1.25	0.2852
Nutrient level	1	101.29	**<.0001**	93.07	**<.0001**
Vol X nutrient level	5	1.35	0.2406	0.77	0.5717
Nutrient level X life stage	1	4.64	**0.0317**	4.61	**0.0322**
Vol X nutrient level X life stage	5	0.78	0.5634	0.80	0.5476
Nutrient type X nutrient level	1	8.53	**0.0036**	4.78	**0.0292**
Vol X nutrient type X nutrient level	5	4.07	**0.0012**	2.48	**0.0311**
Nutrient type X nutient level X life stage	1	5.88	**0.0156**	8.46	**0.0038**
Vol X nutrient type X nutrient level X life stage	5	1.26	0.2787	2.51	**0.0293**
Bench(life stage)	2	11.41	**<.0001**	15.00	**<.0001**

Table 3 Effects of treatments on root allocation from an analysis of covariance for solitary plants of *C. edentula.* Log root mass is the dependent variable and log aboveground biomass (leaf mass + stem mass) is the covariate. Volume, life stage, nutrient type and nutrient level are the independent variables. Bold numbers indicate significant values.

	Log root biomass (g)		
Source	df	F	P
Log aboveground mass	1	860.84	**<.0001**
Volume	5	7.59	**<.0001**
Life stage	1	322.63	**<.0001**
Vol X life stage	5	15.44	**<.0001**
Nutrient type	1	2.19	0.1397
Vol X nutrient type	5	2.89	**0.0138**
Nutrient type X life stage	1	1.93	0.1650
Vol X nutrient type X life stage	5	1.52	0.1807
Nutrient level	1	13.40	**0.0003**
Vol X nutrient level	5	3.75	**0.0024**
Nutrient level X life stage	1	0.00	0.9582
Vol X nutrient level X life stage	5	1.36	0.2367
Nutrient type X nutrient level	1	6.12	**0.0137**
Vol X nutrient type X nutrient level	5	2.27	**0.0464**
Nutrient type X nutrient level X life stage	1	0.31	0.5787
Vol X nutrient type X nutrient level X life stage	5	0.70	0.6248
Bench(life stage)	2	5.39	**0.0048**

Table 4 Effects of treatments on reproductive allocation from an analysis of covariance for solitary plants of *C. edentula*. Log fruit number is the dependent variable and log total biomass is the covariate. Volume, nutrient type and nutrient level are the independent variables. Bold numbers indicate significant values. This analysis was done only on plants from the early-reproductive experiment.

	Log fruit number		
Source	df	F	P
Logtotwt	1	83.95	**<.0001**
Vol	5	1.97	0.0842
Nutrient type	1	0.11	0.7462
Vol X nutrient type	5	0.66	0.6557
Nutrient level	1	6.87	**0.0093**
Vol X nutrient level	5	0.80	0.5486
Nutrient type X nutrient level	1	0.14	0.7056
Vol X nutrient type X nutrient level	5	0.37	0.8685
Table	2	8.30	**0.0003**

Table 5: Summary of predictions derived from the pot size hypotheses stated by Hess and de Kroon (2007).

Prediction	Effect of pot size on biomass or allocation
1	↑ pot size = ↑ root biomass
2	↑ nutrient level = ↑ total biomass
3 WSF	↑ pot size = ↑ total biomass
3 CRF	↑ pot size = no effect on total biomass

4 WSF	↑ pot size = no effect on root allocation
4 CRF	↑ pot size = ↑ root allocation

SUPPLEMENTARY TABLES

Table 1: Table of means for total plant biomass demonstrating nutrient level × life stage, and nutrient level × nutrient type interactions. Data was log transformed for analysis but is presented back-transformed for clarity.

	Life Stage		Nutrient Type	
Nutrient level	Juvenile	Early-reproductive	WSF	CRF
High	.12g, SE=.009	.27g, SE=.020	.26g, SE=.072	.13g, SE=.074
Low	.07g, SE=.005	.12g, SE=.010	.15g, SE=.072	.06g, SE=.081

Table 2: Table of means for total plant biomass demonstrating a nutrient level × life stage × nutrient type interaction. Data was log transformed for analysis but is presented back-transformed for clarity.

	Life Stage-Nutrient Type			
Nutrient level	Juvenile-WSF	Juvenile-CRF	Early-reproductive-WSF	Early-reproductive-CRF
High	.19g, SE=.021	.08g, SE=.007	.34g, SE=.032	.22g, SE=.027
Low	.11g, SE=.011	.05g, SE=.004	.21g, SE=.022	.06g, SE=.009

Table 3: Table of means for total plant biomass demonstrating volume × life stage and volume × nutrient type interactions. Data was log transformed for analysis but is presented back-transformed for clarity.

	Life Stage		Nutrient Type	
Volume	Juvenile	Early-reproductive	WSF	CRF
0.13	.08g, SE=.013	.18g, SE=.027	.13g, SE=.020	.11g, SE=.017
0.24	.14g, SE=.015	.18g, SE=.023	.21g, SE=.024	.12g, SE=.014
0.50	.14g, SE=.013	.18g, SE=.020	.23g, SE=.023	.11g, SE=.011
0.95	.09g, SE=.008	.16g, SE=.017	.18g, SE=.017	.08g, SE=.008
1.95	.09g, SE=.009	.19g, SE=.025	.24g, SE=.029	.07g, SE=.008
3.67	.06g, SE=.010	.18g, SE=.038	.20g, SE=.035	.05g, SE=.011

Table 4: Table of means for total plant biomass demonstrating a volume × nutrient type × nutrient level interaction. Data was log transformed for analysis but is presented back-transformed for clarity.

	Nutrient type-nutrient level			
Volume (L)	WSF-High	WSF-Low	CRF-High	CRF-Low
0.13	.14g, SE=.031	.13g, SE=.028	.18g, SE=.038	.07g, SE=.016
0.24	.23g, SE=.038	.19g, SE=.033	.19g, SE=.032	.08g, SE=.014
0.50	.28g, SE=.038	.19g, SE=.029	.19g, SE=.030	.06g, SE=.008
0.95	.27g, SE=.035	.12g, SE=.017	.12g, SE=.017	.05g, SE=.008
1.95	.41g, SE=.072	.14g, SE=.024	.09g, SE=.016	.05g, SE=.009
3.67	.29g, SE=.074	.14g, SE=.036	.08g, SE=.018	.04g, SE=.013

Table 5: Table of means for root biomass demonstrating nutrient level × life stage, and nutrient level × nutrient type interactions. Data was log transformed for analysis but is presented back-transformed for clarity.

	Life Stage		Nutrient Type	
Nutrient level	Juvenile	Early-reproductive	WSF	CRF
High	.025g, SE=0.002	.071g, SE=0.005	.050g, SE=0.003	.036g, SE=0.002
Low	.015g, SE=0.001	.031g, SE=0.002	.031g, SE=0.002	.015g, SE=0.001

Table 6: Table of means for root biomass demonstrating a nutrient level × life stage × nutrient type interaction. Data was log transformed for analysis but is presented back-transformed for clarity.

	Life Stage-Nutrient Type			
Nutrient level	Juvenile-WSF	Juvenile-CRF	Early-reproductive-WSF	Early-reproductive-CRF
High	.033g, SE=0.003	.019g, SE=0.002	.076g, SE=0.006	.066g, SE=0.007
Low	.020g, SE=0.002	.011g, SE=0.001	.048g, SE=0.005	.020g, SE=0.003

Table 7: Table of means for root allocation demonstrating volume × life stage, volume × nutrient type, and volume × nutrient level interactions. Data was log transformed for analysis but is presented back-transformed for clarity.

	Life Stage		Nutrient Type		Nutrient Level	
Volume (L)	Juvenile	E-reprod.	WSF	CRF	High	Low
0.13	.023, SE=.002	.032, SE=.003	.024, SE=.002	.031, SE=.002	.028, SE=.002	.026, SE=.002
0.24	.022, SE=.001	.032, SE=.002	.024, SE=.002	.029, SE=.002	.027, SE=.002	.027, SE=.002
0.50	.021, SE=.001	.044, SE=.003	.028, SE=.002	.033, SE=.002	.030, SE=.002	.031, SE=.002
0.95	.021, SE=.001	.049, SE=.003	.032, SE=.002	.032, SE=.002	.033, SE=.002	.031, SE=.002
1.95	.019, SE=.001	.077, SE=.006	.035, SE=.002	.042, SE=.003	.044, SE=.003	.034, SE=.002
3.67	.021, SE=.002	.052, SE=.006	.039, SE=.004	.028, SE=.003	.045, SE=.004	.024, SE=.003

Fig. 1 Schematic of a sample tray viewed from the side. Trays were square and contained 9 pots (black rectangles, only three visible in this image). All pots were raised to the same height and held in place by Styrofoam blocks (grey shapes). Pots of different volumes were placed at random within each tray and all pots were centered so aboveground density was kept constant for all treatments

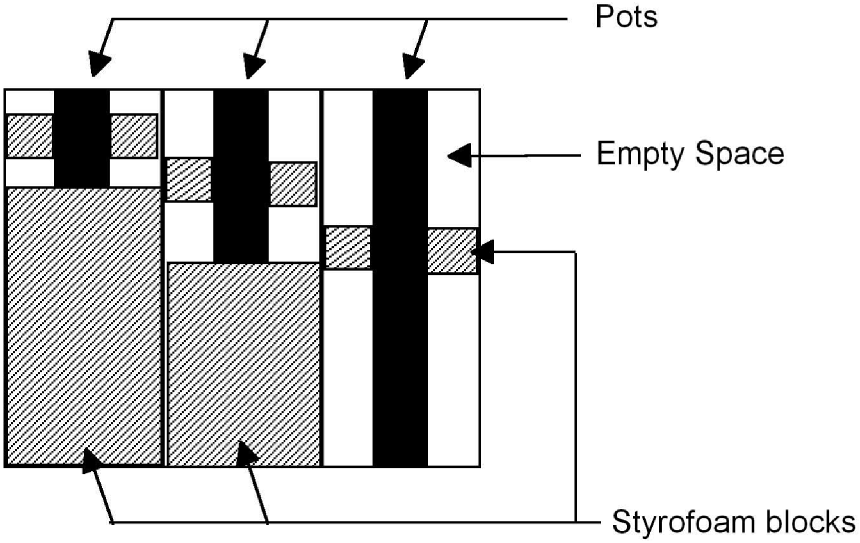

Pots
Empty Space
Styrofoam blocks

Fig. 2 Scatter plot of total plant biomass vs. pot volume for a) juvenile and b) early-reproductive plants. Lines connect plants from the same nutrient type × nutrient level combination. WSF – water soluble fertilizer, CRF – controlled release fertilizer. Bars indicate 1 S.E.

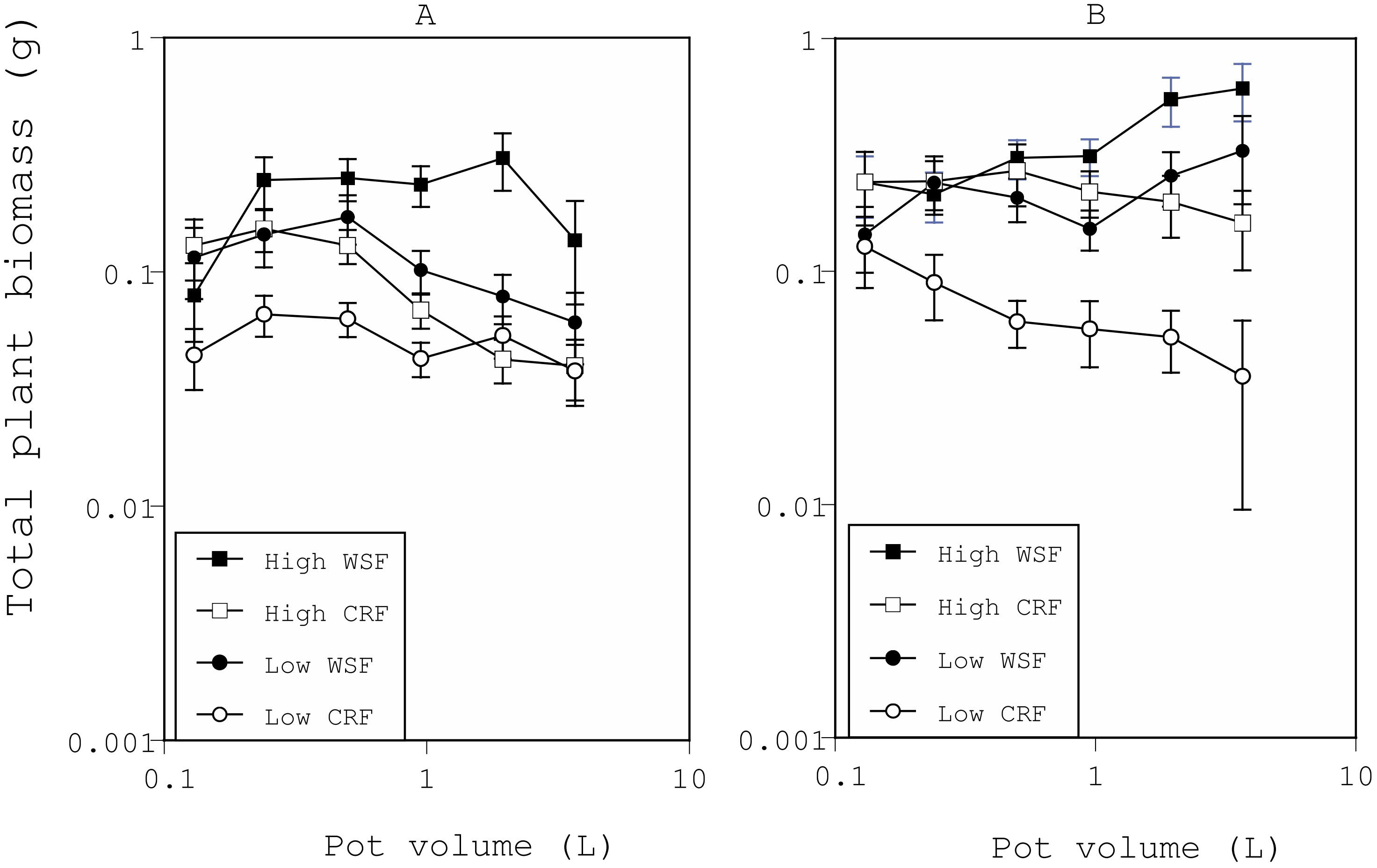

A
B
Total plant biomass (g)
1
0.1
0.01
0.001
0.1
1
10
Pot volume (L)
High WSF
High CRF
Low WSF
Low CRF

Fig. 3 Scatter plot of root biomass vs. pot volume. Lines connect plants from the same life stage. Bars indicate 1 S.E.

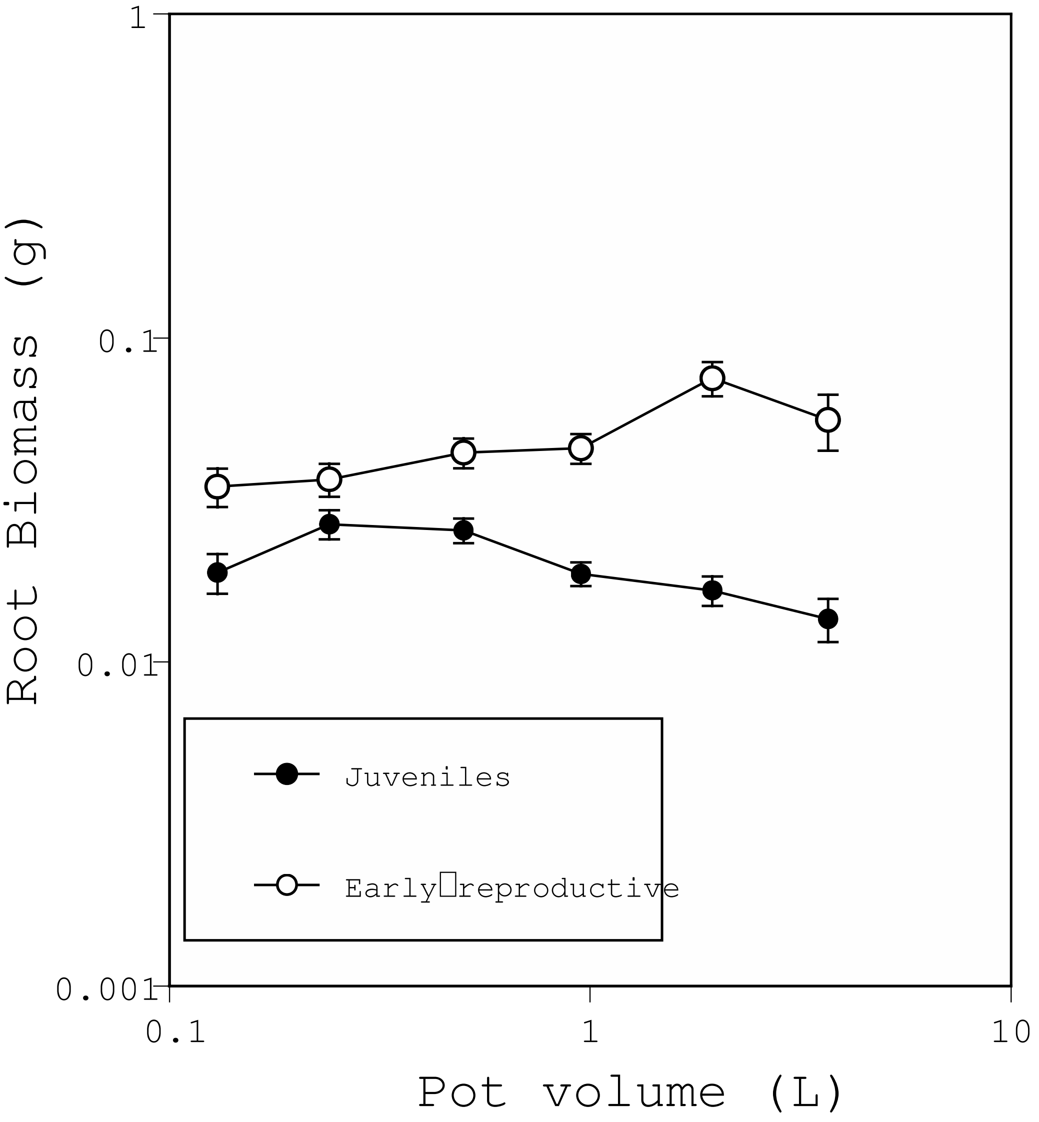

1
0.1
0.01
0.001
Root Biomass (g)
0.1
1
10
Pot volume (L)
Juveniles
Early reproductive

Fig. 4 Scatter plot of root biomass vs. pot volume. Lines connect plants from the same nutrient type × nutrient level combination. WSF – water soluble fertilizer, CRF – controlled release fertilizer. Bars indicate 1 S.E.

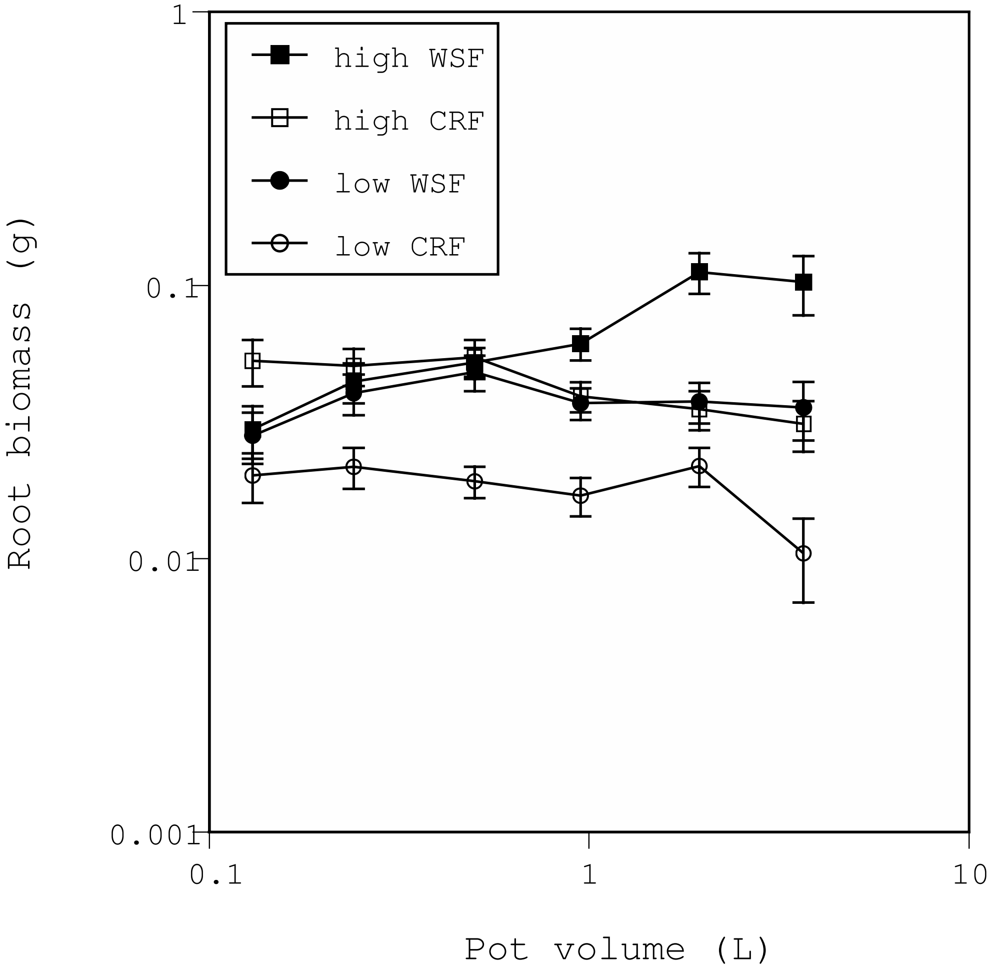
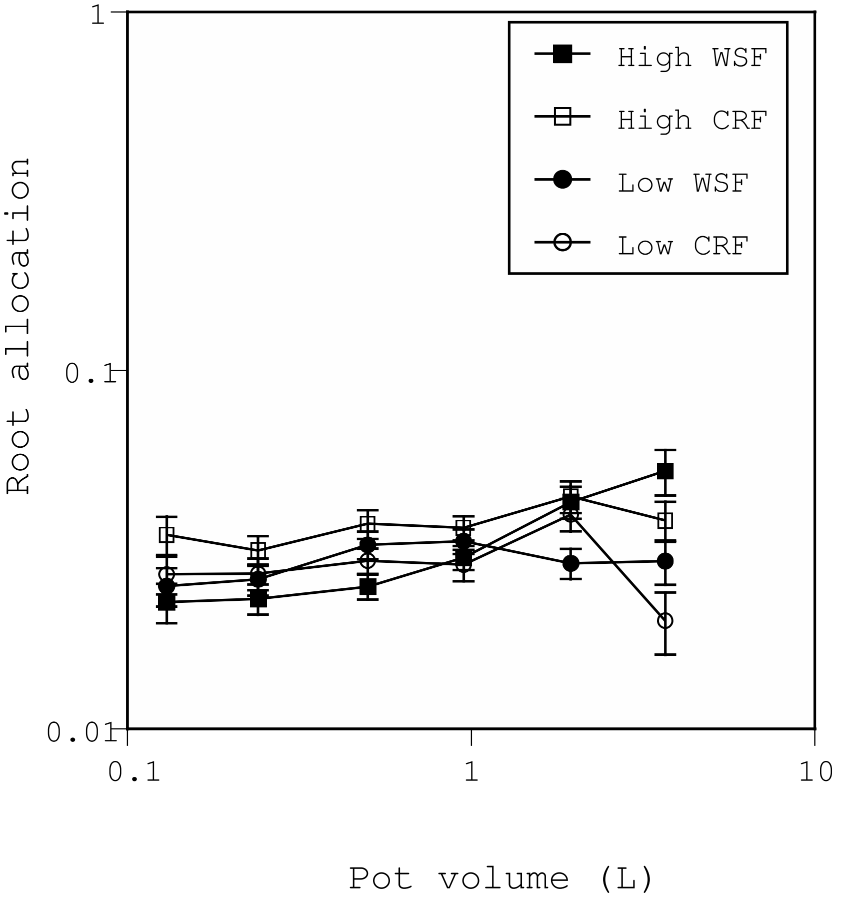
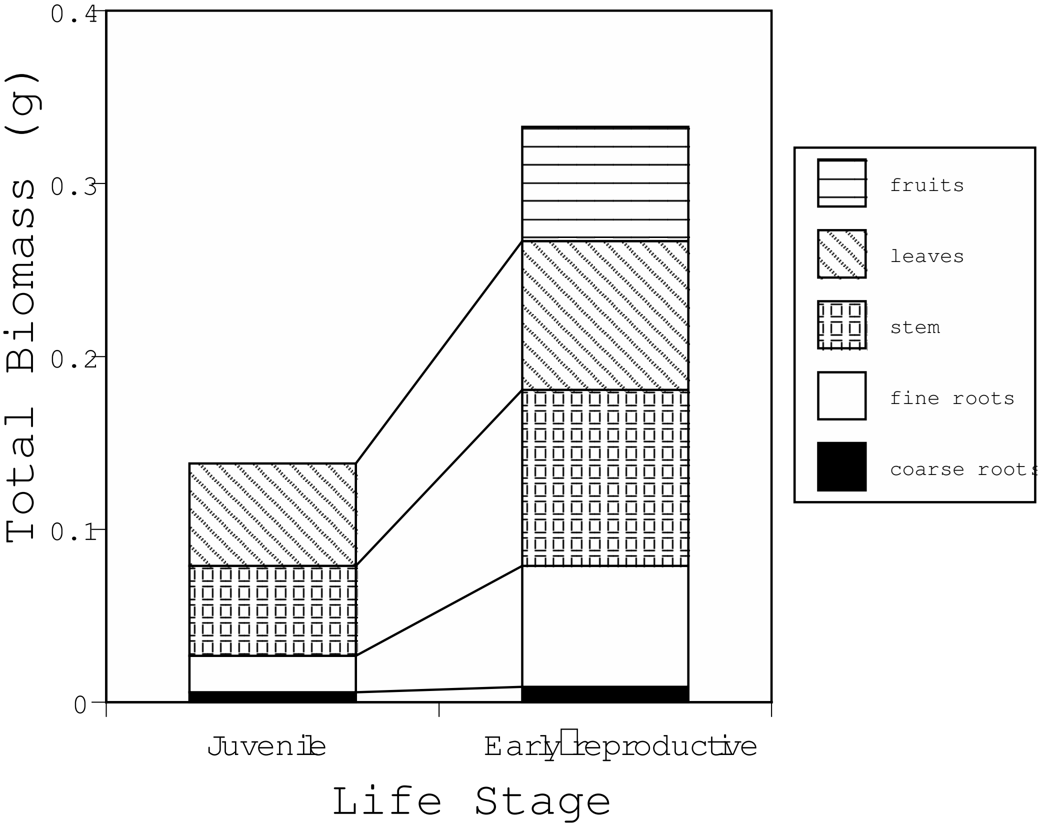

Root biomass (g)
1
0.1
0.01
0.001
0.1
1
10
Pot volume (L)
high WSF
high CRF
low WSF
low CRF

Fig. 5 Scatter plot of root allocation vs. pot volume. Lines connect plants from the same nutrient type × nutrient level combination. WSF – water soluble fertilizer, CRF – controlled release fertilizer. Root allocation values are taken from an analysis of covariance of root mass with aboveground mass (leaves and stem) as the covariate. Bars indicate 1 SE



1
0.1
0.01
0.1
1
10
Root allocation
Pot volume (L)
High WSF
High CRF
Low WSF
Low CRF

Supplementary Fig 1: Stacked bar graph of mean biomass components for *Cakile edentula* measured at two different life stages; juvenile and early-reproductive.


0.4
0.3
0.2
0.1
0
Total Biomass (g)
Juvenile
Early reproductive
Life Stage
fruits
leaves
stem
fine roots
coarse roots

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CHAPTER 4:

COMPARISON OF COMPETITIVE BEHAVIOURS BETWEEN NATIVE AND INVASIVE ECOTYPES OF GARLIC MUSTARD UNDER HIGH, MEDIUM, AND LOW DENSITY CONDITIONS

ABSTRACT

Reduced performance, in terms of size and reproduction, of invasive ecotypes compared to native ecotypes of Garlic Mustard (*Alliara petiolata*) have led to the hypothesis that natural selection may favour reduced competitive ability in invasive plants. Although reduced expression of competitive traits are likely to cause the observed reductions in performance, little is known about which competitive traits may have been selected or assisted in invasive ecotypes of *Alliaria petiolata.* Moreover, how competitive responses would be affected by the changes in density conditions between the native (low density) and introduced (high density) habitats is also unknown. Here we compared competitive responses of North American and European ecotypes of Garlic Mustard to different density conditions. We conducted two experiments, one at the rosette stage and one at the bolting stage of the lifecycle and examined responses in individual competitive traits and performance. We found that both the invasive North American and native European ecotypes of *A. petiolata* responded strongly to density however invasive ecotypes showed overall less competitive phenotypes than native ecotypes. More importantly, the elongation of vertical spacers (internodes and petioles), considered a form of competitive behaviour, was density dependent with North American ecotypes showing reduced elongation in high density compared to European ecotypes. These results provide evidence consistent with evolution of reduced competitive ability in invasive plants and demonstrate how changes in aboveground density conditions can influence competitive responses in invasive plants.

INTRODUCTION

An invasive plant is an introduced species that often forms dense, monospecific stands that suppress the growth of local species resulting in reduced biodiversity (Williamson, 1996). Invasive plants disrupt ecosystem stability by altering biotic and abiotic components thus impacting community species composition, soil characteristics, and nutrient cycling (Vitousek, Dantonio, Loope *et al.*, 1996). Although invasive species become dominant in their new habitats, not all introduced species become invasive and many exotic species are fairly minor components of their home communities (Callaway & Maron, 2006, Williamson, 1996). One thing common to all invasion events is that the new biotic environment encountered by the invader differs from its native environment, exposing the invader to novel selection pressures (Mitchell, Agrawal, Bever *et al.*, 2006). In fact, those species that ultimately invade do so after a considerable lag time (Mack, Simberloff, Lonsdale *et al.*, 2000, Sakai, 2001) suggesting that an adaptation period may be necessary before introduced species can benefit from the conditions of the new environment. Thus, invasion may be conditional on invasive species undergoing adaptive evolution in the new habitat (Bossdorf, Auge, Lafuma *et al.*, 2005, Buswell, Moles & Hartley, 2011, Ellner, Geber & Hairston, 2011).

Rapid evolution on introduced plant species is widespread (Buswell *et al.*, 2011). Introduced plants experience rapid evolution in dispersal ability (Cheptou, Carrue, Rouifed *et al.*, 2008), phenotypic plasticity (Richards, Bossdorf, Muth *et al.*, 2006), mycorrhizal dependence (Seifert, Bever & Maron, 2009), effect of allelotoxins (Prati & Bossdorf, 2004), responses to climate (Maron, Elmendorf & Vila, 2007), performance (size) (Blossey & Notzold, 1995, Bossdorf, Prati, Auge *et al.*, 2004, Leger & Rice, 2003, Siemann & Rogers, 2001, Willis, Memmott & Forrester, 2000), and fitness (Lavergne & Molofsky, 2007, Ridley & Ellstrand, 2009). Because competition is a key process that dominates plant-plant interactions (Novoplansky, 2009), evolution in competitive ability in introduced species has been proposed as a likely mechanism for invasiveness. The "evolution of increased competitive ability" hypothesis (EICA) proposes that because of the release from natural herbivores (Keane & Crawley, 2002), invasive ecotypes can allocate more resources to competition instead of defence and thus become more successful in their new habitats (Blossey *et al.*, 1995). However, the finding that invasive ecotypes of Garlic Mustard (*Alliaria petiolata*), an invasive plant in North America and originally native to Eurasia, are weaker competitors than native ecotypes has led to an alternative hypothesis, the "evolution of reduced competitive ability" (ERCA) (Bossdorf *et al.*, 2004). The ERCA hypothesis argues that if competitive traits are costly, selection may favour reduced competition under intraspecific competitive conditions (Bossdorf *et al.*, 2004). However, no one has yet asked whether and which competitive traits in particular have evolved in North American Garlic Mustard.

Competition for light can be essential for understory species like Garlic Mustard that grow in light limited environments. Plants respond to light competition through phenotypic plasticity in morphological and allocational traits (Maliakal, McDonnell, Dudley *et al.*, 1999, Schlichting & Smith, 2002, Sultan, 2003). Stem and petiole elongation as well as changes in allocation among plant organs (roots, stems, and leaves) have been identified to respond to light competition in plants (Cipollini & Schultz, 1999, Smith, 1995) and are good candidate traits to undergo rapid evolution. In Garlic Mustard, these traits are likely subjected to novel selection pressures in the new habitat because Garlic Mustard grows at higher densities compared to its native habitat and experiences more intraspecific competition due to the formation of monospecific stands. These are adaptive responses (Dudley & Schmitt, 1996) to changes in the colour of the light (low ratio of red to far-red) from light passing through or being reflected off neighbouring plants (Ballaré, Scopel & Sánchez, 1990). These responses are mediated by a family of photoreceptors known as phytochromes (Smith, 1995). This mechanism allows plants to both actively compete for light as well as to respond to impending competition (Ballaré *et al.*, 1990, Ballaré, 1999, Smith, 1995). However, responses to competition can be costly (Cipollini *et al.*, 1999, Schmitt & Wulff, 1993) and could be maladaptive if expressed in the wrong environment (Dudley & Schmitt, 1995). Therefore, asking whether invasive plants have evolved adaptations to aboveground intraspecific competition in high density may be fundamental for understanding the evolution of invasive ecotypes.

To assess evolutionary change it is necessary to compare the responses of the ancestral ecotype and the invasive ecotype to the same novel competitive environment (Richards *et al.*, 2006). We designed two experiments to investigate whether Garlic Mustard has undergone rapid evolution on competitive traits. Because Garlic Mustard is a biennial species, evolution can occur in either first or second year traits. Thus, we tested how North American and European ecotypes of Garlic Mustard respond to density at both the rosette (first year) and bolting (second year) stages. We designed two experiments, one that measured first year traits and one that measured second year traits and fitness. We ask the following questions: 1) Does Garlic Mustard respond to changes in density? 2) Are there intrinsic differences in competitive traits between North American and European ecotypes of Garlic Mustard? 3) Do North American and European ecotypes differ in their competitive responses to density?

MATERIALS AND METHODS

Study Species

Garlic Mustard (*Alliaria petiolata*) is an ideal system to test whether rapid evolution can lead to less competitive but invasive ecotypes. In North America, Garlic Mustard forms dense, monospecific stands that displace local plant species (Anderson *et al*., 1996). Garlic Mustard is a biennial native to Eurasia that was introduced to North America in the late 1700s. It grows as a rosette during the first year and bolts early in spring during the second year (Anderson *et al*., 1996) (See supplementary figure 1 for basic plant parts associated with each life stage of Garlic Mustard). The competitive environment that it experiences in its native habitat differs considerably from the competitive environment in its new habitat. Often found in woody, moist, shady areas (Cavers et al., 1979), in Europe, Garlic Mustard competes for light at lower densities and interspecifically, while in North America, it competes at high densities and intraspecifically.

Experimental Design

Because Garlic Mustard is a biennial plant we designed separate experiments to investigate the differences between North American and European ecotypes in response to density at different life stages. We conducted the first experiment in a greenhouse and grew the plants for a period of three months (from March to June 2009) to look at effects on morphology and allocation on first year traits. The second experiment was done in the field and the plants were grown from May 2008 (European) and June 2008 (North American) until June 2009 to look at effects on morphology and allocation on second year traits as well as reproduction. The differences in planting time in the second experiment were due to later germination of North American ecotypes. All plants were harvested at the same time in June 2009. At time of harvest, North American ecotypes had senesced and European ecotypes were showing the first signs of senescence.

For both experiments Garlic Mustard plants were grown from seed. Seeds were surfaced sterilized with a 10% bleach solution for 10 min. and placed into petri dishes lined with filter paper at 4°C for required stratification.

Experiment 1

North American ecotypes consisted of a single population obtained from Ontario, Canada. European ecotypes consisted of four populations obtained from Netherlands, Slovenia, and Germany in Europe. More than one European population was used to get a better representation of possible ancestral ecotypes. After germination, seedlings were transplanted into plug trays at the cotyledon stage and kept in a growth chamber (Model no. I24L, Conviron Controlled Environments, Winnipeg, Canada) at 25°C for one week. One-week-old seedlings were then transplanted into 10 cm pots containing top soil (Promix, PremierHort, Riviero-du-Loup, Quebec, Canada) and placed in the greenhouse under natural light for three months during spring of 2010. Two additional transplants, into 15 cm and 20 cm pots, were done after one and two months respectively to avoid excessive pot bounding. Plants were fertilized for the first time after one month and were then fertilized weekly with a 20-20-20 NPK water-soluble fertilizer (PlantProd Co., Brampton, Ontario, Canada).

We created the density treatment by planting different amounts of North American or European seedlings into pots of the same size. We planted groups of 5, 10, and 20 seedlings as well as single plants to create 4 density categories. To account for mortality, density categories at the end of the experiment were: solitary (1 plant/pot or 32 plants/m^2), low (2-7 plants/pot or 64-223 plants/m^2), medium (8-14 plants/pot or 255-446 plants/m^2), and high (15-20 plants/pot or 478-637 plants/m^2). Groups of high, medium, and low density were replicated 10 times for each ecotype (North American or European) while we had 42 pots with single plants for each ecotype.

Experiment 2

North American ecotypes were obtained from a single population in Ontario, Canada while European ecotypes were obtained from three populations from Netherlands, Belgium, and Germany in Europe. After germination, seedlings were transplanted into pine cells (Stuewe & Sons Inc., Tangent, Oregon, USA) and grown in the greenhouse for three weeks. At the three-weeks-old stage seedlings were transplanted into the field. Because European ecotypes germinated earlier, they were transplanted into the field during May 2008 while North American seedlings were transplanted during June 2008. Seedlings were fertilized once after transplant with a 5-1-1 NPK fertilizer (Muskie Fish Emulsion

Fertilizer, Green Earth, Nu-Gro IP. Inc., Brantford, Ontario, Canada) and were allowed to grow and over-winter until harvesting started in June 2009.

Because of higher mortality rates in the field and because plants were not confined in a pot, we created the density treatment by varying the distance between plants instead of the number of plants per group. We planted either North American or European seedlings in groups of 36 seedlings at different distances. Groups of seedlings were planted into squares of 6 seedlings by 6 seedlings, either high-density (36 seedlings in 0.09 m^2), medium-density (36 seedlings in 0.36 m^2), or low-density (36 seedlings in 1.44 m^2). Each density treatment was replicated twice.

Data Collection

For experiment 1 (rosette stage), we scored number of leaves per plant, length of the biggest leaf blade as a measure of leaf size, and length of the largest petiole as a measure of height. Biggest leaf blade and largest petiole always coincided as part of the same leaf. We determined aboveground biomass by collecting plant material (leaf blades and petioles) and drying it in an oven for 72 h at 65°C before weighing.

For experiment 2 (bolting stage), we scored number of stalks per plant, length of the longest stalk, length of the first four internodes on the longest stalk, number of leaves per plant, number of branches per plant, and number of pods per plants. We measured biomass of the longest stalk, biomass of the remaining stalks, and biomass of leaves. Because plants were harvested at a final reproductive stage, most leaves had been lost (especially in North American ecotypes) by the time of harvest. Thus we are not using leaf data in our analysis of experiment 2. Because plants were growing in regular soil and roots of neighbouring plants were entangled, root traits could not be measured for either experiment.

Data Analysis

The data were analyzed using SAS statistical software (version 9.2; SAS, Cary, NC, USA). We used a mixed-model ANOVA (PROC MIXED) to test for fixed effects of density, ecotype, and density x ecotype interaction on aboveground biomass, leaf length, and petiole elongation in experiment 1, as well as height, length of internode 4 (first internode to measure more than one cm.), stem biomass, stalk elongation, fecundity and allocation to reproduction in experiment 2. To analyse the effects of density, ecotype and density x ecotype interaction on leaf and brunch number we used a generalized-mixed-model (PROC GLIMIX). Because density and ecotype treatments were applied at the pot level on experiment 1, we considered each pot as a block and thus included block nested within density x ecotype as a random effect in the model. For experiment 2, all density and ecotype treatments were applied within each block and there were two blocks in the experiment, thus block was included as a random effect. Estimates of variables were measured as the least square means from a general linear model for continues variables (LSMEANS option PROC GLM) and a generalized linear model for discrete variables (LSMEANS option PROC GENMOD). We used analysis of covariance to test for differences in allocation and elongation (Coleman, McConnaughay & Ackerly, 1994, McConnaughay & Coleman, 1998). For petiole elongation, stem elongation, and reproductive allocation the dependent variables were length of longest petiole, height of tallest stalk, and number of fruits while the covariates were leaf length, longest stalk biomass, and stem biomass respectively. We analyzed fitness by examining fecundity (number of fruits produced by surviving plants).

RESULTS

Responses to Density

In first year plants, density reduced aboveground biomass, leaf number, and leaf length (Tables 1 and 2). In second year plants, height, stem biomass, branch number, and fecundity (Tables 2 and 3) were also reduced in response to increased density. The longest petiole in first year plants (Fig.1a, Table 1) and the longest stalk (Fig. 2, Table 4) in second year plants elongated in response to density. We measured the first four internodes in the longest stalk to determine if elongation occurred early during plant development and we found that the first internode to measure more than 1 cm in length (internode 4) showed the highest length at medium density (Fig.3a, Table 3).

Differences between native and invasive ecotypes

In first year plants, North American ecotypes had less elongated petioles (Fig.1b, Table 1), and lower aboveground biomass (Eur = 1.20 g, SE = 0.08; NA = 0.81 g, SE = 0.08; Table 1). In second year plants, although we found no difference in elongation of the longest stalk between ecotypes, internode 4 was shorter in North American plants (Fig. 3b, Table 3). North American ecotypes were shorter overall (Eur = 63.23 cm, SE = 4.0; NA = 42.54 cm, SE = 2.93; Table 1), produced less stem biomass (Eur = 0.79 g, SE = 0.13; NA = 0.27 g, SE = 0.05; Table 1), and had lower fecundity (Eur = 3.36, SE = 0.16; NA = 2.90, SE = 0.15; Fig. 4b; Table 1) than did European ecotypes. However, allocation to reproduction was increased in North American compared to European ecotypes (Fig. 4c, Table 4).

Differences between native and invasive ecotypes in response to density

Elongation of the longest petiole differed between North American and European ecotypes in response to density. Elongation of the longest petiole progressively increased with density in European ecotypes while in North American ecotypes elongation increased with density from solitary to low to medium but decreased at high density (Fig 1c, Table 1). Length of internode 4 in the longest stalk of second year plants showed a similar pattern (Fig 3c, Table 3).

DISCUSSION

In this study we show that increases in density cause strong intraspecific competition in Garlic Mustard. Plants responded to competition through density-dependent petiole and stem elongation and showed reductions in performance and fitness. These responses were observed across ecotypes. However, North American ecotypes of Garlic Mustard displayed less competitive phenotypes compared to European ecotypes. A reduction in competitive ability in North American ecotypes may result from processes like inbreeding depression or genetic drift (Barrett & Husband, 1990). However, evidence in Garlic Mustard does not support these processes and instead point towards the evolution of reduced competitive ability (ERCA hypothesis) as a more likely explanation (Anderson, Dhillion & Kelley, 1996b, Meekins, Ballard & McCarthy, 2001, Bossdorf *et al.*, 2004, Durka, Bossdorf, Prati *et al.*, 2005).

At both the rosette and bolting stages, density reduced performance, showing that intraspecific competition is important in Garlic Mustard. Traits that responded to density did so as expected and the responses were maintained across ecotypes. Higher density conditions prompted elongation of petioles and stems and reduced aboveground biomass, height, leaf number, branch number, and fruit production. These responses are consistent with the findings of other experiments (Meekins & McCarthy, 2000, Rebek & O'Neil, 2006, Myers, Anderson & Byers, 2005). They show that, under conditions of competition with limited resources, increases in density will promote the development of more competitive phenotypes by producing more elongated petioles (in the case of rosettes) or stems (in the case of bolting plants) (Figs. 1a and 2d). However the outcome of these competitive responses is an overall reduction on biomass and fitness. Petiole and stem elongation as well as overall height are traits that usually respond to density and are associated with light competition (Cipollini *et al.*, 1999, Smith, 1995, vanHinsberg & vanTienderen, 1997). Elongation has been considered a competitive trait that allows plants to better position their leaves in their struggle to gain access to the light resource, while at the same time providing a way for interference competition by shading neighbours (Smith, 1995). However, elongation can be a costly competitive response (Cipollini *et al.*, 1999, Schmitt *et al.*, 1993) and can be maladaptive if expressed in the wrong environment such as a low density environment (Dudley *et al.*, 1996).

Interestingly, continent of origin was a strong predictor of competitive responses, performance and fitness (Tables 1 and 3). North American ecotypes produced less competitive phenotypes by having less elongated petioles and lower overall height. They also had reduced aboveground biomass and produced fewer fruits. These differences occurred across density treatments with the exception of petiole elongation, which showed a response to density that differed between ecotypes (Fig. 1). Both North American and European ecotypes showed increased petiole elongation in the presence of competitors compared to solitary individuals. However, European ecotypes showed continuous petiole elongation with increasing density while North American ecotypes elongated their petioles as density went from low to medium but then decreased elongation at high density

(Fig.1d). Petiole elongation in response to density occurred during the first year of growth when Garlic Mustard grows only as a rosette. During the second year of growth, when the plants bolted, elongation of the bolting stem responded to density (Fig. 2d) although there was no difference in overall stem elongation across ecotypes. Nevertheless, length of the fourth internode in the bolting stem (first internode to measure more than 1 cm.) showed the same pattern observed for petiole elongation (Fig. 2a-c). Since internode length provides a developmental snapshot in time (Weinig, Johnston, Willis *et al.*, 2007), it thus allows for an assessment of competitive behaviour early in the second season of growth when plants are still actively competing. It is possible that the lack of differences in overall stem elongation between ecotypes at the end of the life cycle is due to plants forgoing competition to allocate all resources into reproduction.

Changes in biomass allocation are also commonly associated with responses to competition. The most common allocation response to density is a shift in allocation of biomass from roots to shoots (Smith, 1995). Because we did not measure belowground traits due to the inability to assign roots to individual plants, we were unable to estimate this parameter. However, data from another experiment indicate that North American ecotypes allocate more to roots compared to shoots and particularly into storage roots (Chapter 5). This suggests a possible change in strategy with plants allocating less to aboveground competition and more to belowground storage. In this study we did measure allocation between petioles and leaf blades in first year plants as well as reproductive allocation in second year plants. Although we found no effects of density or differences between ecotypes in the former, we did find that North American ecotypes of Garlic Mustard allocated more to reproduction that did European ecotypes (Fig. 4c). This shows that although North American ecotypes were smaller and had lower fitness compared to European ecotypes, they invested more into reproduction suggesting a more efficient use of resources.

In North America, Garlic Mustard forms dense monospecific stands, which lead to intraspecific competition. Under these conditions, natural selection should favour the evolution of more competitive phenotypes. However, our results suggest evolution on the opposite direction, towards less competitive phenotypes. One possible explanation is that in the introduced habitat there may be fewer or weaker competitors than in the native habitat. In this case, costly competitive traits will be selected against resulting in the evolution of less competitive phenotypes, which at the same time will reduce intraspecific interactions after the formation of monocultures (Bossdorf *et al.*, 2004). Other possibility however, involves the action of kin selection.

If stands of Garlic Mustard are genetically structured then they already meet a necessary condition for kin selection to occur (Kelly, 1996). Kin selection extends the concept of fitness to include the actions of individuals on genetic relatives (Hamilton, 1964). It proposes that altruistic traits and behaviours can evolve as long as the cost to the altruist is lower than the benefit to the relative weighted by the relatedness between the two (Hamilton, 1964). Then, it is possible that kin selection will favour the evolution of less competitive ecotypes in groups of highly related individuals competing intraspecifically because of benefits that are twofold. First, benefits can be attained through gains in inclusive fitness by reducing interference competition with relatives, and second, by avoiding the costs of producing competitive traits.

A caveat of this study is the relatively low number of populations that were sampled. Since performance and fitness traits can be influenced by differences in latitudinal gradients between North American and European ecotypes (Colautti, et al., 2009), interpretation of those results must be made with caution. However, latitudinal gradients should not influence individual competitive traits and therefore differenced between North American and European ecotypes regarding competitive behaviours can be interpreted from these results.

In summary, we show that there are differences between North American and European ecotypes of Garlic Mustard in regard to competitive traits, performance and fitness. We demonstrate that intraspecific competition due to high density conditions has important consequences for Garlic Mustard and that responses to density differ between North American and European ecotypes. We argue that the observed differences, which are consistent with the evolution of reduced competitive ability in the invasive ecotype, are more likely a product of kin selection than other processes like inbreeding depression or genetic drift.

Finally, we propose that future studies should consider the role of these evolutionary processes by measuring selection at the group level and comparing responses not only between North American and European ecotypes of Garlic Mustard but also between groups of related versus unrelated individuals.

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Table 1 Analyses of variance and covariance for first year traits for greenhouse grown Garlic Mustard (*Alliaria petiolata*)

		Aboveground biomass		Leaf number		Leaf length		Petiole elongation**	
Source	d.f.	*F or Z*	*P*	*F or Z*	*P*	*F or Z*	*P*	*F or Z*	*P*
Density*	3	78.07	**<.0001**	96.86	**<.0001**	53.08	**<.0001**	7.91	**<.0001**
Ecotype*	1	5.44	**0.0218**	2.14	0.1465	0.61	0.4378	15.20	**0.0002**
Den x Eco*	3	0.89	0.4516	0.45	0.7209	1.20	0.3152	4.08	**0.0089**
Pot (Den x Eco)	-	2.71	**0.0034**	5.38	**<.0001**	3.02	**0.0013**	3.98	**<.0001**
Covariate	1	n.a.	n.a.	n.a.	n.a.	n.a	n.a	487.11	**<.0001**

*Treatment tested over pot nested with density x ecotype interaction.

**Petiole elongation was determined from an analysis of covariance with petiole length as the dependent variable and leaf length as the covariate.

Bold numbers indicate significant values.

Table 2: Table of means for first and second year size and performance traits demonstrating responses to density. Data was log transformed for analysis but is presented back-transformed for clarity.

	First year traits			Second year traits			
Density	Aboveground biomass	Leaf number	Leaf length	Height	Stem biomass	Branch number	Fecundity
Solitary	4.59 g, SE =0.69	27.01, SE = 0.03	9.76 g, SE =0.04	n.a.	n.a.	n.a.	n.a.
Low	1.33 g, SE = 0.21	10.41, SE = 0.05	7.41, SE= 0.04	59.2 cm, SE= 3.91	0.74 g, SE = 0.13	1.91, SE = 0.11	3.56, SE = 0.18
Medium	0.6 g, SE = 0.07	6.95, SE = 0.04	5.91, SE= 0.04	49.4 cm, SE= 2.15	0.45 g, SE = 0.05	1.25, SE = 0.10	3.11, SE = 0.11
High	0.2 g, SE = 0.02	4.65, SE = 0.04	4.13, SE= 0.04	47.6 cm, SE= 5.59	0.30 g, SE = 0.09	0.34, SE = 0.54	2.74, SE = 0.24

Table 3 Analyses of variance for second year traits for field grown Garlic Mustard (*Alliaria petiolata*)

		Height		Length internode 4		Stem biomass		Branch number		Fecundity	
Source	d.f.	*F or Z*	*P*	*F or Z*	*P*	*F or Z*	*P*	*F or Z*	*P*	*F or Z*	*P*
Density	2	5.36	**0.0489**	11.84	**<.0001**	5.36	**0.0058**	7.95	**0.0005**	4.71	**0.0106**
Ecotype	1	21.50	**<.0001**	40.25	**<.0001**	21.50	**<.0001**	1.96	0.1641	4.27	**0.0408**
Den x Eco	2	1.68	0.0527	9.64	**0.0001**	1.68	0.1911	2.68	0.0718	1.64	0.1972
Block	1	0.64	0.2614	-	-	0.64	0.2626	0.59	0.2773	0.60	0.2747

Bold numbers indicate significant values.

Table 4 Analyses of covariance for second year traits for field grown Garlic Mustard (*Alliaria petiolata*)

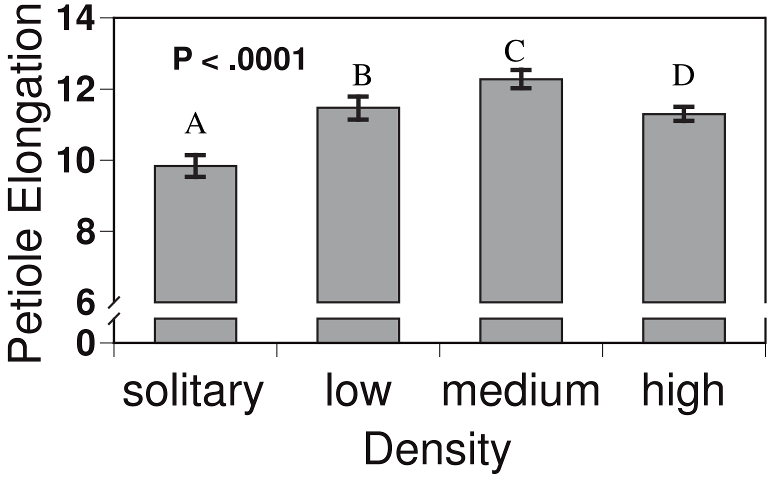
		Stalk elongation*		Allocation to reproduction**	
Source	d.f.	*F or Z*	*P*	*F or Z*	*P*
Density	2	5.63	**0.0045**	0.03	0.9737
Ecotype	1	0.08	0.7794	29.23	**<.0001**
Den x Eco	2	1.10	0.3551	0.03	0.9707
Block	-	-	-	-	-
Covariate	1	1674.37	**<.0001**	830.74	**<.0001**

*Stalk elongation was determined from an analysis of covariance with stalk length as the dependent variable and stem biomass as the covariate.

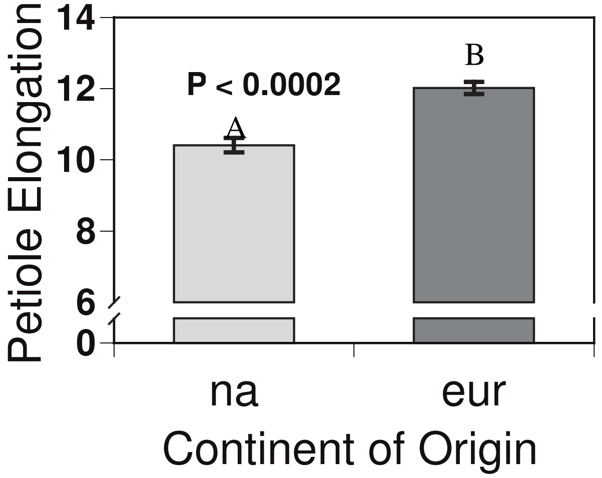
** Allocation to reproduction was determined from an analysis of covariance with pod number as the dependent variable and stem biomass as the covariate.

Bold numbers indicate significant values.

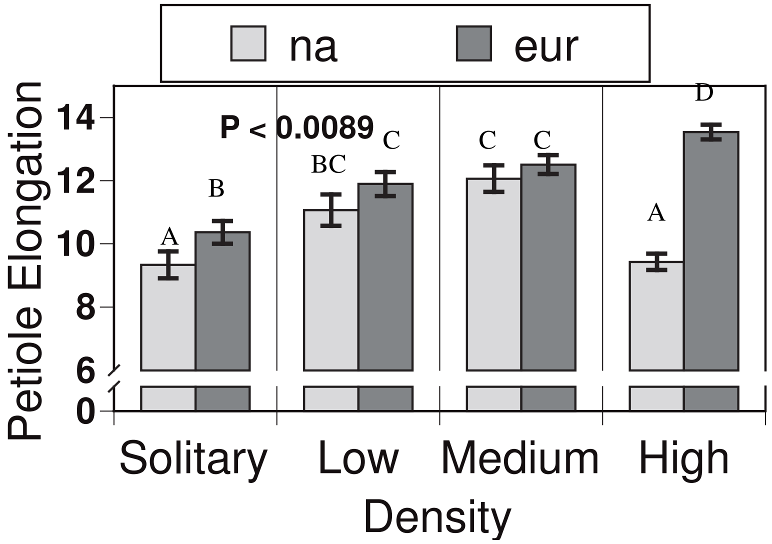
Fig. 1) Petiole elongation response of first year Garlic Mustard to: a) density, b) ecotype, and c) density and ecotype. Petiole elongation was measured from an analysis of covariance with length of the longest petiole as the dependent variable and length of the corresponding leaf blade as the covariate.


Petiole Elongation
14
12
10
8
6
0
P < .0001
A
B
C
D
solitary
low
medium
high
Density

A


Petiole Elongation
14
12
10
8
6
0
P < 0.0002
A
B
na
eur
Continent of Origin

B


na
eur
Petiole Elongation
14
12
10
8
6
0
P < 0.0089
A
B
BC
C
C
C
A
D
Solitary
Low
Medium
High
Density

C

Fig. 2) Elongation response to density of the longest stalk of second year bolting plants of Garlic Mustard. Stalk elongation was measured from an analysis of covariance with length of the longest stalk as the dependent variable and biomass of the longest stalk as the covariate.

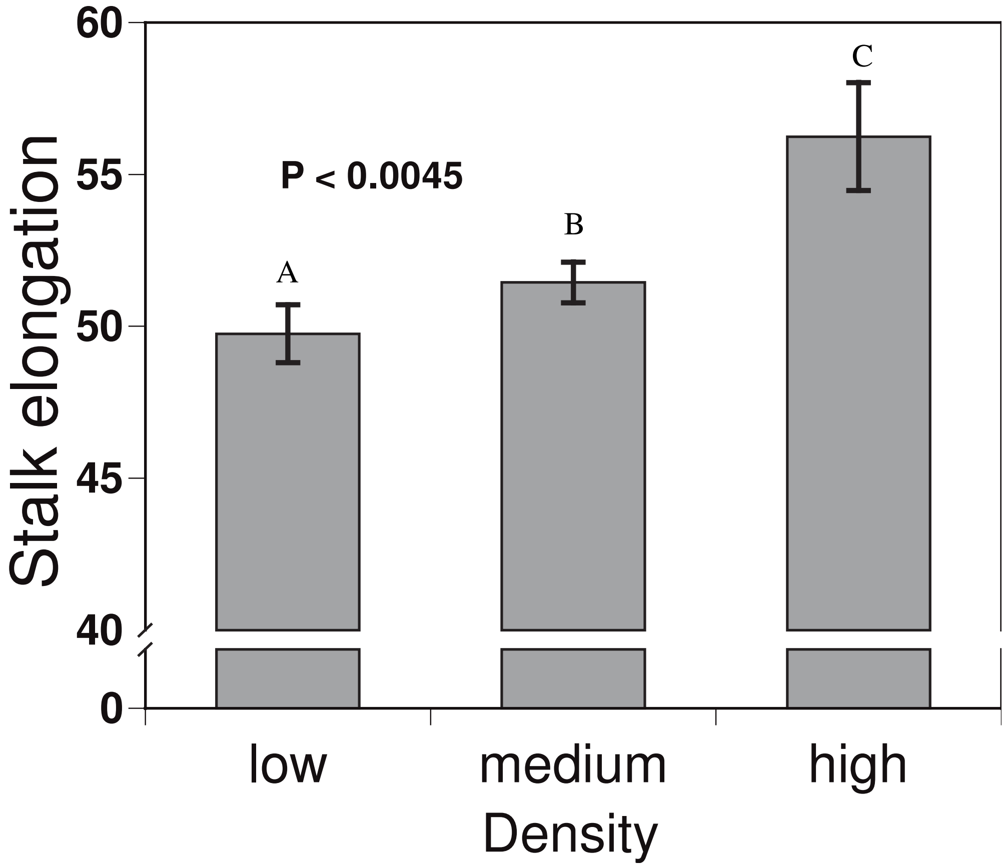
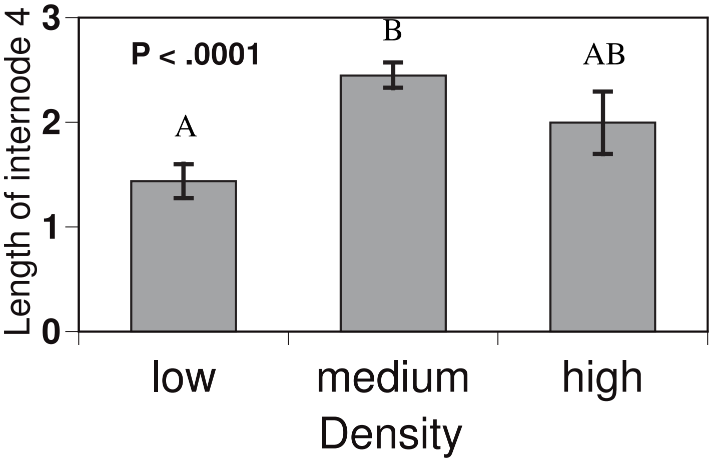
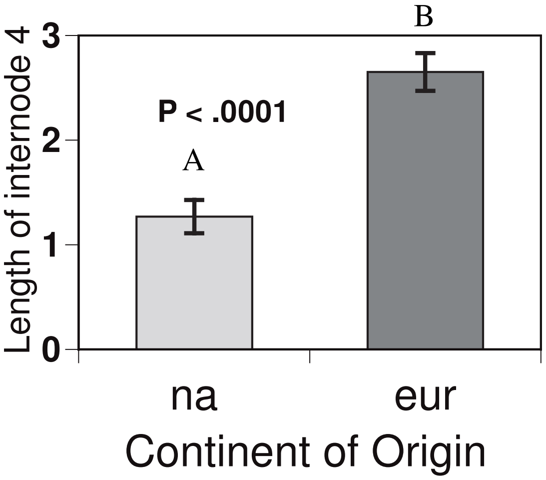

60
55
50
45
40
0
Stalk elongation
P < 0.0045
A
B
C
low
medium
high
Density

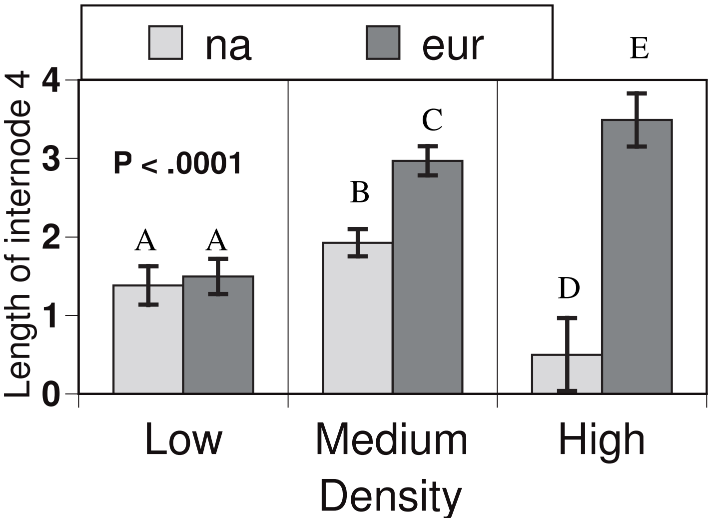
Fig. 3) Responses of the fourth internode on second year bolting plants of Garlic Mustard to: a) density, b) ecotype, and c) density and ecotype. The fourth internode was the first internode to measure more than one centimetre.


Length of internode 4
3
2
1
0
P < .0001
A
B
AB
low
medium
high
Density

A

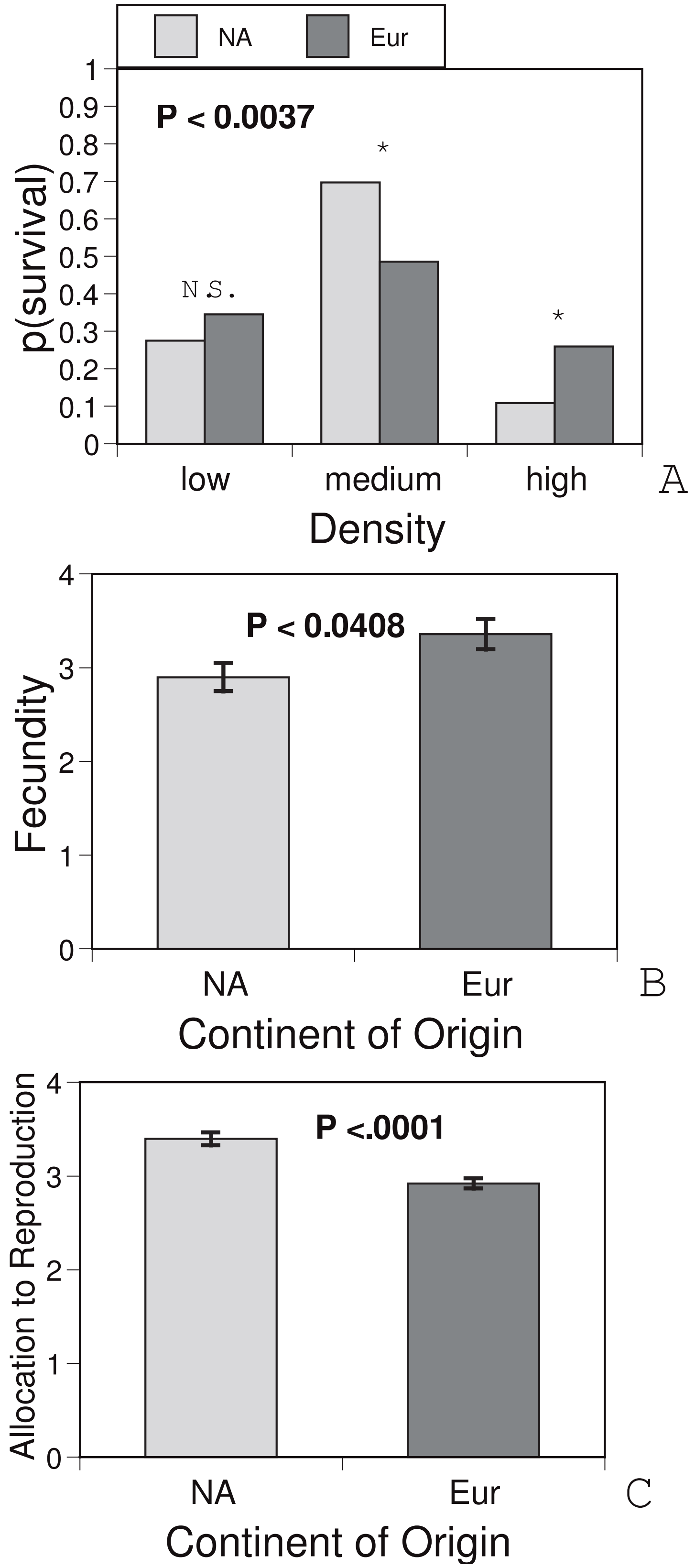

Length of internode 4
3
2
1
0
P < .0001
A
B
na
eur
Continent of Origin

B

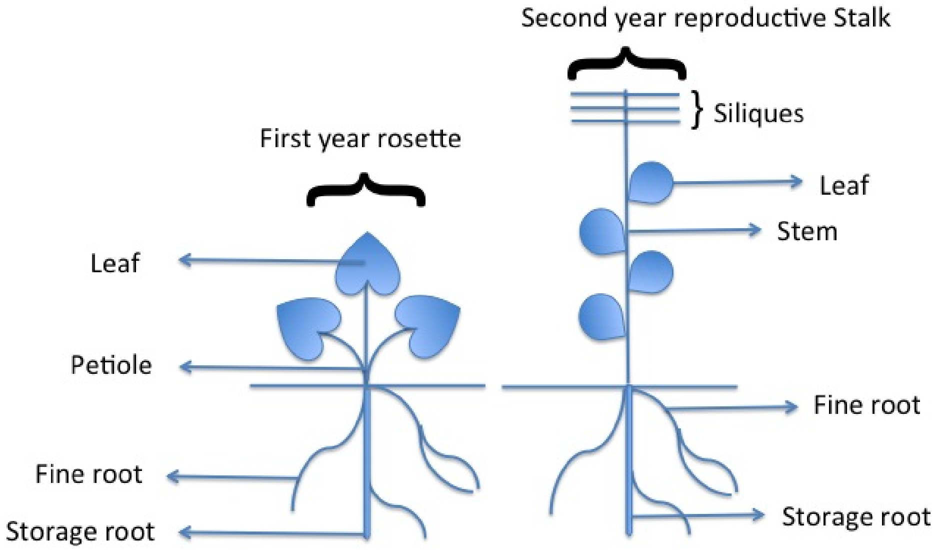

na
eur
Length of internode 4
4
3
2
1
0
P < .0001
A
A
B
C
D
E
Low
Medium
High
Density

C

Fig. 4) A) Probability of survival for second year bolting plants of Garlic Mustard at different densities. B) Differences in fecundity between North American and European ecotypes of Garlic Mustard. C) Differences in allocation to reproduction between North American and European ecotypes of Garlic Mustard. Allocation to reproduction was measured from an analysis of covariance with fruit number as the dependent variable and stem biomass as the covariate.


NA
Eur
p(survival)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
P < 0.0037
N.S.
*
*
low
medium
high
Density
A
Fecundity
4
3
2
1
0
P < 0.0408
NA
Eur
Continent of Origin
B
Allocation to Reproduction
4
3
2
1
0
P <.0001
NA
Eur
Continent of Origin
C

Supplementary Fig. 1) Schematic representation of basic Garlic Mustard morphology during first year (rosette) and second year (bolting) life stages.


Second year reproductive Stalk
Siliques
First year rosette
Leaf
Leaf
Stem
Petiole
Fine root
Fine root
Storage root
Storage root

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CHAPTER 5:

COMPARISON OF COMPETITIVE BEHAVIOURS BETWEEN NATIVE AND INVASIVE GARLIC MUSTARD ECOTYPES IN RESPONSE TO DENSITY, PRESENCE, AND IDENTITY OF NEIGHBOURS

ABSTRACT

Rapid evolution of reduced competitive ability has been shown in invasive plants. It has been hypothesized that this evolution of reduced competitive ability may occur because competitive traits may be costly and invasives may encounter fewer or weaker competitors in the new habitat. However, little is known about which individual competitive traits may evolve in invasive plants and whether and why reduced competitive ability will allow for introduced species to become invasive. Because for many invasive species competitive interactions are likely to involve related neighbours because of limited seed dispersal and high levels of selfing, kin selection may provide a possible explanation by providing benefits through inclusive fitness that will arise from reduced competition with siblings. Here we compared responses of North American and European ecotypes of Garlic Mustard to the presence, identity, and density of competitors. We conducted two experiments, one at the rosette stage and one at the bolting stage of the lifecycle and examined responses in individual competitive traits and performance. Overall, North American ecotypes produced less competitive phenotypes and performed better than European ecotypes. However, increased performance (biomass and

fruit production) only occurred when North American ecotypes were competing with siblings belowground. These results provide evidence in support of the evolution of reduced competitive ability hypothesis in Garlic Mustard and are consistent with the action of kin selection.

INTRODUCTION

In nature, plants interact with neighbouring plants. The presence of neighbours reduces the availability of limited resources (Casper & Jackson, 1997). Plants compete aboveground for light (Smith, 1995) and belowground for water and mineral nutrients (Casper & Jackson, 1997; Novoplansky, 2009). The ability to respond to resource availability and to sense competitors allows plants to adapt to different competitive environments (Ballaré, Scopel, & Sánchez, 1990; S. A. Dudley & Schmitt, 1995; Franklin & Whitelam, 2005; Marschner, 1995; Novoplansky, 2009; Schlichting & Smith, 2002; Sultan, 2003) by responding through phenotypic plasticity in competitive traits (Callaway, 2002; Novoplansky, 2009). Thus, competitive traits that confer competitive ability may allow exotic plants to invade new habitats by outcompeting local species and may be under strong natural selection in the introduced habitat (Bossdorf et al., 2005; Mooney & Cleland, 2001; Sakai, 2001). However, little is known about how individual traits contribute to competitiveness in invasive plants.

Invasive plant species are species that when introduced into new habitats can form monospecific stands that displace local species (Williamson, 1996). In their native habitats, invasive species are often minor components of the community and do not form monospecific stands (Callaway & Maron, 2006; Williamson, 1996). Therefore, invasive species experience a different competitive environment in their new habitats compared to their native habitats, with more interspecific interactions in their native communities and more intraspecific interactions in their new communities. Thus, competitive traits in invasive plants may experience different selection pressures in the new habitat compared to their native habitats. The evolution of competitive ability in exotic plants has been hypothesized to be a cause of invasiveness (Blossey & Notzold, 1995; Bossdorf, Prati, Auge, & Schmid, 2004). However, how competitive ability is hypothesised to evolve depends on the competitive environment that invasive plants encounter. The "evolution of increased competitive ability" hypothesis (EICA) predicts that invasive plants will evolve to be more competitive in their new habitat as they are under relaxed selection for herbivore defense (Blossey & Notzold, 1995). However, the "evolution of reduced competitive ability" hypothesis (ERCA) predicts the opposite because there may be fewer or weaker competitors in the new habitat and thus costly competitive traits will not be selected for (Bossdorf et al., 2004). In some species invasive ecotypes outperform native ecotypes in competition while in other species they do not (Bossdorf et al., 2005) providing only partial support for either hypotheses. However, the interpretation of comparisons of performance and fitness traits between invasive and native ecotypes may be misleading because fitness outcomes may be influenced by unaccounted environmental factors such as latitudinal gradients (Colautti, Maron, & Barrett, 2009). Therefore, a trait-based approach that examines putative competitive traits together with performance and fitness will be more appropriate to elucidate possible evolutionary changes that might have occurred in invasive species (File, Murphy, & Dudley, 2011). However, few studies (Chapter 4) have yet examined which specific traits have evolved in invasive species that can lead to changes in competitive ability.

In Garlic Mustard (*Alliaria petiolata*), a species native to Europe and highly invasive in North America, North American ecotypes lost in competition against European ecotypes (Bossdorf et al., 2004). North American ecotypes of Garlic Mustard, compared to European ecotypes, showed reduced petiole elongation and internode elongation in high density (Chapter 4). Since elongation of vertical spacers is a competitive trait, because it allows plants to gain access to the light (Huber, Fijan, & During, 1998; Weijschede, Berentsen, de Kroon, & Huber, 2008; Weijschede, Martinkova, de Kroon, & Huber, 2006), this result is consistent with the ERCA hypothesis. However, the study described in chapter 4 only examined differences in competitive responses among ecotypes of Garlic Mustard aboveground. To date no one has examined ecotypic differences in competitive responses belowground.

Aboveground, plants sense the presence of competitors and respond by allocating resources to shoots (Cipollini & Schultz, 1999) while belowground, they respond by changing allocation to roots (Gersani, Brown, O'Brien, Maina, &

Abramsky, 2001; Maina, Brown, & Gersani, 2002; O'Brien, Gersani, & Brown, 2005). In nature however, cues that signal aboveground and belowground competition are likely to occur simultaneously (Murphy & Dudley, 2007). Thus, if resources are limited, then a tradeoff would be expected between the ability of plants to compete aboveground and belowground (although see Murphy & Dudley 2007). Therefore, to understand the evolution of competitive ability in invasive plants, it is necessary to examine responses to competitors in both aboveground and belowground traits.

Plants respond differently to the presence of root neighbours depending on the identity of neighbouring roots. Plants have been shown to differentiate self from non-self roots (Falik, Reides, Gersani, & Novoplansky, 2003; Gruntman & Novoplansky, 2004; Semchenko, John, & Hutchings, 2007), siblings from strangers (Bhatt, Khandelwal, & Dudley, 2010; Biedrzycki, Jilany, Dudley, & Bais, 2010; S.A. Dudley & File, 2007; Murphy & Dudley, 2009), and neighbours of the same species from different species (Maestre, Callaway, Valladares, & Lortie, 2009; Mommer et al., 2010; Semchenko, John, et al., 2007). Many invasive species like Garlic Mustard are highly selfing and have limited seed dispersal (Anderson, Dhillion, & Kelley, 1996), characteristics that allow invasives to form monospecific stands in their new habitats (Williamson, 1996). These monospecific stands are likely composed of highly related individuals, which increases the opportunity for kin selection (Kelly, 1996). When interactions with siblings are predictable (File et al., 2011; Kelly, 1996) or if plants can recognize siblings (Waldman, 1988), kin selection can favour the evolution of altruistic traits and behaviours (Hamilton, 1964). In terms of plant competition, altruism towards siblings can involve reduced competitive behaviours (S.A. Dudley & File, 2007). Thus, kin selection may favour the evolution of reduced competitive ability in invasive plants.

Here we designed two experiments to test whether and which putative competitive traits have evolved in North American ecotypes of Garlic Mustard. We considered both aboveground and belowground traits and looked at differences between responses to siblings and strangers. Because Garlic mustard is an obligate biennial, we looked at effects at both the rosette (first year growth) and bolting (second year growth) stages of the life cycle. We asked the following questions: 1) Are there differences in competitive responses between North American and European ecotypes of Garlic Mustard? 2) Are competitive responses found in above or belowground traits? 3) Are competitive responses dependent on whether plants sense neighbours aboveground or belowground? 4) Do competitive responses depend on whether neighbours are siblings and strangers?

MATERIALS AND METHODS

Experimental Design

We designed two experiments that compared the responses of North American and European ecotypes of Garlic Mustard to competition with conspecifics. One experiment compared the responses of first year traits (rosette stage) of each ecotype to different cues of neighbour identity while the other compared responses of second year traits (bolting stage) of each ecotype to different cues of neighbour identity at two different densities. The experiment designed to examine first year traits was conducted in a greenhouse while the experiment designed to examine second year traits was conducted in the field. For both experiments Garlic Mustard plants were grown from seed. Seeds were surfaced sterilized with a 10% bleach solution for 10 min. and placed into petri dishes lined with filter paper at 4°C for required stratification. North American ecotypes were obtained from two populations in Ontario, Canada while European ecotypes were obtained from four populations in Europe.

Experiment 1

After germination, seedlings were transplanted into plug trays at the cotyledon stage and kept in a growth chamber (Model no. I24L, Conviron Controlled Environments, Winnipeg, Canada) at 25°C for one week. One-week-old seedlings were then transplanted into cuboid-shaped board pots (Zipset Plant Bands, bleached board, light weight; Stuewe & Sons, Corvallis, Oregon, USA) with constant cross-sectional area in a mix of 3:1 sand and turface (Profile Products, Buffalo Grove, Illinois, USA). The plants were grown for a period of 3 months from July to October 2010 and fertilized weekly with a 20-20-20 NPK water-soluble fertilizer (PlantProd Co.,Brampton, Ontario, Canada) until the end of the experiment. The experiment was a factorial design with treatments that consisted of continent of origin (North American or European), and neighbours (solitary, siblings, or strangers). The experiment consisted of 3 blocks. Each block had 2 trays and each tray contained 64 plants. All experimental treatments were applied within each block.

The neighbours treatment consisted of either a solitary treatment, a group of 4 plants each growing alone in a pot (3.8 × 3.8 × 35.5 cm), or a neighbours treatment, a group of 4 plants planted together in a pot of four times the cross-sectional area (7.6 × 7.6 × 35.5 cm). Each group of four was then composed of siblings, 4 seedlings from the same family (maternal sibship), or strangers, 4 seedlings from different families. Within each block, half of the groups of four in the competitive conditions treatment were composed of plants from North American families and the other half from European families, thus creating the continent of origin treatment. All families were equally represented within each block. Groups of four for each treatment were randomly placed within each block. It should be noted that because aboveground density, rooting depth, and nutrient availability per plant were constant for all treatments, the neighbour treatment is not designed to test the effects of competition but instead the responses to the presence/absence and identity of neighbouring plants.

Experiment 2

After germination, seedlings were transplanted into pine cells (Stuewe & Sons Inc., Tangent, Oregon, USA) and grown in the greenhouse for five weeks. At the five-weeks-old stage seedlings were transplanted into the field. The plants were grown for a period of one year under natural conditions from June 2008 to July 2009.

The experiment was a factorial design with treatments that consisted of continent of origin (North American or European), presence of neighbours (solitary or neighbours), relatedness of neighbours (siblings or strangers), and density (medium or low). The experiment consisted of 6 blocks. Each block contained 28 groups of four plants arranged in a checkerboard pattern for a total of 112 plants per block. All experimental treatments were applied within each block except for density that was applied at the block level.

The neighbours treatment consisted of either a solitary treatment, a group of 4 plants each growing in its own buried PVC pipe 1.5 m long and 5 cm in diameter or a neighbour treatment, a group of 4 plants sharing a buried PVC pipe 1.5 m long but 13 cm in diameter. Each pot in the neighbour treatment was matched with four pots in the solitary treatment, so that sample size was equal for each treatment. Utilizing these dimensions ensured that a solitary plant had the same soil depth and average soil volume as a plant with root neighbours.

The relatedness treatments were created by making groups of four plants that were either siblings (maternal sibships) or strangers (unrelated conspecifics). A total of eight families, four North American and four European, were used and were equally represented in every block. Half of the groups of four were composed of plants from North American families and the other half from European families, thus creating the continent of origin treatment. Groups of four for each treatment were randomly placed within each block.

Placing the groups of four plants at different distances from each other created the density treatment. PVC pipe was buried in the field prior to planting at either 0.5 cm from each other (medium density treatment) or 20 cm from each other (low density) treatment. It is important to note that the density treatment varied the distance between groups of four plants while density within groups of four plants remained constant at all times.

Data Collection

For experiment 1 (rosette stage), we measured number of leaves per plant, and for the largest leaf, the length of the leaf blade as a measure of leaf size, and length of the petiole as a measure of height. We determined biomass components by collecting plant material (leaf blades, petioles, and roots) and drying it in an oven for 72 h at 65°C before weighing. Roots were then separated into storage root (main tap root of the plant and any other root more than 1 cm in diameter) and fine roots (roots less than 1 cm in diameter).

For experiment 2 (bolting stage), we scored number of stalks per plant, length of the longest stalk, length of the first four internodes on the longest stalk, number of leaves per plant, and number of pods per plants. Because plants were harvested at a final reproductive stage, most leaves had been lost by the time of harvest. Thus we did not use leaf data in our analysis of experiment 2. Instead, we measured biomass of all stalks as a measure of plant size. Because plants were growing in regular soil and roots of neighbouring plants were entangled, root traits were not be measured for experiment 2.

Data Analysis

The data were analyzed with SAS statistical software (version 9.2; SAS Institute, Cary, North Carolina, USA). We used PROC GLM to carry out analyses of variance and covariance. We used analysis of covariance to test for differences in allocation and elongation (Coleman, McConnaughay, & Ackerly, 1994; McConnaughay & Coleman, 1998). We used contrast statements to compare the effects of presence versus absence of neighbours in experiment 1 (CONTRAST option, PROC GLM) For experiment 1, we included family nested within continent of origin and block as independent variables to measure family and block effects. For experiment 2, we included family nested within continent of origin as an independent variable to measure family effects. To measure block effects in experiment 2, because blocks were assigned to different density treatments, F -ratios for density effects were tested over the mean square for blocks, nested within the density effect, in the denominator.

Because of the difficulty of separating the roots of plants in the root neighbours treatment in experiment 1, the experimental unit for belowground traits and total biomass were groups of four plants, either the four in a large pot, or four adjacent plants in single pots. For aboveground traits in experiment 1 and for all traits in experiment 2, the experimental unit was the individual. The data was log-transformed to ensure that the residual variance was homoscedastic, and the distribution of the residuals did not differ significantly from normality. Parameters are presented untransformed for clarity.

Elongation was measured as the least square mean (lsmean) from an analysis of covariance with petiole length as the dependent variable and leaf length as the covariate for experiment 1 (LSMEANS option, PROC GLM). Because individual weights of stalks were not measured elongation could not be estimated in experiment 2. Allocation to roots compared to shoots was measured as the least square mean from an analysis of covariance with root biomass as the dependent variable and aboveground biomass (sum of petiole mass and leaf blade mass) as the covariate. Allocation to storage roots was determined using fine roots as the dependent variable and storage roots as the covariate. For allocation to leaves, leaf mass was the dependent variable and petiole mass the covariate. We determined allocation to reproduction in experiment 2 by using number of fruits as the dependent variable and stem biomass as the covariate. We analyzed fitness by examining fecundity (number of fruits produced by surviving plants).

RESULTS

Effects of continent of origin

At the rosette stage, North American ecotypes of Garlic Mustard had less elongated petioles (EUR = 7.40 cm, SE = 0.32; NA = 6.75 cm, SE = 0.11; Table 1), increased specific leaf area (EUR = 12.45 cm^2/g, SE = 0.42; NA = 13.5 cm^2/g, SE = 0.18; Table 1), allocated more to roots compared to shoots (EUR = 0.32 g, SE = 0.01; NA = 0.41 g, SE = 0.02; Table 1), and allocated less to fine roots compared to storage roots (EUR = 0.21 g, SE = 0.007; NA = 0.16 g, SE = 0.007; Table 1) than European ecotypes. These differences in competitive traits, leaf morphology, and allocation did not depend on the presence or identity of neighbouring plants.

At the bolting stage, while North American plants were taller than European plants (EUR = 26.06 cm, SE = 2.48; NA = 31.25 cm, SE = 2.33; Table 3) and allocated more to reproduction (EUR = 26.06 cm, SE = 2.48; NA = 31.25 cm, SE = 2.33; Table 3) independently of other factors, differences in stem biomass, fecundity, and allocation to stalks were dependent on the presence and identity of neighbours, density conditions, or a combination of these. North American plants had higher stem biomass than Europeans but only in the presence of root neighbours and when those neighbours were siblings (Fig. 1, Table 3). Fecundity was affected by two kinds of interactions. First, North American plants had higher fecundity than Europeans but only in the presence of root neighbours that were siblings (Fig.2, Table 3). Second, North American and European plants did not differ in fecundity in high density. However in low density, North American plants had higher fecundity than Europeans when plants were growing in shared pots (Fig. 3, Table 3). Finally, North American plants allocated more to stalk production than European plants when they grew alone in pots and under low density conditions. However, they allocated less than Europeans when sharing pots at low density and when growing alone in high density (Fig. 4, Table 3).

Effects of presence of neighbours

At the rosette stage, in the presence of belowground neighbours, Garlic Mustard plants were taller (solitary = 6.49 cm, SE = 0.31; siblings = 7.55 cm, SE = 0.33; strangers = 6.98 cm, SE = 0.35; Table, 2) had lower specific leaf area (solitary = 13.71 cm^2, SE = 0.31; siblings = 12.76 cm^2, SE = 0.26; strangers = 12.47 cm^2, SE = 0.30; Table, 1) and allocated more to petioles compared to leaves (solitary = 0.043 g, SE = 0.0007; siblings = 0.046 g, SE = 0.0007; strangers = 0.045 g, SE = 0.0008; Table, 1). Moreover, plants with belowground neighbours produced more leaf biomass (solitary = 0.064 g, SE = 0.006; siblings = 0.095 g, SE = 0.009; strangers = 0.094 g, SE = 0.010; Table, 2), petiole biomass (solitary = 0.033 g, SE = 0.002; siblings = 0.049 g, SE = 0.003; strangers = 0.048 g, SE = 0.04; Table, 2) as well as more total biomass (solitary = 1.61 g, SE = 0.10; siblings = 2.01 g, SE = 0.09; strangers = 2.01 g, SE = 0.13; Table, 2). There were no effects of belowground neighbours on root biomass (data not shown).

At the bolting stage the presence of belowground neighbours affected stem biomass, allocation to stalks, and fecundity in combination with other treatments and mediated the responses of North American and European plants to the relatedness of their neighbours (Figs. 1, 2, and 4; described above). However, there was a main effect of root neighbours in allocation to reproduction. Plants that had root neighbours allocated less to reproduction than solitary plants (solitary = 12.49, SE = 0.63; root neighbours = 11.28, SE = 0.59; Table, 3).

Effects of relatedness of neighbours

At the rosette stage the only trait that responded to the relatedness of neighbours was petiole elongation. When Garlic Mustard plants were grown in groups of siblings they elongated their petioles more than plants growing with strangers or solitary plants (solitary = 6.86 cm, SE = 0.16; siblings = 7.45, SE = 0.15; strangers = 6.91 cm, SE = 0.16; Table, 1).

At the bolting stage plants were shorter when growing with siblings compared to strangers (siblings = 26.15, SE = 2.29; strangers = 31.09 cm, SE = 2.48; Table, 3). Stem biomass and fecundity were both affected by the relatedness treatment but the responses depended on continent of origin and the presence of neighbours. Stem biomass increased in North American plants growing with siblings in the presence of root neighbours and decreased in European under those conditions (Fig. 1, Table 3). The same pattern was observed for fecundity, with North American plants producing more fruits with siblings in the presence of root neighbours and European plants producing less (Fig. 2, Table 3).

DISCUSSION

In this study we found that North American ecotypes of Garlic Mustard produced overall less competitive phenotypes than European ecotypes but performed better in the presence of root neighbours when those neighbours were siblings. While differences in competitive phenotypes were independent of competition cues (i.e. they occurred across density and neighbour treatments), differences in performance and fitness were dependent on density conditions, the presence of root neighbours, and the identity of those roots, whether siblings or strangers. North American ecotypes responded less competitively in both above and belowground traits at the rosette stage by producing less elongated petioles and allocating less to fine roots. At the bolting stage, they performed better by showing increased stem biomass and fruit production in the presence of root neighbours when those neighbours were siblings. We argue that these responses, along with changes in morphology and allocation, provide evidence for the evolution of reduced competitive ability in Garlic Mustard from a trait-based perspective. We propose that changes in performance and fitness, although harder to interpret, indicate the action of kin selection in North American ecotypes of Garlic Mustard because they occurred only in the presence of sibling neighbours.

The evolution of reduced competitive ability in invasive plants has been hypothesised as a possible cause of invasiveness (Bossdorf et al., 2004). Bossdorf et al. (2004) showed that North American ecotypes of Garlic Mustard lost in competition with their ancestral European ecotypes and proposed that selection may favour reduced competitive ability in invasive plants because invasives may find weaker or fewer competitor in the new habitat. Under those conditions, and assuming that competitive traits are costly, less competitive phenotypes may be advantageous. However, little is know about which individual traits may evolve in invasive ecotypes. Here we found that aboveground, North American ecotypes of Garlic Mustard produced less elongated petioles at the rosette stage than European ecotypes. Elongation is considered a costly competitive trait (Cipollini & Schultz, 1999; Schmitt & Wulff, 1993) that allows for access to resources and interference competition aboveground (Smith, 1995). Reduced elongation was also found in a previous study (Chapter 3), where density was varied and aboveground responses measured. Belowground, North American ecotypes also produced less competitive phenotypes than Europeans. They allocated more resources to roots compared to shoots but fewer resources to fine roots, the resource gathering (Givnish, 1986) and hence the competitive portion of the root system compared to coarse roots. Taken together, these results provide strong evidence in support of the evolution of reduced competitive ability hypothesis in Garlic Mustard. However, whether and why the evolution of less competitive traits may contribute to introduced ecotypes becoming or remaining invasive is still unclear.

At the rosette stage, North American and European ecotypes differed in competitive traits, with North American showing less competitive phenotypes, but there were no differences in performance (biomass components). Differences in performance and fitness occurred at the bolting stage. North American ecotypes had more stem biomass and higher fecundity than European ecotypes. However, these differences occurred only when North American ecotypes were in the presence of root neighbours and when these neighbours were siblings (Figs. 1 and 2). These results have several implications. First, they indicate that, for Garlic Mustard, the success of a strategy that involves producing less competitive phenotypes during the rosette stage of the life cycle depends on the presence and identity of neighbours belowground. Second, they indicate that belowground interactions may be crucial because North American ecotypes did not perform better when they grew each in their own pot, regardless of the identity of neighbours. Third, and most importantly, that producing less competitive phenotypes will only provide an advantage in groups of siblings.

These results differ from those in chapter 3, where North American ecotypes of Garlic Mustard also produced less competitive phenotypes than Europeans at the rosette stage but showed overall reduced performance and fitness at the bolting stage. However, two key differences exist between this study and the one in chapter 3. First, groups of plants in chapter 3 were always composed of mixed families, the equivalent to the stranger treatment here in which we found no differences in performance and fitness between North American and European ecotypes. Second, plants in chapter 3 were subjected to different levels of competition by manipulating density conditions. Here, we examined responses to the presence and identity of belowground neighbours while maintaining the level of belowground competition constant. We maintained resources per plant constant by increasing pot size proportionally with number of plants per pot thus preventing responses to resource depletion. This methodology was adapted from studies that looked at responses to the presence of root neighbours (Gersani et al., 2001; Maina et al., 2002; Murphy & Dudley, 2007; O'Brien et al., 2005). Although the manipulation of pot size has been challenged (Hess & de Kroon, 2007; Schenk, 2006; Semchenko, Hutchings, & John, 2007; although see O'Brien & Brown, 2008) I demonstrated that the methodology is valid (Chapter 2). Thus, it is not surprising that differences in performance and fitness between North American and European ecotypes of Garlic Mustard will differ between these two studies.

Bossdorf et al. (2004) proposed that less competitive phenotypes may be beneficial if competitive traits are costly and introduced species encounter few or weak competitors in the new habitat. An alternative explanation suggests the action of kin selection (Chapter 3), with less competitive phenotypes providing gains through inclusive fitness and avoiding the cost of competition. The differences in competitive responses between North American and European ecotypes of Garlic Mustard did not depend on the presence or identity of neighbours. However, differences in performance and fitness did. These findings are consistent with the action of kin selection, which will favour the evolution of altruistic traits in groups of siblings (Hamilton, 1964). For kin selection to act it is necessary that plants interact predictably with siblings (File et al., 2011; Kelly, 1996) or that they can recognize siblings from strangers (S.A. Dudley & File, 2007; Waldman, 1988). Because seed dispersal is stochastic the likelihood of plants predictably interacting with siblings is low (File et al., 2011). However, a feature of invasive plants is that they form high-density monospecific stands in the introduced habitats (Williamson, 1996). If these stands are composed of highly related individuals because of limited seed dispersal and high levels of self-fertilization, then a genetically structured populations can be formed that can meet the requirements for kin selection (Kelly, 1996). Another way that kin selection may influence the evolution of competitive traits in invasive plants is if plants can distinguish neighbours based on whether they are siblings or strangers. This ability has been recently shown in plants (Bhatt et al., 2010; S.A. Dudley & File, 2007; Murphy & Dudley, 2009) and responses to siblings and strangers seem to be mediated by root exudates (Biedrzycki et al., 2010). Our results support the hypothesis that Garlic Mustard plants can recognize siblings. We found several traits at both the rosette and bolting stages that responded differently to siblings and strangers. At the bolting stage, our results are clearly in accordance with predictions from kin selection theory. Plants showed less competitive behaviours (less height) and increased performance and fitness (more stem biomass and fruit production) when growing with siblings. At the rosette stage however, our results are harder to interpret. We found that plants elongated their petioles more when growing with siblings than when growing with strangers. Because petiole elongation is considered a competitive trait, this result could be interpreted as an increased competitive response towards siblings instead of the reduced competitive response predicted by kin selection theory. However, a very similar result has been found in another species, *Impatiens pallida*, and has been argued that changes in aboveground canopy architecture, by means of stem elongation, may reduce mutual shading and serve as a form of cooperation among siblings (Murphy & Dudley, 2009).

In addition to showing differences in competitive traits and performance, North American and European ecotypes of Garlic Mustard also showed differences in morphology and allocation. North American ecotypes produced wider and thinner leaves as indicated by increases in specific leaf area. They also allocated more resources to roots and specifically to storage roots. These responses may also contribute to invasiveness of Garlic Mustard in North America. Increased specific leaf area allows for better light capture and thus may serve as a mean to offset costs associated with the observed reduced elongation response. In addition, allocation of resources to storage roots may allow for better performance at the bolting stage during the second year of growth.

The original ERCA hypothesis was based on a study that showed that North American Garlic Mustard had reduced size and fitness in intraspecific conditions than did European Garlic Mustard (Bossdorf et al., 2004). Here we find support for the ERCA hypothesis in putative competitive traits but we found that North American ecotypes performed better than European ecotypes in terms of size and fitness. Similar increases in performance have been interpreted as evidence for the EICA hypothesis (Blossey & Notzold, 1995). However, overall differences in performance may be hard to interpret (Colautti et al., 2009; File et al., 2011). Fitness measurements may be influenced by unaccounted abiotic environmental factor (Colautti et al., 2009) as well as by biotic characteristics of the environment like the genetic identity of neighbours (File et al., 2011; Hamilton, 1964). Therefore, in this study we used a trait based approach in addition to the more traditional fitness based approach to look for evidence of evolution of competitive ability in invasive plants (File et al., 2011). However, although accounting for abiotic factors like differences in latitudinal gradients between North American and European populations (Colautti et al., 2009) was beyond the scope of this study, we specifically examined how performance differed depending on the identity of neighbours in a group. We found that North American ecotypes of Garlic Mustard only performed better in groups of siblings in shared pots. We argue that these results are consistent with the action of kin selection in North American ecotypes and propose that kin selection pressures may lead to the evolution of less competitive traits and convey an advantage in monospecific stands of related individuals.

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Table 1 Analysis of covariance for first year traits for greenhouse grown Garlic Mustard (*Alliaria petiolata*)

		Petiole elongation		Specific leaf area		Petiole to leaf allocation		Root to shoot allocation		Fine to storage root allocation	
Source	d.f.	**F**	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Continent	1	4.08	**0.0441**	5.12	**0.0243**	0.17	0.6823	8.69	**0.0041**	26.44	**<.0001**
Relatedness	2	6.36	**0.0020**	7.94	**0.0004**	9.39	**0.0001**	0.02	0.9850	0.82	0.4453
C x R	2	0.02	0.9822	1.23	0.2951	0.75	0.4721	0.68	0.5070	1.32	0.2734
Fam (con)	7	9.30	**<.0001**	1.19	0.3096	5.18	**<.0001**	na	na	na	na
Block	2	4.92	**0.0079**	18.39	**<.0001**	12.12	**<.0001**	1.81	0.1696	8.59	**0.0004**
Covariate	1	549.37	**<.0001**	5397.74	**<.0001**	6713.15	**<.0001**	432.77	**<.0001**	550.22	**<.0001**
Contrast	d.f.	**F**	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Solitary vs shared	1	2.93	0.0880	15.77	**<.0001**	15.93	**<.0001**	na	na	na	na
Siblings vs strangers	1	7.62	**0.0061**	1.10	0.2950	0.81	0.3505	na	na	na	na

Bold numbers indicate significant values.

Table 2 Analysis of variance for first year traits for greenhouse grown Garlic Mustard (*Alliaria petiolata*)

		Height		Leaf biomass		Petiole biomass		Total biomass	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Continent	1	0.02	0.8837	0.09	0.7626	0.11	0.7400	1.69	0.1977
Relatedness	2	5.96	**0.0029**	9.29	**0.0001**	13.45	**<.0001**	4.49	**0.0140**
C x R	2	1.11	0.3318	0.59	0.5549	0.65	0.5215	0.54	0.5844
Fam (con)	7	6.79	**<.0001**	4.23	**0.0002**	4.71	**<.0001**	na	na
Block	2	0.49	0.6101	0.43	0.6521	0.44	0.6460	0.05	0.9532
Contrast	d.f.	**F**	*P*	*F*	*P*			*F*	*P*
Solitary vs shared	1	6.84	**0.0093**	16.81	**<.0001**	25.66	**<.0001**	8.73	**0.0040**
Siblings vs strangers	1	2.98	0.0855	0.02	0.8973	0.02	0.8826	0.00	0.9896

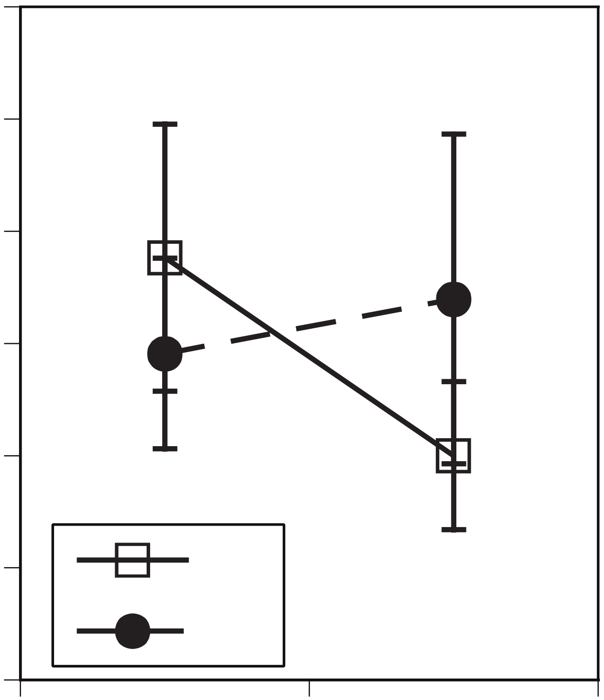
Bold numbers indicate significant values.

Table 3 Analyses of variance and covariance for second year traits for field grown Garlic Mustard (*Alliaria petiolata*)

		Height		Stem Biomass		Fecundity		Allocation to Reproduction		Allocation to Stalks	
Source	d.f.	*F*	*P*	**F**	*P*	*F*	*P*	*F*	*P*	*Chi-square*	*P*
Density	1	1.45	0.2301	1.13	0.2882	1.52	0.2184	0.00	0.9943	1.12	0.2904
Relatedness	1	4.33	**0.0386**	2.26	0.1338	1.66	0.1993	1.20	0.2740	0.09	0.7591
Neighbours	1	0.99	0.3199	2.28	0.1328	2.12	0.1466	4.32	**0.0389**	0.51	0.4771
Continent	1	4.40	**0.0371**	1.99	0.1602	5.72	**0.0176**	11.19	**0.0010**	0.06	0.8138
Den x Rel	1	0.11	0.7458	0.02	0.8855	0.21	0.6465	1.02	0.3133	0.16	0.6885
Den x Nei	1	1.39	0.2394	0.42	0.5156	0.53	0.4677	0.72	0.3984	13.14	**0.0003**
Den x Con	1	2.15	0.1440	0.65	0.4222	0.30	0.5847	0.87	0.3527	1.50	0.2207
Rel x Nei	1	0.01	0.9382	0.07	0.7874	0.00	0.9683	0.03	0.8741	1.90	0.1676
Rel x Con	1	1.59	0.2089	1.65	0.1998	1.64	0.2020	0.75	0.3862	0.36	0.5474
Nei x Con	1	1.96	0.1629	4.49	**0.0352**	5.47	**0.0203**	1.73	0.1893	0.07	0.7966
Den x Rel x Nei	1	0.06	0.8088	0.01	0.9152	0.64	0.4677	0.97	0.3270	0.96	0.3272
Den x Rel x Con	1	2.65	0.1050	1.48	0.2259	0.00	0.9890	1.04	0.3089	0.65	0.4194
Den x Nei x Con	1	1.15	0.2853	0.58	0.4470	3.93	**0.0487**	1.10	0.2955	5.31	**0.0213**
Rel x Nei x Con	1	2.32	0.1289	10.47	**0.0014**	8.52	**0.0039**	0.02	0.8779	2.26	0.1326
Den x Nei x Rel x Con	1	0.44	0.5094	0.01	0.9419	1.25	0.2651	3.64	0.0577	3.68	0.0550
Fam (Con)	6	2.00	0.0669	2.81	**0.0118**	4.69	**0.0002**	2.25	**0.0400**	17.95	**0.0064**
Block (Den)	4	1.87	0.1161	2.44	**0.0480**	3.11	**0.0162**	1.14	0.3400	2.22	0.3294
Covariate	1	na	na	na	na	na	na	1642.43	**<.0001**	75.04	**<.0001**

Bold numbers indicate significant values.

Fig. 1) Differences in stem biomass between North American and European ecotypes of second year Garlic Mustard in response to the presence and identity of belowground neighbours.



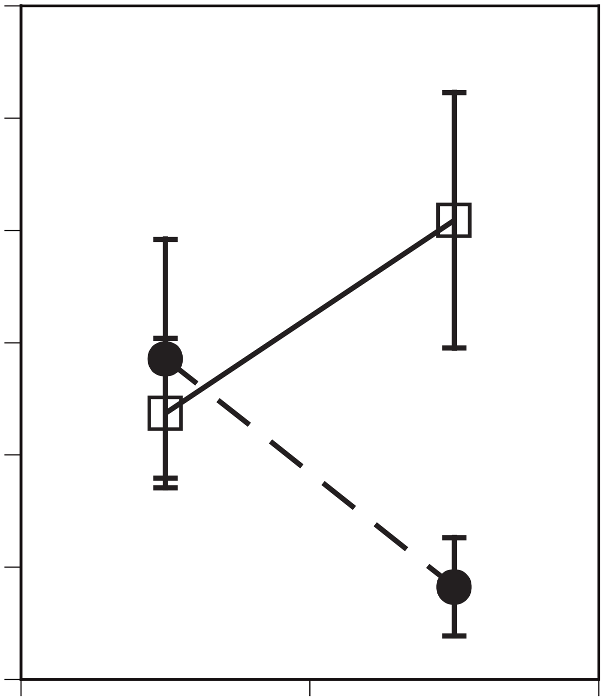


Fig. 2) Differences in fecundity between North American and European ecotypes of second year Garlic Mustard in response to the presence and identity of belowground neighbours.

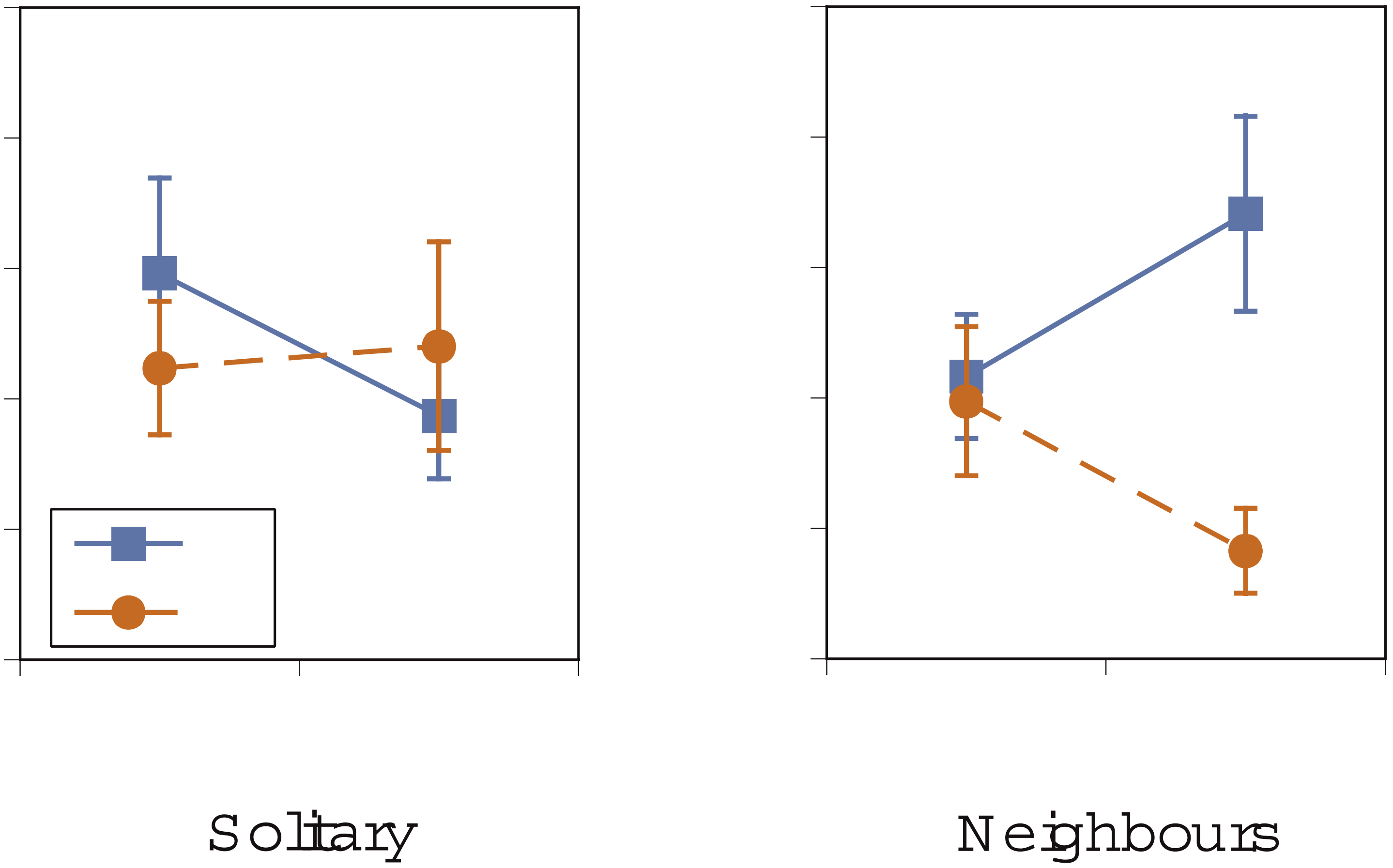

Solitary
Neighbours

Fig. 3) Differences in fecundity between North American and European ecotypes of second year Garlic Mustard in response to the presence of belowground neighbours under high and low aboveground density.

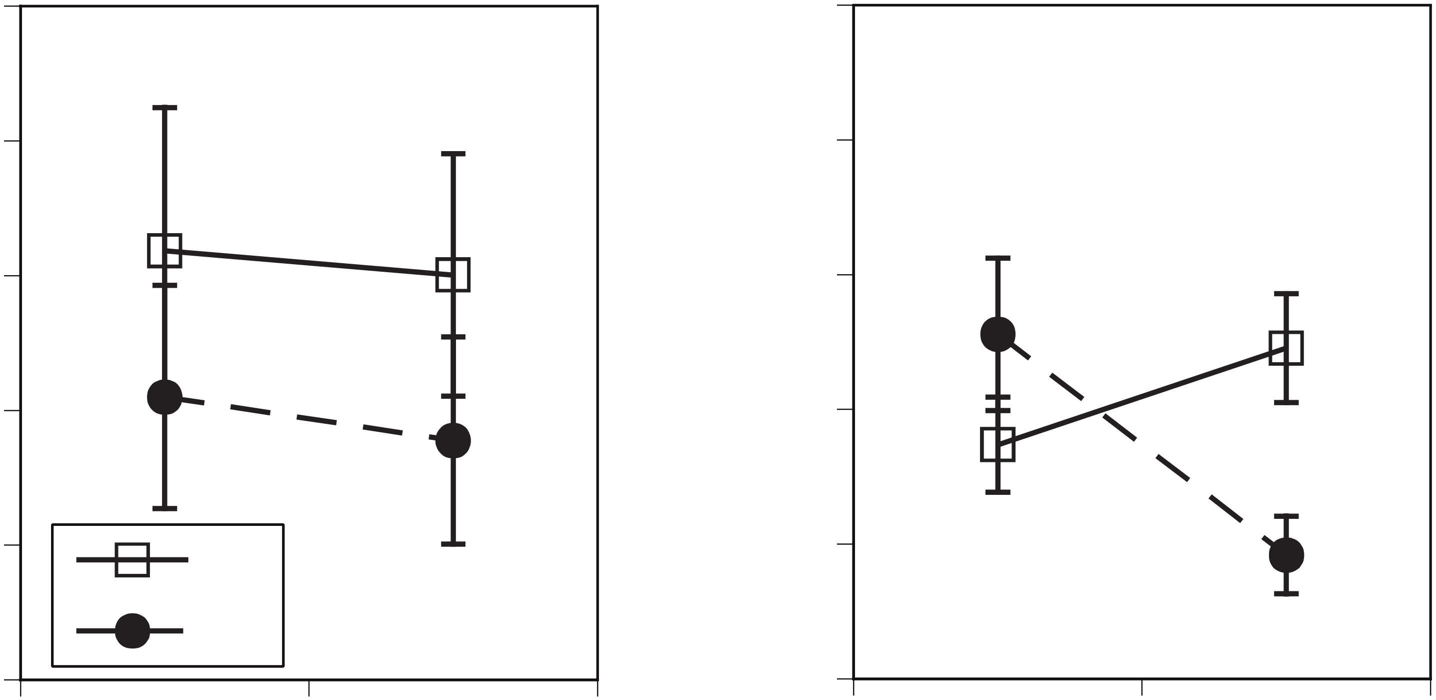
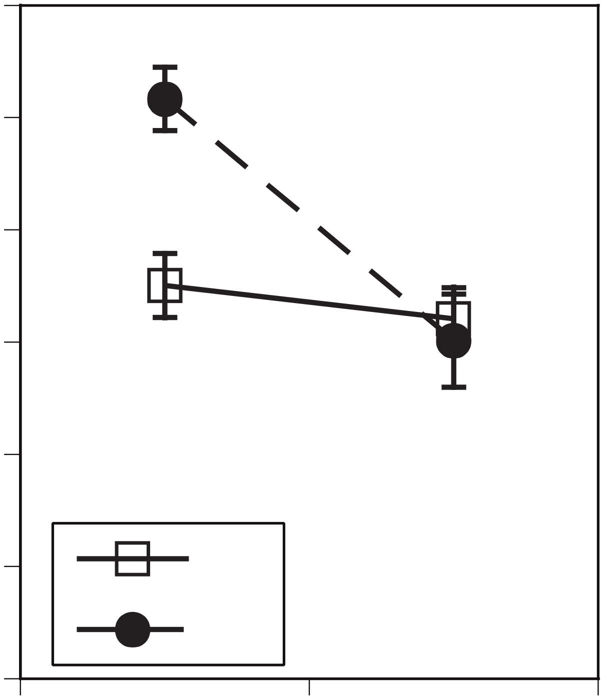
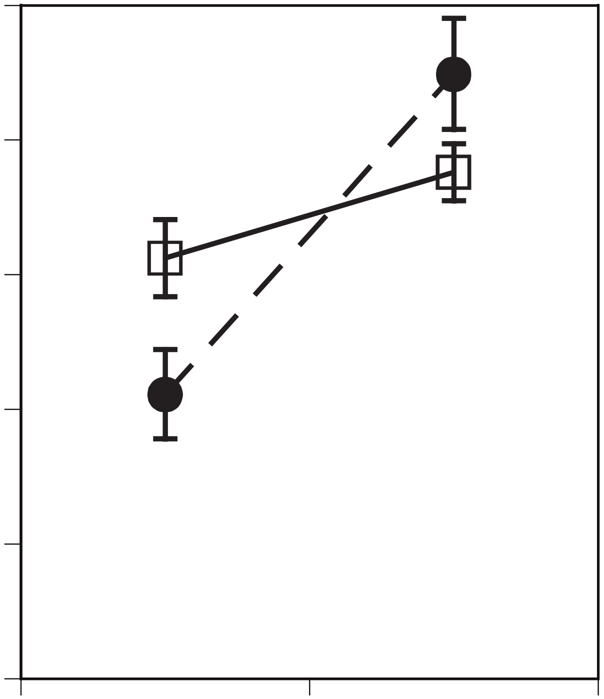


Fig. 4) Differences in allocation to stalk production between North American and European ecotypes of second year Garlic Mustard in response to the presence of belowground neighbours under high and low aboveground density. Allocation to stalk production was measured from an analysis of covariance with number of stalks as the dependent variable and stem biomass as the covariate.





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CHAPTER 6:

GENERAL CONCLUSION

Here I have presented four studies with the objective to further our understanding of the competitive behaviours of plants. This body of work encompassed six experiments, five that examined the responses of *I. pallida* and *A. petiolata* to cues of competition and to the identity of competitors (Chapters 2, 4, and 5) and one that tested empirically the validity of the methodology used in those studies (Chapter 3).

In Chapter 2, I examined the responses of *I. pallida* to the cues of above and belowground competition applied simultaneously and assessed whether those responses would depend on the identity of neighbours (siblings or strangers). The study provided evidence that *I. pallida* was capable of kin recognition. *I. pallida* responded differently to the cue of belowground competition (presence of neighbouring roots) when growing with siblings and strangers. Although this study supported the idea that plants are capable of kin recognition, the responses to siblings and strangers of *I. pallida* were different from those found previously in *C. edentula* (Dudley & File, 2007). This way, the study presented in chapter 2, demonstrated that responses to siblings were species-specific and in accordance to the ecology of the species. Moreover, for the first time, I showed evidence that of a response to the cue of belowground competition (presence of root neighbours) can be mediated by the presence of the cue of aboveground competition (low R:FR ratio). Taken together these results showed the complexity of competitive responses that plant may have in response to impending competition from neighbours and to the identity of those neighbours (Chapter 2).

To examine responses to the presence of neighbours separately from resource depletion belowground, I used a methodology that involved increasing rooting volume (pot size) proportionally with number of plants per pot (Chapters 2 and 5). This allowed to maintain belowground resources per plant constant while manipulating the presence and absence of neighbouring roots in a pot. However, a paper by Hess and de Kroon (2007) proposed that plants could perceive these changes in rooting volume and that some of the results obtained form studies that used this methodology could be artefacts of pot size manipulations. This argument was theoretically based and was further formalized with a set of hypotheses. However these hypotheses have never been empirically tested before (Hess & de Kroon, 2007). In chapter 3, I tested the hypotheses proposed by Hess and de Kroon (2007) empirically and showed that the methodology used in chapters 2 and 5 is valid.

Chapters 4 and 5 examine the responses of the invasive species *A. petiolata* to density conditions, presence of root neighbours and identity of root neighbours. In chapter 4, I designed two experiments with the objective of elucidating whether and how *A. petiolata* would respond to density and whether differences in competitive responses between native and invasive ecotypes would depend on density. I found that native and invasive ecotypes of *A. petiolata* responded strongly to density however invasive North American ecotypes showed overall less competitive phenotypes than native European ecotypes. More importantly, the elongation of vertical spacers (internodes and petioles) in response to density, considered a form of competitive behaviour, was lower in invasive ecotypes compared to native ecotypes. These results provided evidence in support of the evolution of reduced competitive ability hypothesis in invasive plants however why the evolution of reduced competitive phenotypes in invasive ecotypes would be advantageous was still unclear. Given the ecology of invasive species in their introduced habitats and in particular that of *A. petiolata* that can form monospecific stands of closely related individuals, I proposed that kin selection could be a likely explanation (Chapter 4).

In chapter 5, I examined *A. petiolata* competitive responses to the presence and identity of neighbours (siblings or strangers) and found that invasive North American ecotypes again showed less competitive phenotypes than native European ecotypes. However, performance and fitness were increased in groups of siblings that were growing in shared pots. These results provide further evidence in support of the ERCA hypothesis in invasive plants, and are consistent with the action of kin selection.

The comparison of invasive and native ecotypes of invasive species in common gardens has been extensively used as a tool to elucidating evolutionary changes in invasive species. However, this approach is not without limitations. Most studies that use this approach, including those presented here, do not account for sources of among-population variation that can affect performance and fitness outcomes (Colautti, Maron, & Barrett, 2009). Although a trait-based approach that examines putative competitive traits instead of just performance and fitness traits provides a more robust comparison, future studies should consider sources of among-population variation such as latitudinal gradients (Colautti et al., 2009) to get a more accurate picture of the role of rapid evolution in biological invasions.

After invasive species are introduced into new habitats, they experience different biotic and abiotic conditions compared to their native habitat. These differences among habitats will impose novel selection pressures on the invasive ecotype. However, little is yet know about these selection pressures especially those imposed by changes in the competitive environment.

What exactly constitutes competitive ability is also unclear as the ability of plants to compete is determined by both competitive response and competitive effect (Cahill, Kembel, & Gustafson, 2005). Therefore, more research is needed to elucidate whether competitive ability as a whole will evolve in invasive species by examining competitive responses as well as the effect that those responses will have on competitors. Moreover, whether the evolution of competitive ability can be the main factor that allows plants to become invasive, as originally suggested by the EICA and ERCA hypotheses, or just a component that adds to invasive characteristics is yet to be determined. Based on the evidence presented here I advocate the later because kin selection is more likely to operate once monospecific stand of relative have been established. However, more research is clearly needed to answer this question.

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