

**THE ROLE OF THE NOVEL WEAPONS AND ENEMY RELEASE  
HYPOTHESES ON THE INVASIVENESS OF KNAPWEEDS, *CENTAUREA*  
SPP., IN NORTH AMERICA.**

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial  
fulfillment of the requirements for the degree of Master of Science.

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## ABSTRACT

Knapweeds, *Centaurea* spp., are among the most successful grassland invaders in North America. Despite similar life histories, only certain members of this genus have become problem species, while others remain as benign, naturalized exotics. Two ideas which have been proposed to explain the success of exotic plants are the novel weapons (allelopathy) and enemy release hypotheses. In this study, both hypotheses were examined by comparing highly invasive knapweeds to their non-invasive kin. Leachates collected naturally from the soils of both highly invasive and non-invasive knapweeds had no noticeable allelopathic effects on indicator species. Though allelopathy has been reported in invasive knapweeds, it is likely the result of specific environmental factors. In terms of herbivory, non-invasives were fed on more by a generalist feeder, and incurred significantly more herbivory than invasives in a common garden. This indicates that invasive knapweeds may possess novel defenses which deter local herbivores.

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## CHAPTER ONE: GENERAL INTRODUCTION

### *Exotic Plant Invasions*

The intrusion of aggressive, exotic plants into North American natural habitats has often been the result of the purposeful or accidental introduction of species as a result of human activity (Mack et al., 2000). As humans began to move around the globe, many species were purposely introduced as crops or for medicinal purposes, as ornamentals, or as a means to prevent soil erosion (D'Antonio and Vitousek, 1992; Weber, 2003; Forseth and Innis, 2004). Other species have arrived by accident as contaminants of seed crops (Watson and Renney, 1974, Story et al., 2001) or through the release of ballast water (Mills et al., 1996). Multiple introductions and the founding of several populations of a species can promote the establishment of plant populations within natural communities (Moody and Mack, 1988). While the majority of introduced plants fail to establish or remain at stable population sizes (Williamson and Fitter, 1996), a small fraction of species eventually move beyond a latent state within the environment and invade local habitats displacing native species and interrupting normal ecosystem functioning (D'Antonio and Vitousek, 1992; Mack et al., 2000).

Exotic, invasive plants pose many threats to the natural habitats in which they invade. Aquatic invaders such as alligatorweed (*Alternanthera philoxeroides*) and hydrilla (*Hydrilla verticillata*) can form dense monocultures, which can block irrigation systems, inhibit light flow to underlying aquatic plants (ultimately preventing photosynthesis), and cause the build-up of debris in waterways which may lead to flooding (Holm et al., 1997). Vines, such as kudzu (*Pueraria montana*), can

smother native vegetation thereby altering the biodiversity in the environment in which they invade (Sun et al., 2006). Other terrestrial invaders may alter soil chemistry through allelopathy (Heisey, 1996; Ridenour and Callaway, 2001), displace native species (Meekins and McCarthy, 2001), disrupt natural fire regimes (D'Antonio and Vitousek, 1992) or pose major threats to agricultural communities and grazing animals (Broughton, 2000). Species such as Japanese barberry (*Berberis thunbergii*) and Japanese stilt grass (*Microstegium vimineum*) have been shown to change the soil community around their rhizospheres, altering the bacteria to fungal ratios from that found under native plants (Kourtev et al., 2002).

The establishment of exotic plant species within natural areas is often predicted by the “power of ten rule”. Of all species that are introduced, only about 10% will escape into the natural environment, and only 10% of those will actually become invaders within their new habitats (Williamson and Fitter, 1996). Lockwood et al. (2001) obtained similar proportions when they evaluated non-indigenous plant species in California, Florida and Tennessee, finding that 5.8%, 9.7% and 13.4%, respectively, had become aggressive weeds. For the large majority that do not become established, barriers such as low initial population size, inappropriate climatic conditions, and biotic resistance (Maron and Vilà, 2001) from competition with local flora and herbivory by local fauna may play a combined role.

### ***Life-History Traits of Invasive Plants***

Many life-history characteristics have been found to be common amongst invasive plant species. Daehler (1998), found that many natural-areas invaders include plant species that can reproduce clonally, are aquatic (i.e. alligatorweed), or

which are climbing vines (*Clematis* spp.). Clonal growth has been reported as a means by which many woody species, including salt cedar (*Tamarix* spp.) in the United States (Gaskin and Schaal, 2002) and willows (*Salix* spp.) in Australia (Adair et al., 2006), have become invasive. In addition, nitrogen fixers were also highly represented amongst the invasive species studied by Daehler (1998). These included *Acacia* spp. and the highly invasive kudzu vine (*Pueraria montana*).

Novel mutualistic interactions between introduced plants and native flora and fauna have aided in the success of some exotics. These relationships include pollination by insects, mycorrhizal formation and seed dispersal by animals (Richardson et al., 2000). Asteraceous species have been shown to be favoured by pollinators, giving exotic asters an added advantage within habitats (Morales and Aizen, 2002). *Centaurea maculosa* seeds have been shown to be favourably dispersed by native ants which feed on elaiosomes located on the seeds without consuming the seeds (Jensen and Six, 2006).

A study comparing invasive and non-invasive pines (*Pinus* spp.) found that, in general, the invasive members have quick germinating seedlings as well as more frequent production of large seed crops (Rejmànek and Richardson, 1996). In addition, many of the aggressive invaders produced smaller seeds that were more easily dispersed (Rejmànek and Richardson, 1996). Rejmànek (1996) also described the invasive success of exotic plant species in terms of phylogeny. He pointed out that many of the highly invasive weeds within North America belonged to genera which were absent from the continent including *Ailanthus*, *Hydrilla*, *Melaleuca*, *Pueraria*, *Tamarix*, and *Salix*. These species may possess characteristics not present in the

native flora, hence promoting their invasion. Moreover, herbivores and pathogens within the native range will be less likely to shift over to such plants, as the exotic plants are phylogenetically distant from their normal hosts (Rejmànek, 1996). In addition, exotics representing life forms (i.e. annuals, trees, etc.) which are not present within the introduced habitat are more likely to become invasive (Mack, 2003). Exotic species originating from areas with similar climates as their introduced ranges also have a higher chance of becoming invasive, though differences in habitat structure may still restrict invasion (Mack, 1996; Richardson and Thuiller, 2007).

Phenotypic plasticity to both biotic and abiotic factors has also been documented in invasive plant species (Callaway et al., 2003). For example, the invasive grass *Microstegium vimineum* allocates more resources to the production of leaf biomass in shaded environments than in open plots, and will increase flower production when the soil is fertilized (Claridge and Franklin, 2002). This plasticity allows it to occupy a range of habitats. Alligatorweed, *Alternanthera philoxeroides*, which generally reproduces asexually, has been found to allocate more resources to its roots when nutrients and water are limiting to ensure that the root system survives the winter so that clonal growth can occur in the spring (Geng et al., 2007).

Exotic plants are often opportunistic, and take advantage of freshly disturbed sites. Morales and Aizen (2002) found that only five out of 15 of their studied exotic plants occurred in undisturbed habitats, whereas 13 of 15 native species occurred in both undisturbed and disturbed sites. Exotic plants tend to easily invade freshly disturbed sites, but their numbers tend to decline as succession from old fields back to natural forests progresses (Meiners et al. 2002). Many of the exotic annuals and

biennials are displaced by woody native species due to shading; however, shade-tolerant invasive plants such as garlic mustard, *Alliaria petiolata* (Meekins and McCarthy, 2001), and honeysuckles, *Lonicera* spp. (Meiners et al., 2002), often persist and remain aggressive even in non-disturbed habitats. The success of exotics including knapweeds (*Centaurea* spp.) within undisturbed sites is often attributed to more complex interactions with native flora and fauna. These interactions include plant – plant competition through allelopathy, a reduction in the number of natural enemies (herbivores) within the introduced ranges, and the evolution of increased competitive ability as a result of a lack of herbivores.

***Interactive Hypotheses of Invasion Success: Allelopathy, Enemy Release, and EICA***

Allelopathy, or the natural release of phytotoxic compounds from one plant which inhibits the growth of neighbouring vegetation, has been gaining momentum as one the leading explanations as to why some exotics become invasive (Callaway and Ridenour, 2004). Allelopathic potential has been reported in numerous invasive species including garlic mustard, *Alliaria petiolata* (Prati and Bossdorf, 2004), *Lantana camara* (Gentle and Duggin, 1997), and in knapweeds, *Centaurea* spp. (Callaway and Aschehoug, 2000; Ridenour and Callaway, 2001; Callaway et. al., 2006). The novel weapons hypothesis (Inderjit et al., 2006) predicts that allelopathy in the introduced range is more detrimental to native plant species because they are not adapted to the presence of the novel phytochemicals within the soil, hence giving the intruder an increased competitive advantage against its new neighbours. This advantage is absent within the intruder's native range, as local species are presumed

to have adapted to the chemistry (Callaway and Aschehoug, 2000). In addition to the direct effects on plant competition, the persistence of allelopathic compounds in soils can alter the natural succession of plants within a habitat (Bazzaz, 1979) as well as be an indicator of novel herbivore defensive chemistry present in the intruder (Kelsey and Locken, 1987).

The presence of novel defenses in an exotic plant may make it less palatable to non-adapted native herbivores and hence give it a competitive advantage against highly attacked natives. Keane and Crawley (2002) proposed the enemy release hypothesis to explain the invasive success of some exotic plants. Having left their herbivore fauna behind, exotic plants suffer less damage and hence have more energy to spend for growth and reproduction than the native vegetation which is attacked regularly. Such species can become invasive if local herbivore faunas are unable to detoxify these compounds and hence avoid feeding on these plants. Some aggressive exotics, including *Silene vulgaris*, are less attacked in their introduced ranges as compared to their native range (Wolfe, 2002), while others such as *Vincetoxicum rossicum*, *Alliaria petiolata*, and *Centaurea maculosa* experience significantly lower levels of damage than native species and non-invasive kin within their introduced habitats (Agrawal and Kotanen, 2003; Cappuccino and Carpenter, 2005; Carpenter and Cappuccino, 2005). In a review of highly invasive and non-invasive exotic plants, Cappuccino and Arnason (2006) found that invasives were more likely than non-invasives to contain phytochemicals which were not present in native North American taxa. Many of these compounds have been reported as having some biocidal effects.

The enemy release hypothesis can be considered the precursor to the evolution of increased competitive ability (EICA) hypothesis, which was proposed by Blossey and Nötzold (1995), and states that the invasiveness observed in some exotics is due to a genetic shift from defensive traits to vegetative growth in the absence of herbivores. Comparing purple loosestrife, *Lythrum salicaria*, acquired from an introduced, invasive, herbivore-free population in North America and a native, herbivore-loaded population from Switzerland, they found that introduced plants were larger than those from the native range. Moreover, root-feeding larvae performed better on the North American plants than those from Switzerland, indicating a reduction in defense in the introduced populations (Blossey and Nötzold, 1995). Populations of California poppy, *Eschscholzia californica*, from introduced ranges are often more robust and produce more seeds than those from native populations, but only when competition is minimal, such as in disturbed habitats (Leger and Rice, 2003). Siemann and Rogers (2003) found that leaves from introduced *Sapium sebiferum* were preferred more than those from the native population by a generalist grasshopper, indicating a potential decrease in defense in the introduced populations. Since the introduced population is rarely fed upon, they equate its ability to invade on the relocation of resources from defense to growth and reproduction (Siemann and Rogers, 2001). In another study, Rogers and Siemann (2005) found that introduced populations of *Sapium sebiferum* are able to compensate more than native populations after both mechanical and natural defoliation, indicating a shift towards growth in the invasive population. However, there was not a significant difference between the introduced and native populations in herbivory

caused by a native generalist herbivore. To fully support EICA, a difference in phytochemistry between populations and a preference of the generalist herbivore for the introduced population would have to be shown. Joshi and Vrieling (2005) found that invasive populations of tansy ragwort (*Senecio jacobaea*) did allocate more resources to growth and hence were larger in their introduced ranges, however, a trade-off with levels of defensive chemicals was not found. Ragwort within introduced ranges produced equal or higher amounts of pyrrolizidine alkaloids as feeding deterrents for generalists; however, defense against specialists was reduced (Joshi and Vrieling, 2005).

Many hypotheses have been proposed to explain the success of exotic plants within their introduced ranges; however, finding the common thread that ties this success together is often difficult. In order to establish why members of a certain group of plants become invasive while other congeners either fail to establish or remain low in number, it is important to compare the species in a common environment. Moreover, comparing a small group of related species (i.e. those from the same genus) controls for characteristics due to phylogeny that are present when species are not closely related. One group of exotics which exhibit this dichotomy are the knapweeds (*Centaurea* spp.), with several members becoming highly invasive within North America, while others either remain benign as garden plants or as naturalized members of the plant community.

The knapweeds are a good study system for understanding exotic plant invasiveness since both highly invasive and non-invasive members are present within North America. In this thesis, I have examined two major hypotheses centering on

why certain knapweeds have become aggressive natural-areas invaders, while others have not. The novel weapons hypothesis focuses on the key role of allelopathy in plant invasions, and has been reported in several members of this group. By comparing leachates collected from the soils of the aggressive invaders and their non-invasive kin on the growth of indicator species, it will be possible to determine if allelopathy is a driving force in knapweed success. Secondly, I have examined the role that release from natural enemies plays on *Centaurea* invasion. By comparing herbivory levels between invasives and non-invasives in a common garden as well as in no-choice feeding experiments, it is possible to deduce the degree to which aggressive invaders escape herbivory.

#### ***Natural History and Biology of Knapweeds (Centaurea spp.)***

Knapweeds (*Centaurea* spp.) are members of the Asteraceae, and belong to the thistle tribe (Cardueae, subtribe Centaureinae) which consists of 83 genera and approximately 2500 species (Bremer, 1994). There are approximately 500 species of *Centaurea*, most of which are native to Eurasia but which have been widely introduced world-wide (Bremer, 1994). The USDA Plants Database (2007) lists 38 *Centaurea* species as being present within the United States, only three of which are native: *C. americana*, *C. rothrockii* and *C. transalpine*. The Centaureinae are considered to be the most chemically diverse group within the Cardueae, having a variety of flavanones, lignans, and sesquiterpene lactones (Heywood et al., 1977). For the purposes of this project, three invasive knapweeds (*C. maculosa* Lam., *C. diffusa* Lam., and *C. solstitialis* L.) and four non-invasive knapweeds (*C. cyanus* L., *C. jacea* L., *C. scabiosa* L., and *C. uniflora* Turra spp. *nervosa* (Willd.) Bonnier & Layens),

were compared in order to examine the potential factors which promote invasion in a small sub-set of this group.

Spotted knapweed, *C. maculosa*, is a deep tap-rooted biennial or short-lived perennial (3 to 9 years), which was first identified in North America in 1893 and most likely arrived as a crop contaminant (Story et al., 2001). It is native to central Europe where it is found as far east as central Russia and Siberia (Sheley et al., 1999a).

*Centaurea maculosa* flower heads are generally purple but can vary to pink or white with a few small spines present on the bracts (Watson and Renney, 1974). Spotted knapweed is a self-compatible species, producing upwards of 40 000 seeds per plant, which are dispersed by movement of the floral heads that propel seeds away from the parent (Watson and Renney, 1974). In addition, spotted knapweed can spread vegetatively through lateral shoots beneath the soil (Watson and Renney, 1974).

*Centaurea maculosa* is an aggressive invader of rangelands and old fields in British Columbia and Alberta, but is a fairly benign species within Ontario (Watson and Renney, 1974). It is also highly invasive within the western United States, reaching densities of up to 400 plants/m<sup>2</sup> (Weber, 2003). It is estimated that over 2.8 million hectares of land are currently infested, with the majority found in Montana (1.9 million hectares) (Story et al, 2001). The presence of spotted knapweed reduces foraging potential in fields, and promotes soil erosion and water run off (Jacobs and Sheley, 1998). Native plant diversity is also negatively affected. *Centaurea maculosa* is a stronger competitor for water and other nutrients than several native species (Hill et al., 2006). In addition, *C. maculosa* potentially disrupts native floral communities through the release of allelochemicals into the soil (Ridenour and Callaway, 2001).

Diffuse knapweed, *C. diffusa*, like spotted knapweed, is a biennial or triennial species which also possesses a deep taproot (Watson and Renney, 1974). This species is also native to Europe, spanning from the Mediterranean into western Asia and is very common in Ukraine (Roché and Roché, 1999). The floral heads of this species are generally white with bracts covered in many small, sharp spines which probably protect the flower from herbivory. Diffuse knapweed is also capable of self-pollination, but vegetative growth is rare (Watson and Renney, 1974). Plants can produce up to 40 000 seeds/m<sup>2</sup>, which are dispersed by wind when the plant breaks off and becomes a tumbleweed (Roché and Roché, 1999). *Centaurea diffusa* was first recorded in North America in 1907 in an alfalfa field in Bingen, Washington (Watson and Renney, 1974). It currently occupies over 1.2 million hectares of undisturbed grasslands in the Pacific Northwest of the United States and Western Canada (Sheley et al., 1997). Much like spotted knapweed, diffuse knapweed monocultures reduce native plant biodiversity, promote soil erosion, and lower the foraging quality of pastures (Roché and Roché, 1999).

Yellow starthistle, *C. solstitialis*, is a deep taprooted winter annual native to south-eastern Europe (Weber, 2003). Floral heads are bright yellow with bracts consisting of many stout spines which are roughly 1-2 inches long. It is largely self-incompatible and relies on insect pollination for reproductive success (Maddox et al., 1996). Yellow starthistle produces two types of achenes: those with a pappus, which disperse away from the plant and generally have higher rates of germination, and non-pappus seeds which generally fall beneath the parent plant (Young et al. 2005). A single plant is capable of producing upwards of 150 000 seeds (Sheley et al., 1999b).

It is believed that yellow starthistle arrived in North America as a contaminant in agricultural seed in the early 1800's, reaching California (its main invasive range in North America) in the middle to late 1800's (Sheley et al., 1999b). It is a prominent invader of natural grassland communities in the western United States, infesting over eight million acres alone in California (Sheley et al. 1999b). The large genetic diversity found in *C. solstitialis* indicates that the colonizing population was not subjected to a founder effect and most likely arose from a large introduction of genetically different seeds (Sun, 1997). Yellow starthistle is also very toxic to livestock (Roy et al., 1995), thus contaminating many grazing lands.

Brown knapweed, *Centaurea jacea*, is a non-invasive, naturalized species within North America. It is a long-lived perennial with purple flowers, which is capable of clonal growth through the production of side rosettes from its root system (Jongejans and De Kroon, 2005). Brown knapweed is found naturally in many parts of Europe including Germany and Belgium (Vanderhoeven, 2002). *Centaurea cyanus*, or bachelor's button, is a common exotic in North America. It is a short-lived annual which produces one to multiple blue flowers atop its sole stem (pers. obs.). *Centaurea scabiosa*, greater knapweed, is a woody perennial with a purple flower that has a wide distribution across Europe (Kumarasamy et al., 2003). Both *C. scabiosa* and *C. cyanus* were traditionally used to treat coughs (Kumarasamy et al., 2003).

Singleflower knapweed, *Centaurea uniflora*, is fairly uncommon in North America and is mainly present as a garden plant. It is a perennial species which has hairy leaves (as compared to the other knapweeds studied) and generally produces few,

large purple flowers (pers. obs.). Unlike the invasive knapweeds, the non-invasive knapweeds tend to have floral heads which lack large spines (pers. obs.).

## CHAPTER TWO: ALLELOPATHY AND THE NOVEL WEAPONS HYPOTHESIS

### INTRODUCTION

#### *Allelopathy and the Novel Weapons Hypothesis*

The success of exotic plants in introduced ranges has been attributed to numerous characteristics including vegetative reproduction (Daehler, 1998), production of large number of seeds (Watson and Renney, 1974), increased competitive ability (Blossey and Nötzold, 1995), and the release from natural enemies (Keane and Crawley, 2002). Another mechanism that is recently receiving much attention in invasive plant biology is allelopathy and the novel weapons hypothesis (Callaway and Ridenour, 2004; Vivanco et al., 2004; Inderjit et al., 2006). This hypothesis states that some plant species become invasive because they possess chemicals that are novel to their introduced ranges and to which local plant species have yet to adapt. Allelopathy, or the release of phytotoxic chemicals from a plant into the environment, has been proposed as a trait common amongst some invasive plants. A literature review done by Sanchez-Moreiras et al. (2003), found that several members of the Poaceae (including the invasives *Agropyron repens* and *Poa pratensis*) exhibited allelopathic effects on target species (such as radish and lettuce) ranging from inhibition of root and shoot growth to inhibition of photosynthesis. In addition, the effects of allelopathy can be more prominent in introduced ranges since native species have yet to adapt to the invader's chemical properties (Hierro and Callaway, 2003; Callaway and Ridenour, 2004; Vivanco et al., 2004).

Allelopathy was first coined by H. Molisch and is derived from the roots “allelon” meaning mutual and “pathos” meaning harm. It is generally used to describe

the negative effect that one organism (plant) has on another through the release of toxic compounds into the environment (Putnam and Duke, 1978). Numerous plant species have been reported as being allelopathic including black walnut, *Juglans nigra* (Jose & Gillespie, 1998), and rice, *Oryza sativa* (Kato-Noguchi, 2004).

Considered one of the worst allelopathic plants, *Juglans nigra*, which is native to North America, has been found to affect the growth of many plant species through the release of juglone from its roots, especially in crop fields where it is often planted for agroforestry (Jose & Gillespie, 1998). Momilactone B, the active allelochemical isolated from *Oryza sativa*, has been found to inhibit the growth of neighbouring plant species. This finding has led to the selection of allelopathic rice cultivars that are able to combat other weedy species (Kato-Noguchi, 2004).

Allelopathy has been reported as a mechanism through which many exotic plant species become invasive in their new environments. Two invasive species that have demonstrated allelopathic activity are tree-of-heaven, *Ailanthus altissima* (Heisley, 1996), and spotted knapweed, *Centaurea maculosa* (Ridenour and Callaway, 2001; Bais et al., 2003), through the release of ailanthone and (-) - catechin respectively. Water extractions of the leaf and root material of *Lonicera maackii* were found to inhibit germination of tested species (Dorning and Cipollini, 2006). Plants grown in soil contaminated with root exudates and litter from the aggressive invader *Lantana camara* exhibited decreased seedling germination and biomass compared to those in plots that had been treated with carbon, a substance that absorbs organic phytochemicals (Gentle and Duggin, 1997). Soils from the rhizosphere of garlic mustard, *Alliaria petiolata*, reduced the germination of native and introduced *Geum*

spp. by 14% as compared to carbon-treated soils (Prati and Bossdorf, 2004). The native *Geum* appeared to benefit more from the addition of carbon to soils than the introduced *Geum* from *A. petiolata*'s native range (Prati and Bossdorf, 2004).

Allelochemicals can be released into the environment through several mechanisms. The shrub *Salvia leucophylla* releases vaporized terpenes (cineole and camphor) from its leaf tissues, which are easily absorbed by leaf waxes (cuticle and suberin) and dry soil particles, and which have been shown to reduce biomass in other plant species (Muller, 1966). Leaf litter from *Helianthus annuus* (Hall et al., 1982), *Chenopodium murale* (Inderjit, 2006), and various woody tree species (Lodhi, 1976) inhibit the germination and growth of local herbaceous species. Plant functional group also plays a role in the phytotoxicity of decaying plant material, as litter from nitrogen fixers tends to be more allelopathic than that of forbs and woody species, which in turn tends to be more allelopathic than grasses (Bonanomi et al., 2006). However, this may be due to the microbial breakdown of the litter, which often causes an increase in acidity in the environment (Inderjit, 2006). Misting or soaking leaves, mimicking the leaching of substances through rain, has been used to examine potential allelopathic compounds in Florida rosemary, *Ceratiola ericoides* (Hunter and Menges, 2002), and *Centaurea maculosa* (cnicin) leaves (Kelsey and Locken, 1987) as well as to identify the allelopathic compounds produced by *Dittrichia viscosa* trichomes (Levizou et al. 2004). Elemental allelopathy, or the accumulation and concentration of high levels of inorganic compounds around the rhizosphere of a plant, has also been proposed as a potential means by which some plants can inhibit the growth of their neighbours (Boyd and Martens, 1998).

The most commonly studied allelopathic system is the exudation of chemicals from roots. This is often surveyed through direct root chemical extraction, root exudate collection and/or soil experimentation. The release of allelopathic compounds from roots into the rhizosphere can occur passively through membrane diffusion or actively through ion channels and vesicle transport (Bertin et al., 2003). The “find-and-grind” approach, which involves the physical destruction of root material and chemical extractions to release any potential root chemicals, has been used to identify phytotoxins within plant roots. However, these methods are generally considered weak techniques in allelopathic studies since the chemicals isolated are often not naturally released into the environment or persist at levels which are not toxic. More sound approaches in the study of root allelopathy involve the direct collection of exudates from plants grown hydroponically (Dorning and Cipollini, 2006), or testing the soils in which the plant has grown either through direct transplant of test species (Ridenour and Callaway, 2001; Prati and Bossdorf, 2004) or the collection and application of soil leachates (Newman and Rovira, 1975; Callaway et al., 1991). These methods have been frequently used to assess the allelopathic capabilities of invasive knapweeds.

#### ***Allelopathy in Knapweeds***

Allelopathic compounds have been identified in several of the highly invasive knapweed species. The roots of Russian knapweed, *Acroptilon repens* (formerly *Centaurea repens*) contain 7,8-benzoflavone, a phytotoxin that inhibits root growth and causes cell death in several test species including itself (autotoxic) and other related species (*C. maculosa* and *C. diffusa*) (Stermitz et al., 2003). However, the

chemical was isolated via direct extraction from roots instead of through natural root exudation, therefore, its classification as an allelochemical remains to be determined. Russian knapweed is also suspected of being an elemental allelopathic plant by concentrating Zn levels in the top layers of soil as a result of hyperaccumulation in plant tissues, and the subsequent release through litter decomposition (Morris et al., 2006).

*Centaurea diffusa* was found to have much more negative effects on the growth of North American species than on species from its native European range when grown in the same environment (Callaway and Aschehoug, 2000). This effect was eliminated when activated carbon was added to the pots, indicating the existence of a potential phytotoxin/allelochemical in the soil. The compound supposedly responsible was later identified as 8-hydroxyquinoline, a chemical not previously identified as being produced in natural systems (Vivanco et al., 2004). More recently, simulated herbivory on *C. solstitialis* was found to have an adverse effect on native species grown in the same soil (Callaway et al., 2006). When activated carbon was added to the systems, the indicator species improved in performance.

*Centaurea maculosa* is the most widely studied knapweed in terms of allelopathic potential. Fletcher and Renney (1963) identified the presence of phytotoxic compounds from dried *C. repens* (now *Acroptilon repens*), *C. diffusa* and *C. maculosa* leaf material. Using barley and lettuce, they found significant decreases in germination as well as in root and shoot growth when the indicator species were treated with knapweed solutions. The compound responsible was later found to be cnicin, a sesquiterpene lactone that has high concentrations in leaf tissues as

compared to roots (Kelsey and Locken, 1987) and that is most likely released into the environment through plant decomposition. Callaway et al. (1999) reported that feeding of a bio-control moth on the roots of *C. maculosa* did not have a negative effect on knapweed biomass. However, in pots where *C. maculosa* was planted alongside a native grass (*Festuca idahoensis*) feeding damage by the moth actually caused an unexpected, indirect decrease in grass biomass, potentially through the release of phytotoxic root compounds (Callaway et al., 1999). This phenomenon was again reported in 2001, when *Festuca* root growth was suppressed in soils contaminated with *C. maculosa* roots, but was significantly higher when the soils were treated with activated carbon in order to remove organic compounds (Ridenour and Callaway, 2001). The allelopathic compound was later identified as (-)-catechin, an enantiomer of the much more common (+)-catechin molecule (Bais et al., 2002).

### ***Testing for Allelopathy***

The classification of a plant as being allelopathic requires careful consideration. A compound may be phytotoxic; however, unless it is released naturally by the plant, be it aerially, through leaf leachates, or root exudates, it should not be considered allelopathic (Inderjit and Duke, 2003). To fully understand the effects of allelopathy in the field, it is important to utilize tests that involve intact plants as well as mimic situations that can occur naturally in the field (Inderjit and Weston, 2000). It is important to show that the allelochemical is present in the environment in concentrations high enough to inhibit the growth of a target species (Inderjit and Weiner, 2001). Activated carbon is often used in allelopathy studies due to its ability to absorb allelochemicals and other phytotoxins from soils and leachates

without interfering with nutrient concentrations (Inderjit and Callaway, 2003). This technique has frequently been used to assess allelopathy in *Centaurea maculosa* (Callaway et al., 1999; Callaway and Aschehoug, 2000). By amending soils with activated carbon it is possible to detect whether the interference between invasive and native plants is due to a compound present in the soil or from standard resource competition.

In this study, three highly invasive and three non-invasive exotic knapweeds were analyzed for their potential allelopathic effects on other plant species. Evidence indicates that invasive *Centaurea* species should possess allelopathic compounds that are more detrimental to plant communities in their introduced ranges than in their native range. The non-invasive *Centaurea* species may not possess these so-called “novel weapons”, and hence have no added advantage against North American plant communities. They either possess chemistry that is ordinary or common within introduced habitats, or that lacks the strong biocidal properties found in their invasive kin. It was hypothesized that leachates collected naturally from the soils of the invasive species would exert more negative effects on the fitness of indicator species than their non-invasive kin. This effect should be nullified when the leachate is treated with activated carbon, an absorbent that has been shown to remove allelochemicals from the environment. The presence of reduced germination and/or growth in non-carbon-treated leachate as compared to carbon-treated leachate should thus be an indicator of allelopathic potential.

## MATERIALS AND METHODS

### *Centaurea species*

Seeds of the non-invasive *C. cyanus* and *C. uniflora* var. *nervosa* were obtained through VanDusen Botanical Garden, Vancouver, BC. Seeds of *C. scabiosa* and *C. jacea* were obtained locally from Gatineau, QC and Shirley's Bay in Ottawa, ON respectively. *Centaurea maculosa* seeds were obtained from R. Callaway from wild populations in Missoula County, Montana, USA. Seeds of *C. solstitialis* were obtained from G. Kyser from populations in Yolo County, California, USA. *C. diffusa* seeds were obtained through A. Kulmatiski from populations in Castle Rock, Colorado, USA. Lettuce, *Lactuca sativa* L. var. iceberg, and Radish, *Raphanus sativus* L. var. early scarlet globe, were obtained from the Ontario Seed Company through Richie's Feed and Seed, Ottawa, ON. Juglone, 5-hydroxy-1,4-naphthoquinone (batch # 08612HA) and lignan-based activated carbon (Darco®, 4-12 mesh, granular; batch # 07207HD) were obtained from Sigma-Aldrich. A lignan-based carbon was used based on its allelochemical absorptive properties (G. Thelen, pers. com.).

### *Activated Carbon – Juglone Verification*

To verify the ability of activated carbon to remove allelopathic chemicals from leachates, the effects of juglone and carbon-filtered juglone on the germination and growth of radish seedlings was first examined. Juglone is a known allelopathic compound that interferes with cell elongation and growth of target species (Böhm et al., 2006). Activated carbon has been documented as removing a variety of organic compounds from soils and leachates (Inderjit and Callaway, 2003), therefore a

positive result of adding carbon to juglone on the growth of an indicator species would validate that the carbon used was truly active.

A 2 mM juglone solution was prepared by dissolving 0.28 g of juglone in 0.8 L of warm, distilled water. Half of this solution (0.4 L) was filtered into a 1 L Mason jar through a funnel containing filter paper and an excess amount (approx. 100 g) of activated carbon in order to remove the dissolved juglone from the solution. This procedure was also repeated for distilled water, which would serve as a comparative control.

Fifty radish seeds, divided into sets of five forming ten Petri dish replicates, were used in each treatment. On Day 0, each dish was watered with 10 mL of the corresponding solution (distilled water, carbon-treated distilled water, 2 mM juglone, or carbon-treated 2 mM juglone), using individual 10 mL sterile pipettes to avoid cross-contamination. Each dish was watered again with 3 mL of solution on Day 3 following the same protocol in order to prevent seedling desiccation.

Germinating seeds were counted daily for seven days in order to determine the speed of germination. Speed of germination was recorded using the formula  $\sum(N_i/1 + N_2/2 + \dots + N_I/I)$ , where  $N_1$  = number of seeds germinating on day 1,  $N_i$  = number of seeds germinating on day "I", etc. The higher the number, the faster the seeds germinated. This is a more sensitive measure than percent germination for allelopathic studies since it gives more "weight" to earlier germinating seeds and hence can account for delays (Allaie et al., 2006). Speed of germination was calculated for each Petri-dish in a given trial, and an average taken from all ten replicates.

At the end of Day 7, all seedlings were scanned using an Epson Perfection flatbed scanner so that shoot and root measurements could be taken using Scion Image (Beta 4.0.2). Shoot and root measurements within a given dish were then averaged, giving a total of ten replicates for each trial. After seedlings were scanned, all plant material was dried and the total dry weight of radish within a dish was measured, and average weight per plant calculated based on the number of seeds that had germinated.

Differences between speed of germination and seedling weight were analyzed using an ANOVA as the data fit the assumptions of parametric tests (Levine's test for homogeneity of variance  $p > 0.05$ ; normal distribution of residuals; random scatter of residuals). Non-parametric Kruskal-Wallis tests were used to compare shoot and root data since a lack of homogeneity of variance between treatments was observed (Levine's test for homogeneity of variance,  $p < 0.05$ ). Tukey-Kramer HSD post-hoc tests were used to identify pair-wise differences in parametric variables, whereas the Dunn's post-hoc test was performed to identify pair-wise differences in non-parametric data.

All statistical tests were performed using JMP-IN version 5.1 (SAS Institute, Cary, NC).

#### ***Allelopathic Potential of Centaurea Soil Lechates***

Seeds of three invasive (*Centaurea diffusa*, *C. maculosa*, and *C. solstitialis*) and three non-invasive (*C. cyanus*, *C. jacea*, and *C. uniflora* var. *nervosa*) knapweed species were planted in 8 inch diameter pots containing Pro-Mix BX in the greenhouses at Carleton University in early April 2005. Once seedlings had emerged,

individual plants were transplanted into 10x10 cm pots and were grown in a greenhouse during the spring/summer of 2005. No fertilizers were added to any knapweeds during this time.

Leachate was collected from twenty individuals (groups of four plants creating five replicates – A to E) of each of the six *Centaurea* spp. at the time of bolting with the exception of *C. jacea*, which was sampled during the rosette stage. A control leachate was obtained by filtering greenhouse water through pots containing only Pro-Mix BX soil to eliminate soil contaminants as potential confounding factors. Two hundred milliliters of water was poured into each pot containing one plant and the resulting leachate (from the four plants within the group) was funnelled into the corresponding jar creating one replicate batch of leachate of approximately 500 mL. Following collection, leachate was divided, with one half (approx. 250 mL) remaining untreated and the other treated with an excess amount of lignan-based activated carbon in order to remove any potential allelochemicals.

Two hundred lettuce seeds were placed in sets of ten into each of 20 Petri dishes between two 90 mm filter papers (this was repeated separately using radish as the indicator). Ten dishes were watered directly with the non-treated leachate and the remaining with carbon-treated leachate. Two dishes in each set-up were watered with leachate from a different replicate batch. Each dish was initially watered (Day 0) with 10 mL of the corresponding leachate using a sterile 10 mL pipette within one hour of the time that leachate was collected. Separate pipettes were used for each batch of leachate to prevent cross-contamination. In order to compensate for water loss, each dish was re-watered with 3 mL of freshly collected leachate on Day 3 and Day 7

within one hour of collection. The top filter papers of each dish were replaced on Day 3 and Day 6 due to deterioration over time. This process was repeated for all *Centaurea* species.

Germination was recorded daily for all seeds over ten days. Seeds were examined under a dissecting microscope for the emergence of the root radicle from the seed coat. Once a seed had been documented as germinated, it was placed aside in the dish in order to avoid potential damage from further manipulation. Speed of germination of lettuce and radish was calculated using the equation mentioned in the juglone experiment. All seeds watered with a given leachate batch (20 seeds) were counted as one replicate.

After ten days, all germinated seeds were scanned and root and shoot length measurements were taken using Scion Image imaging software (Beta 4.0.2). Average dry weight of germinated seedlings per Petri dish was also recorded using a fine balance (Mettler-Toledo AB135-S). Average shoot and root lengths as well as average weight were calculated for each dish within a given treatment, and average values for the two dishes watered with the same batch of leachate were obtained. This resulted in five replicates per treatment for all measurements. Seedlings that had incurred damage due to physical manipulation, or that had germinated and died due to unknown reasons, were omitted from analysis.

Student t-tests or Wilcoxon-ranked sums tests were performed to compare carbon and non-carbon treatments for each *Centaurea* species and the control on lettuce and radish growth parameters. The non-parametric Wilcoxon-ranked sums test was used for comparisons that failed to meet the criteria of homogeneity of variance

(Levene's test:  $p < 0.05$ ). Since each *Centaurea* species was tested at different times throughout the season, direct comparisons among *Centaurea* leachate treatments were not made since they may be confounded by varying lab conditions and a decrease in viability of the indicator species' seeds over time.

All statistical tests were performed using JMP-IN version 5.1 (SAS Institute, Cary, NC).

## RESULTS

### *Activated Carbon – Juglone Verification*

Juglone was found to have strong negative effects on the growth of radish seedlings; however, these effects were ameliorated when the juglone was filtered through a carbon funnel. Only five of 50 seeds germinated in the 2 mM juglone dishes while the remaining seeds had turned black indicating seedling mortality. Those five which had germinated demonstrated stunted growth as well as obvious signs of necrosis. Seedlings growing in all remaining treatments appeared healthy.

Treatment was a significant predictor of germination (ANOVA:  $F_{3,39} = 84.7$ ,  $p < 0.0001$ ; Figure 1). Speed of germination was significantly lower for juglone-treated seedlings than for seedlings treated with carbon-filtered juglone which performed equally as well as seedlings treated with carbon-treated water. Seedlings watered with untreated distilled water had the highest speed of germination.

Root and shoot lengths also varied among treatments (Kruskal-Wallis:  $\chi^2 = 23.9$ ,  $df = 3$ ,  $p < 0.0001$  and  $\chi^2 = 28.1$ ,  $df = 3$ ,  $p < 0.0001$  respectively) (Figure 2 and Figure 3). Both were found to be lowest in the pure 2 mM juglone dishes when compared to all other treatments. Similarly to the result for germination, both carbon-

treated juglone and carbon-treated water had statistically indistinguishable shoot and root growth. Seedlings watered with pure distilled water had slightly higher growth rates than that of both carbon treatments.

Radish weight was negatively affected by juglone (ANOVA:  $F_{3,39} = 162.7$ ,  $p < 0.0001$ ). No differences in radish weight between carbon-treated juglone, carbon-treated water, or pure distilled water were observed (Figure 4).

### ***Centaurea Allelopathy***

In all treatments, both lettuce and radish seedlings appeared to undergo normal growth. All seedlings that germinated appeared healthy and there was no obvious visual detection of an effect of any leachate (water or *Centaurea* spp.) on the health of either indicator species.

Speed of germination did not vary between the non-carbon and carbon-treated leachate dishes for either the water control or any of the *Centaurea* spp. leachate treatments for lettuce (Figure 5; Table 1) or radish (Figure 6; Table 2).

For all carbon, non-carbon comparisons for a given leachate treatment, no differences in root (lettuce: Figure 7; Table 3; radish: Figure 8, Table 4) or shoot (lettuce: Figure 9; Table 5; radish: Figure 10, Table 6) growth were observed for lettuce or radish with the exception of water-treated lettuce. In this case there was a slight increase in stem growth when treated with carbon ( $t = -2.36$ ,  $df = 8$ ,  $p = 0.046$ ); however there was no difference in root growth between the carbon and non-carbon treatments.

Similarly to germination and shoot/root elongation, weight was also not affected by the addition of carbon to the leachate. For any given treatment, there was

no statistical difference in weight between untreated and carbon-treated lettuce (Figure 11; Table 7) or radish (Figure 12; Table 8).

Differences amongst germination and growth rates of lettuce and radish between different plant leachate treatments were observed in this experiment. Growth was generally lower in *C. jacea* and *C. diffusa* treatments as compared to all other treatments, and lettuce germination and growth was found to be at its highest in the water control. Though this may indicate potential species differences, it is most likely due to the timing of the experiments as *C. jacea* and *C. diffusa* were tested later in the fall and the lettuce seeds may have lost some viability during this time. Similarly, a new batch of lettuce seeds (from the same supplier) were used for the water control, and these appeared to be much more vigorous than the previously used lettuce.

## DISCUSSION

Allelopathy has been recently reported in several members of the *Centaurea* genus including *C. diffusa*, *C. maculosa*, and *C. solstitialis*, all of which are invasive in North American natural habitats. Based on the results obtained in this experiment, no noticeable inhibitory allelopathic effect of soil leachates collected naturally from the invasive species on radish or lettuce growth was observed as compared to carbon treated controls. Moreover, there was no apparent difference between the highly invasive and the non-invasive *Centaurea* in terms of allelopathic potential. This is contradictory to my prediction that allelopathy is a common trend promoting the invasive success of certain knapweeds as there was no apparent evidence that they differed from their non-invasive kin. Non-carbon treated and carbon-treated control

leachates collected by running water through soil in which no plants were grown did not differ in effect on lettuce or radish seedling growth. This indicates that the application of carbon did not have a positive or negative effect on plant growth.

The allelopathic potential of *Centaurea maculosa* as reported by the Callaway group has been met with criticism. Blair et al. (2005) were unable to isolate concentrations of (-)-catechin equal to that reported by the Callaway group using the same methodology. In addition, they were unable to find toxic levels of (-)-catechin under natural stands of *C. maculosa* in the field, or to elicit a negative effect on Idaho fescue unless they used 10X the concentration that was reported as being toxic. Inderjit et al. (2006) reported that the lack of effect observed by Blair et al. (2005) was mainly due to the time of soil collection (season) and the delay between sampling and testing, as (-)-catechin production peaks in early spring and is non-apparent in other seasons. However, in several of the studies completed on *Centaurea*, soil was collected and stored for periods of up to several weeks without chemical fixation and an effect was still observed (Perry et al., 2005). To eliminate time as a potential variable in this study, all leachates were collected and applied to seeds within one hour on three separate occasions. That an absence of effect was found in this situation implies that even immediate application after collection of *Centaurea* leachate may not produce a response in indicator species. The effect of (-)-catechin on *C. maculosa* itself has also had contradictory results within the Callaway group. Perry et al. (2005) report that *C. maculosa* seedling recruitment is balanced via (-)-catechin, which serves as an auto-inhibitor in the field; however, it had been reported earlier that *C. maculosa* was highly resistant to its own (-)-catechin in the soil unless it was directly

injected back into the plant cells since it is not readily re-absorbed by the roots (Bais et al., 2002; Veluri et al., 2004).

In this experiment, I took the approach of looking for an effect of leachates in lieu of testing specific plant compounds for allelopathic effects. Since I was concerned with comparing the allelopathic capabilities of highly invasive knapweeds to their non-invasive relatives, identifying the suspected compounds was not a primary concern. Leachates in this experiment were collected by running water through the soil and root systems of the *Centaurea* species. This is in contrast to some of the work done on *Centaurea diffusa* and *Centaurea maculosa*, which involved the controversial method of extraction of root exudates from intact plants or soils using hexane (Bais et al., 2002), ethyl acetate (Vivanco et al., 2004), and methanol (Perry et al., 2005; Thelen et al., 2005) to isolate the suspected allelopathic compounds. This methodology, though useful in finding potential compounds with phytotoxic effects, does not necessarily mimic natural plant-plant interactions. Organic solvents can extract multiple compounds from plant tissues. Some of these can be phytotoxic; however, they may never be released naturally by the plant itself (Putnam and Duke, 1978). Given high enough concentrations, almost any compound can act as a phytotoxin. It should, however, not be considered allelopathic unless it is naturally released by the suspected allelopathic plant. To maximize the effect in the field, allelochemicals should be able to disperse away from the originating plant in order to elicit a negative effect on neighbours. Logically, water run-off should play a role in this translocation, which is why leachates were collected solely through the application of water to knapweed pots.

The method used in this experiment followed a simple, natural approach of root exudation and subsequent movement of compounds through the soil by means of water. Since no effect of any leachate was observed, it is likely that any allelochemicals that may be produced by *Centaurea* spp. are not capable of dispersing away from the parent plant, therefore their activity may be limited to the soil and rhizosphere of the exuder. This would limit the effect of the compound in the environment. Stowe (1979) examined the allelopathic inhibitory effects of seven co-occurring introduced plants against each other and found general inhibitory effects (as well as autotoxicity) in several allelopathy laboratory bioassays (ground tissue, leaf leachate, decomposed tissue and root leachate) of all species. However, there was no evidence in the field of plants which inhibited each other forming small scale distribution patterns in response to allelochemicals within the soil (Stowe, 1979). Stowe concluded that almost any plant species can be identified as having allelopathic properties in laboratory bioassays, but this does not mean positive results should be expected in the field.

The collection and application of leachates (fluids collected by running water through soils, leaf litter, etc.) has been a common method for testing the allelopathic capabilities of different plant parts (Callaway et al., 1991; Ahmed and Wardle, 1994; Kong et al. 2004). This is often considered a more natural approach than chemical extraction and crude extracts (i.e. "find-and-grind") as it can mimic the natural flow of compounds through the soil (Inderjit and Callaway, 2003). For example, leachates collected from pots containing live roots of blue oak, *Quercus douglasii*, were found to reduce the biomass of a local grass (*Bromus diandrus*) more than leachates collected

from pots containing only soil (Callaway et al., 1991). Ahmed and Wardle (1994) used this technique to collect leaf and root exudates from vegetative (rosette) and flowering ragwort (*Senecio jacobea*) to test for allelopathic effects on legumes (e.g. *Trifolium* spp.). Their results indicated that in both stages (vegetative and flowering) root leachates have inhibitory effects on seedling emergence and growth, whereas only leaf leachates from flowering ragwort have inhibitory effects on the test species.

The allelopathic potential of black walnut (*Juglans nigra*) has been known for some time. Juglone has been shown to reduce root growth in soybean (*Glycine max*) through premature cell wall lignification of radicles (Böhm et al., 2006) as well as reduce net photosynthetic rates and impair root and leaf respiration (Jose and Gillespie, 1998). Since this is a known allelopathic compound, it can be used to assess the absorptive capabilities of activated carbon.

In this study, juglone was used to assess the reliability of activated carbon to remove organic, allelopathic compounds from leachates. As was expected, the application of juglone to radish seeds resulted in reduced germination and growth over a seven day span. This effect was negated when the juglone sample was filtered through carbon with the radish seedlings performing equally as well as in carbon-filtered water and non-filtered water. For this reason, it should be safe to assume that the treatment of leachates from *Centaurea* plants with activated carbon will remove similar organic, allelopathic compounds.

Though no potential allelopathic effects of any *Centaurea* spp. were observed on radish or lettuce in vitro, this does not necessarily mean that these species lack allelopathic capabilities. Environmental stressors have been linked to the

production of allelopathic chemicals by plants. Mechanical defoliation of *C. solstitialis* was found to intensify its negative effects on local grasses than when it was left unclipped (Callaway et al., 2006); however, whether this was due to added resource depletion or allelopathy still remains unclear. Similarly, root herbivory on *C. maculosa* by the biological control agents *Agapeta zoegana* and *Trichoplusia ni* has been reported to cause a decrease in biomass and flowering of the native grass *Festuca idahoensis* when the two species are grown together, indicating an increased production or release of allelochemicals in response to root herbivory (Callaway et al., 1999). Whether the chemical released was the result of an induced herbivore defense or was already present within the root is still unclear. *Helianthus annuus* was found to produce more phenolics within its leaves while under nutrient stress in the environment, which resulted in greater inhibition of *Amaranthus retroflexus* germination when *H. annuus* leaf litter was added to the soil (Hall et al., 1982). Environmental stressors such as drought, temperature, and amount of sunlight can also influence the production rate of root exudates by plant species (Pramanik et al., 2000; Bertin et al., 2003). Soil composition and pH (Inderjit and Bhowmik, 2004), as well as soil biotic communities (Blum, 1998; Inderjit and Weiner, 2001), can alter the concentrations of allelochemicals available to be taken up by plants in the environment therefore the effects observed in one habitat may be less pronounced than in another.

In this study, all *Centaurea* species were grown and maintained in a greenhouse under natural light conditions, without competition, and were well watered daily. Since the highly invasive *Centaurea* spp. often dominate in natural

rangelands and grasslands with low precipitation (LeJeune and Seastedt, 2001; Weber, 2003), they may not have been physiologically stressed and hence compound production may have been reduced. Powell et al. (1997) found that neither *C. maculosa* nor *C. diffusa* had an effect on the growth of conifer seedlings in a well hydrated forest habitat, but implied that under water stress the results may have varied. In addition, seedlings were grown in an aerobic environment within the Petri-dishes in the absence of soil and were exposed to light during germination checks. It has been shown that the phytotoxicity of root material declines quickly in aerobic situations, but remains somewhat stable in anaerobic environments (Bonanomi et al., 2006) and that some allelochemicals such as (-)-catechin are photo-sensitive (Weir et al., 2003). Lack of effect of *Centaurea* leachates may have been due to this reason.

Soil was not used in this experiment as the main growth medium for the lettuce and radish seedlings. Instead, seeds were placed between moistened filter papers in Petri-dishes so that they could be more easily scored for germination, as well as to limit root damage during handling. A lack of effect of leachate may have been due to the absence of interaction with soil molecules or soil biota that could influence alleochemical activity (Inderjit and Weiner, 2001).

*Centaurea maculosa* has been found to alter mycorrhizae composition in its introduced ranges. Mycorrhizae can significantly affect the competitiveness of the plants they are associated with (Hartnett et al., 1993). *Centaurea*-invaded habitats have been found to have lower diversities of arbuscular mycorrhizae (Mummey and Rillig, 2006) as well as unique communities (Callaway et al., 2004b). This is likely due to the production of (+)-catechin by this species, which serves as an anti-fungal

after it is exuded by the root (Veluri et al., 2004). In the presence of *C. maculosa*, mycorrhizal fungi within field plots tend to develop smaller hyphae and produce lower levels of glomalin, a glycoprotein that aids in soil aggregation and moisture retention, than in neighbouring plots of native species (Lutgen and Rillig, 2004; Mummey and Rillig, 2006). It is therefore possible that some of the negative effects of *Centaurea* are due to the loss of beneficial mycorrhizae of the native plant species, as opposed to direct chemical inhibition through allelopathic compounds. It is possible that the highly invasive species contain more anti-fungal compounds than their non-invasive kin. Had the indicator species in this experiment been grown in soil media, potential allelopathic interactions may have been more noticeable.

Plants that are connected by mycorrhizal networks can often exchange carbon through source-sink dynamics (Francis and Read, 1984). This phenomenon has been reported in *Centaurea maculosa*, which was found to have accumulated upwards of 15% of its above-ground carbon from the native grass *Festuca idahoensis* based on carbon isotope signatures (Carey et al., 2004).

Plant species can vary in their responses to root exudates, with some being more affected than others (Muller, 1966; Newman and Rovira, 1975). Keeley et al. (1985) found that foliar leachate from living *Adenostoma fasciculatum* tissue only inhibited the germination of three of 20 species tested, but promoted germination in eight species. Some species are also able to neutralize the effects of phytotoxins in the soil through the exudation of their own compounds. Two native species, *Lupinus sericeus* and *Gaillardia grandiflora* are able to block the effects of catechin via the release of oxalate from their roots (Weir et al. 2006).

Lettuce and radish are typical species which are used in allelopathy bioassay experiments since they are most likely not resistant to allelochemicals. Since no effect was observed on either indicator species, it is unlikely that the suspected allelopathic compounds can move freely through the soil or are present in high enough concentrations to negatively affect these species. It may have been more appropriate to test *Centaurea leucata* on plant species that naturally occur in the areas where knapweeds invade (Hierro & Callaway, 2003) as this may have given different results. The initial aim of this study was to examine the effect of *Centaurea* spp. on native plants; however, due to the lack of evidence which was observed in the pilot allelopathy study on lettuce and radish, and the amount of time it would have taken to run a long term study on the effects on native species, this was not feasible.

To fully understand the effects of allelopathy in the field, it is important to utilize tests that involve intact plants as well as mimic situations that can occur naturally in the field (Inderjit and Weston, 2000). The collection of leachates in this experiment via direct flow of water through the soils and roots of intact *Centaurea* spp. follows this criterion as it represents a natural movement of solutions that can occur during rainfall. No chemicals were used to force allelopathic extracts, therefore only compounds which were present in the rhizosphere of the knapweeds were collected and applied. Since no difference was observed between the carbon and non-carbon treatments of any *Centaurea* spp. in terms of growth parameters of either lettuce or radish, it is unlikely that any allelochemicals can be naturally leached from the rhizosphere of these plants. That no differences in effect were observed between the reportedly allelopathic, invasive and the non-invasive knapweeds does indicate

that alleopathy may not be the sole driving force in *Centaurea* invasions and that reported allelopathic capabilities of these plants may be directly related to a combination of abiotic and biotic factors within a given habitat.

## CHAPTER THREE: HERBIVORY AND THE ENEMY RELEASE HYPOTHESIS

### INTRODUCTION

#### *Enemy Release Hypothesis*

The invasiveness of some exotic plants can be attributed to many physiological characteristics including vegetative growth, rapid and high volume seed production, competitive nutrient acquisition and phenotypic plasticity within different habitats. Members of the genus *Centaurea* which have become invasive have been documented as having several of these characteristics (Watson and Renney, 1974; Sheley et al., 1999b; LeJeune and Seastedt, 2001; Hill et al., 2006); however, these traits are also present in some native species as well as in some non-invasive *Centaurea* so they are unlikely to be the main forces driving knapweed invasion. One such hypothesis that has been proposed to explain why certain exotic species are able to out-perform their native counterparts is the enemy release hypothesis (ERH).

The enemy release hypothesis states that some exotic plants become invasive in their introduced ranges because they have left their natural herbivore faunas (especially specialist feeders) behind in their native ranges (Keane and Crawley, 2002). In addition, exotic plants with fewer relatives in their introduced ranges may have a further escape from native fauna, as they may be less likely to accumulate herbivores than a species from a common genus or family (i.e., Asteraceae) (Rejmànek, 1996; Keane and Crawley, 2002). This hypothesis is not solely restricted to plant invasions and has been used to understand the success of exotic animals. Torchin et al. (2003) reported that animals in their introduced environments support

only half as many parasites as they did in their native ranges, and that on average only four new parasites infected them in their introduced ranges.

***Testing the ERH: Comparison of Herbivores in the Native and Introduced Ranges and Phylogenetic Studies in the Introduced Habitat***

Testing of the enemy release hypothesis can take two approaches. The first involves comparing damage and herbivore loads on an invasive species within its native and introduced ranges. In this case, it is predicted that herbivory will be lower in the introduced range. Wolfe (2002) took this approach when he compared *Silene vulgaris* in its exotic North American range to non-invasive populations in Europe. As predicted, North American populations were found to have significantly lower levels of attack from aphids, fungal disease, and floral and fruit predators than European populations (Wolfe, 2002). One of the major contributors to *Silene vulgaris* control in Europe was a specialist moth fruit predator, *Hadena rivularis*, which destroyed upwards of 25% of all fruit in the native range, yet no such predator has filled this niche in North America (Wolfe, 2002). Mitchell and Power (2003) found that, on average, plants within their introduced ranges have lower fungal and viral loads than in their native ranges, but more importantly, those that were reported as having the most release from pathogens tended to be the most noxious and invasive. Fenner and Lee (2001) found only one seed predator amongst 7800 floral heads of 13 introduced Asteraceae in New Zealand; coincidentally, it was found in a species with native relatives in the area. In Britain, the native range of all the species studied, 487 floral heads were found to be infected (10 of 13 species). Native seed predators have yet to utilize these species in New Zealand.

In some instances, introduced plants encounter equal levels of natural enemies in their new ranges as in their native range. A study examining invasive European bunchgrass, *Ammophila arenaria*, found that though sterilization of soils to remove pathogens and fungi improved overall plant performance (biomass), there was no difference in biomass between plants grown in untreated soil from its native versus its introduced ranges, and that the soil biota diversity between sites was comparable (Beckstead and Parker, 2003). However, they did not compare soil pathogen loads with other species growing in the area. Comparing herbivory levels between the old and new ranges does allow for a general understanding of enemy release; however, a more appropriate assessment involves comparing exotics to native confamilial or congeneric species in the introduced ranges as these are the species with which the exotics are competing.

The phylogenetic approach for examining enemy release can provide an increased understanding of the role of herbivores within the introduced ranges, as feeding preference for natives and avoidance of exotic relatives can give the exotics a competitive edge within the environment. In addition, phylogenetically based comparisons can control for characteristics which are common amongst related members. Yela and Lawton (1997) found that caterpillar loads on introduced *Quercus cerris* in England were lower but not statistically different from those on the native *Quercus robur*; however, they did not equate herbivore load to levels of herbivory. A phylogenetically controlled experiment was performed by Agrawal and Kotanen (2003) to address the enemy release hypothesis for exotic plants. Using a common garden, they planted 15 congeneric or confamilial pairs and exposed them to natural

levels of herbivory. In general, the exotic plants suffered more leaf herbivory than their native counterparts (11 out of 15 pairs). This result was counter-intuitive to the predictions of enemy release; however, with the exception of *Vincetoxicum rossicum* (which had lower levels of herbivory compared to *Asclepias syriaca*) none of the exotics used were potent invaders of natural areas (Agrawal and Kotanen, 2003). In 2005, the Agrawal group re-ran the same study and found the reverse effect, introduced plants experienced 22% less leaf herbivory than natives; however, the herbivore communities between taxonomic pairs did not differ (Agrawal et al., 2005). In addition, non-natives experienced 50% less reductions in mass than natives in relation to damage by soil biota.

The accumulation of herbivores on introduced species appears to occur rapidly and is independent of time. Strong et al. (1977) found that time since introduction was not an important factor in the accumulation of native herbivores on sugarcane, and that overall area of cultivation was more important. Herbivores can readily accept new plants into their diet if they possess the appropriate detoxification pathways, and this may limit exotic plant establishment and invasiveness. The species that are not consumed by local fauna may increase and eventually fully invade the habitat. Carpenter and Cappuccino (2005) found that levels of leaf herbivory were lower on introduced versus native plants but that time since introduction did not explain the amount of herbivory experienced by introduced plants. More interestingly, exotic plants that were classified as being highly invasive were found to have much lower levels of herbivory than non-invasive exotics. This finding was supported a year later when phylogenetically distinct introduced invasive and non-invasive

exotics were compared in natural habitats (Cappuccino and Carpenter, 2005). Again, invasives suffered lower levels of insect herbivory than their non-invasive relatives within natural habitats.

In this experiment I have taken the approach of comparing levels of herbivory between highly invasive knapweeds (*C. diffusa*, *C. maculosa* and *C. solstitialis*) and their non-invasive kin (*C. cyanus*, *C. jacea*, *C. scabiosa* and *C. uniflora*) in order to address the enemy release hypothesis. It was hypothesized that the highly invasive knapweeds have an advantage over non-invasives within natural habitats because they suffer lower levels of herbivory and hence can put more resources into growth, reproduction, and dispersal. It was predicted that within a common garden, above ground herbivory (leaf damage) will be lower on the invasives than on the non-invasives, and that feeding damage by local insects, in no-choice experiments, will be higher on non-invasive plants.

## **MATERIALS AND METHODS**

### ***Centaurea* Biology, Chemistry, and Herbivores**

Knapweeds are members of the large Asteraceae family, belonging to the thistle tribe (Cardueae: Centaureinae). There are approximately 500 species of *Centaurea* worldwide; however, only three are listed as being native within North America. The seven *Centaurea* species used in this experiment all vary in life-history, from annuals (*C. cyanus* and *C. solstitialis*) to short and long lived perennials (*C. diffusa*, *C. jacea*, *C. maculosa*, *C. scabiosa*, and *C. solstitialis*). The three invasive knapweeds: *C. diffusa*, *C. maculosa*, and *C. solstitialis* all invade rangelands and open fields within North America. They have been documented as being prolific seed

producers (Watson and Renney, 1974) and strong nutrient competitors (LeJeune and Seastedt, 2001; Hill et al., 2006). Allelopathy has also been reported as being a common trait within this group (Callaway and Aschehoug, 2000; Ridenour and Callaway, 2001; Callaway et al., 2006); however, other researchers have been unable to verify this trend (Blair et al., 2005).

Knapweeds have a very diverse set of chemical components. Caffeic acid (a phenolic), choline (an alkaloid), and centaurein (a flavonoid), are common amongst most members of this group (Heywood et al., 1977). Other compounds, including the sesquiterpene lactones jenerin (*C. uniflora* and *C. solstitialis*) (Flamini et al., 2004) and cnicin (*C. diffusa* and *C. maculosa*) (Kelsey and Locken, 1987), are found in only a small subset of the genera. Several of the highly invasive members have unique chemicals including (-)-catechin in *C. maculosa* (Bais et al., 2002), solstitialin in *C. solstitialis* (Heywood et al., 1977) and 8-hydroxyquinoline in *C. diffusa* (Vivanco et al., 2004), which are fairly unique in the plant kingdom. These compounds may serve as added feeding deterrents in these species.

Within their native ranges, knapweeds are readily fed upon by many herbivores. Feeding damage of the weevil, *Ceratapion basicorne*, on the roots and foliage of *Centaurea solstitialis* and *C. cyanus* has been documented in wild populations within Turkey (Smith et al., 2006). The root weevil, *Cyphocleonus achates*, attacks the root crown of both *Centaurea maculosa* and *C. diffusa* causing an overall decrease in knapweed fitness as well as an increase in plant mortality (Corn et al., 2006). The root feeding moth, *Agapeta zoegana*, is found infesting the roots of *Centaurea maculosa* in Austria and Hungary (Smith and Story, 2003). Seed head

feeders are also common insects found on *Centaurea*. The larva of two tephritid flies, *Urophora quadrifasciata* and *U. affinis*, and the flower head weevil, *Bangasternus fausti*, all feed within the capitula of knapweeds, killing flower heads and reducing overall seed production (Smith and Mayer, 2005).

### ***No-Choice Herbivory Trials***

#### *Goldenrod beetles (Trirhabda virgata)*

The goldenrod beetle *Trirhabda virgata* LeConte (Coleoptera: Chrysomelidae) is a specialist feeder on members of the genus *Solidago* (Asteraceae) (Blatt et al., 1999). It is a univoltine species: eggs overwinter in the soil under their host plants and larvae emerge in early May to feed on goldenrod (Herzig and Root, 1996). Larvae go through three instars before they return to the soil to pupate (Wise et al., 2006). After about two weeks, adults emerge and once again start consuming leaf material before they mate and disperse (Wise et al., 2006). Females begin to lay eggs in the late summer in soil or within dead plant material, and these eggs remain dormant until the next spring (Wise et al., 2006). This species was tested due its high abundance within the experimental garden plot and its feeding preference for Asteraceae, to which the genus *Centaurea* is also a member.

*Trirhabda virgata* beetles were collected from a goldenrod population in Brewer Park, Ottawa, ON in June, 2006. Adult beetles were kept in a cage and fed goldenrod prior to experimentation. No-choice feeding tests using six knapweed species (*C. scabiosa* was omitted due to a lack of leaf material) were performed. Beetles were removed from the holding cage and placed in a fasting cage for 24 hrs before testing. Twenty-five beetles were tested for each plant species. Leaf cuttings of

approximately the same size were taken from 25 individual greenhouse-grown plants of each species on the day of the feeding trial. Beetles were placed individually in Petri-dishes containing a damp filter paper and a leaf cutting from one of the six plant species. Trials were run for 48 hrs, following which leaves were scanned and scored for leaf herbivory using imaging software.

*Red-Legged Grasshoppers (Melanoplus femurrubrum)*

The red-legged grasshopper, *Melanoplus femurrubrum* DeGreer (Orthoptera: Acrididae), is a generalist feeder found throughout North America (Swan and Papp, 1972). Adults are generally one inch in length, with olive-green to reddish brown bodies and are characterized by their bright red tibiae (Swan and Papp, 1972). This species prefers mixed grass-herbaceous habitats (Cantrall, 1943), where it often feeds on a variety of plants including grains, alfalfa, and other forbs such as *Solidago* spp., *Rudbeckia hirta*, and *Daucus carota* (Swan and Papp, 1972; Beckerman, 2002). *Melanoplus femurrubrum* is a univoltine species (Beckerman, 2000). Juveniles emerge in early June to July and go through five or more instars (Swan and Papp, 1972). Adults mate throughout the summer, and females lay eggs until September or October, which over-winter until the next spring (Beckerman, 2000).

Grasshoppers were collected from the Carleton University experimental field in Ottawa, Ontario using a sweep net during the months of June and July 2006. All individuals were stored in a 15 X 16 X 18 in. plywood and plexi-glass cage with a 60W incandescent lightbulb to support thermoregulation. The cage was stored in the laboratory at room temperature (approx. 22 °C). Grasshoppers were fed a diet of local

vegetation (grasses, *Inula helenium* and *Arctium* spp.) supplemented with organic lettuce and carrot. Only adults were used in the feeding trials.

Prior to the feeding experiments, grasshoppers were removed from the main storage cage and were placed in a holding cage for a 24 hr fasting period. Twenty-five young rosette leaves of approximately the same size were collected from each of the seven plant species on the day of each feeding experiment. For each plant species, only one leaf was cut from each plant in order to reduce the effects of individual plant chemical differences and to eliminate the potential confounding factor of induced chemical defenses (Exception: *Centaurea scabiosa*, since there were only five individuals, five leaves were taken from each plant).

All leaves were scanned prior to being presented to grasshoppers so that initial leaf area could be measured using imaging software (Scion Image). Each grasshopper was provided with its own feeding arena, which consisted of a 1 L Mason jar and wire mesh to which the leaf material was attached with a paper clip. Grasshoppers were placed in the arenas for 2 hrs at an ambient temperature of 26 °C. At the end of this period, grasshoppers were removed and returned to the feeding cage. Leaves were removed and were re-scanned to determine remaining leaf area. The amount of material eaten by each grasshopper was calculated as the initial leaf area minus the final leaf area. Average damage was calculated for each plant species and means were compared using a standard ANOVA and species differences assessed using a Tukey HSD post-hoc comparison.

### ***Common Garden Field Experiment***

Twenty individuals of seven knapweed species consisting of three invasives (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) and four non-invasives (*C. cyanus*, *C. jacea*, *C. scabiosa*, and *C. uniflora*) were planted in the experimental garden at Carleton University, Ottawa, on 15 June, 2006. This site was chosen due to its proximity and its high abundance of asteraceous species, which harboured a pool of herbivorous insects that could potentially move onto the knapweeds. Prior to planting, all greenhouse species were acclimatized to UV by first placing them outside within mesh cages for seven days in a shaded area within the garden, then for an additional seven days in the sun. The cages prevented most herbivores from eating the knapweeds prior to planting in the garden. One plant of each species was randomly assigned a position within a group of seven plants using a random number generator in Microsoft Office Excel 2003. This was performed twice for a given row, resulting in 14 plants per row (seven species X two random allotments) for a total of 10 rows (140 plants) within the common garden.

Throughout the summer, periodic weeding was performed in order to minimize the likelihood of plant death due to competition and crowding from local plant species (i.e. *Vincetoxicum rossicum*). Plants that died early in the summer (usually as a result of mammal herbivory / digging) were replaced by new greenhouse plants.

All above-ground biomass was collected for each plant in early September. Leaves were removed from all plants, cleaned, and then pressed and dried in the laboratory with the aid of a space heater. The number of leaves and the number of

leafless petioles (where only the petiole remained) found on each plant were recorded. All seed heads were removed from the invasive plant species prior to the disposal of the plant tissues.

Following drying, twenty leaves / leafless petioles were randomly selected from each individual plant and were scanned using a scanner. If a plant had fewer than twenty leaves, then all available leaf material was scanned. If leafless petioles were present, a random number generator was used to determine the number of these petioles that would be used to score herbivore damage (based on the percentage of leafless petioles collected). Percent leaf damage was calculated for all leaves using imaging software. Leafless petioles were all assigned 75% damage levels based on approximations made using Scion Image. Average percent damage per plant was calculated. If a plant had suffered 100% herbivory by small mammals (groundhogs – which was the common occurrence for *C. cyanus*), damage was scored as 100% for that individual. Plants which had died, or for which reason of disappearance was unknown, were omitted from analysis. At the end of the growing season 20 *C. cyanus*, 20 *C. jacea*, 7 *C. scabiosa*, 19 *C. uniflora*, 18 *C. maculosa*, 16 *C. diffusa*, and 19 *C. solstitialis* plants were accounted for in the garden. A Kruskal-Wallis analysis was used to compare percent leaf herbivory between knapweed species since there was a lack of homogeneity of variance between *Centaurea* species (Levine's test for homogeneity of variance  $< 0.0001$ ). A Dunn's post-hoc test was performed to establish where differences lay among the species.

## RESULTS

### *Trirhabda virgata*

As was somewhat expected, the specialist goldenrod beetles did not feed on any leaves of the knapweed species during the 48hr exposure. A small amount of damage was noted on one *C. jacea* leaf, however, the damage was minimal and did not warrant further analysis.

### *Melanoplus femurrubrum*

Grasshoppers fed on all species of plants during the two-hour feeding trials (Figure 13). Average damage, from highest to lowest, was found to be as follows: *C. cyanus*, *C. solstitialis*, *C. scabiosa*, *C. uniflora*, *C. jacea*, *C. diffusa*, and *C. maculosa*. Damage was found to be statistically different among species ( $F_{6,174} = 8.47$ ,  $p < 0.0001$ ). *C. cyanus* (non-invasive) and *C. solstitialis* (invasive), incurred similarly high levels of herbivory by the grasshoppers as compared to the other species tested in the trials (means comparison using Tukey's HSD post-hoc,  $p < 0.05$ ). Two highly invasive species, *C. diffusa* and *C. maculosa*, suffered the lowest amounts of herbivory, but were only statistically different from *C. cyanus* and *C. solstitialis*.

### *Common Garden Herbivory*

Herbivory levels in the field were found to vary among all *Centaurea* species ( $\chi^2 = 66.1796$ ,  $df = 6$ ,  $p < 0.0001$ ). The four non-invasive knapweeds all experienced significantly higher levels of herbivory than their invasive kin (Figure 14). *Centaurea cyanus* suffered the highest amount of damage (80.7%) as a result of complete herbivory by small mammals, as was determined from the presence of severed stems. Mammalian herbivory was also a factor in the high damage observed on *C. jacea*

(42.9%), as many leaves had been bitten off by the time of collection. *Centaurea scabiosa* and *C. uniflora* had equivalent amounts of leaf herbivory, 28.6% and 21.4% respectively. It is possible that damage on *C. scabiosa* may have been higher as fewer of these plants were accounted for in the field and may have been entirely eaten, however unlike *C. cyanus*, this could not be verified. It is more likely that the *C. scabiosa* plants were out-competed by native vegetation in the field as they tended to be the least hardy of all *Centaurea* within the greenhouse.

The three invasive knapweeds, *C. maculosa*, *C. diffusa*, and *C. solstitialis* all experienced the lowest observable amount of leaf herbivory in the field (16.8%, 10.6%, and 2.95% respectively). These plants were the most prominent growers of all the knapweeds with individual *C. solstitialis* plants reaching heights upwards of one meter. Most members of these species were observed to be flowering at the time of collection in September, whereas the only non-invasive flowering was *C. uniflora* (*C. cyanus* did flower earlier in the summer, but most of these succumbed to mammal herbivory). Although *C. solstitialis* had the lowest amount of visible herbivory at the end of the growing season, it was observed that its rosette leaves had suffered substantial levels of damage early in the season. These leaves had senesced by the time of collection.

## DISCUSSION

Escape from herbivory has been proposed as one of the methods by which certain exotic plants become invasive. In this study, the highly invasive knapweed species were less fed upon by herbivores than their non-invasive kin, potentially giving them a competitive advantage in their introduced ranges. The lack of herbivory

on the highly invasive knapweeds as compared to their non-invasive congeners illustrates the importance of herbivores in the control of introduced plant species. Within the common garden, local fauna readily accepted the non-invasive species into their diets, and avoided the aggressive, invasive knapweeds, which were also within “striking range”. This finding supports one aspect of the enemy release hypothesis, that when compared to plants grown within the same habitat, the level of herbivory observed on exotic, aggressive invaders is lower, hence giving them a competitive advantage over other vegetation. A meta-analysis done by Levine et al. (2004) involving herbivory studies, found that native herbivores have a strong negative effect on the establishment and performance of introduced plants, and that herbaceous species were much more negatively affected than woody species.

The importance of insect herbivory on invasive plants, and hence the role of enemy escape, is often illustrated through studies involving biological control agents. In areas where both floral gall flies *Urophora quadrifasciata* and *U. affinis* have been introduced for knapweed control, *U. affinis* will often dominate; however, *U. quadrifasciata* will attack additional plants that have not been galled by the other species, thus further reducing seed production (Harris and Shorthouse, 1996; Coombs et al., 2004). *Clematis vitalba* in New Zealand has been positively controlled by both the leaf mining fly, *Phytomyza vitalbae*, and the fungal pathogen, *Phoma clematidina* (Paynter et al., 2006). Damage by the fly often leads to the infection of the plant with the fungal pathogen, and this combined action by both agents resulted in higher leaf area damage than was produced by either agent alone (Paynter et al., 2006). Ragwort, *Senecio jacobaea*, has been successfully controlled by two introduced herbivores: the

cinnabar moth, *Tyria jacobaeae*, and the flea beetle, *Longitarsus jacobaeae* (James et al., 1992). The moth attacks early in the season, feeding primarily on leaf tissues, whereas the flea beetle appears in the late summer with the larva feeding on the roots and the emerging adults on the leaves (James et al., 1992). The production of leaves and flowers, and seed viability of ragwort were all significantly lowered when both biocontrols were present than when one was excluded from the system (James et al., 1992). Since both insects fed during different stages of plant development, there was no overlap in feeding niches and the ragwort was prevented from compensating for the loss of photosynthetic tissue.

The importance of leaf feeders and defoliators in controlling introduced species has been highlighted in several plant species. Waterhyacinth populations are significantly reduced through the actions of the leaf feeding beetles *Neochetina eichhorniae* and *N. bruchi*, which damage developing petioles and decrease the clonal spread of the species (Center et al., 2005). Control of dense stands of St. Johnswort, *Hypericum perforatum*, has occurred in Australia, South Africa, and North America due to several agents including the chrysomelid leaf beetle, *Chrysolina quadrigemina*, which can completely defoliate plant populations (Gordon and Kluge, 1991; Coombs et al., 2004). In this experiment, the non-invasive knapweeds incurred significantly more leaf herbivory than the invasives within a natural habitat, with one species, *C. cyanus*, experiencing almost complete extirpation from the garden. This rapid acceptance by local fauna may provide the biotic resistance required to prevent certain exotics from becoming abundant in their new ranges; however, why other exotics are avoided is still unclear.

Insect feeding was observed on all plants within the common garden; however, its overall level was masked by mammalian herbivory especially on the non-invasive *Centaurea*. *Centaurea cyanus*, *C. jacea* and *C. uniflora* were the most heavily eaten by mammals, with many individuals losing significant amounts of leaf and stem material. Parker et al. (2006) found that mammalian herbivory on introduced plants had three to five times the effect on plant survival than insect herbivory, indicating the importance of feeding acceptance by mammals as a means of control.

Overall *C. maculosa* above-ground damage in the field was found to be 16.8%. This is much higher than that reported by Cappuccino and Carpenter (2005), who measured an average insect damage of 0.32% on wild *C. maculosa* in natural habitats, which was significantly lower than the 13.1% observed on *C. jacea*. Newingham and Callaway (2006) found that total leaf herbivory of over 40% by *Trichoplusia ni* larvae was needed to cause a significant reduction in *C. maculosa* biomass, any less and the plants were able to compensate. However, even at high levels of defoliation, reproductive output remained constant. A study comparing invasive *Lonicera japonica* to its native congener *L. sempervirens* found that herbivory levels of the native vine were significantly higher than on the invasive, and that the invasive was more able to compensate for tissue loss than the native (Schierenbeck et al., 1994).

The goldenrod beetle, *Trirhabda virgata*, did not feed on any of the *Centaurea* spp. tested in a no-choice herbivory trial. This was somewhat expected as this species is considered a specialist herbivore on *Solidago* spp.; however, it has been reported as being able to feed on asters. Blatt et al. (1999) found that although *T. virgata* preferred *Solidago canadensis*, and more openly chose this species in the field,

larvae and adults were capable of full development on both *S. graminifolia* and *Aster laterifolius* (another member of the Asteraceae) though growth was somewhat reduced. Though *Centaurea* is also a member of the Asteraceae, it is unlikely that *T. virgata* will ever use it as a host due to the lack of specialization to knapweed chemistry. Since goldenrod was common amongst the local vegetation within the garden plot, and individual *T. virgata* were noticed on the knapweeds during the summer, testing for a potential host-shift of *T. virgata* was not unwarranted.

The presence of confamilial or congeneric species within an invaded habitat may influence the amount of herbivory observed on introduced species. Prieur-Richard et al. (2002) found that the exotic species *Conyza bonariensis* and *C. canadensis* (Asteraceae) suffered higher levels of herbivory and decreases in fitness when present in fields consisting of other Asteraceous species than in fields of Poaceae. They attribute this to the species richness of Asteraceae within the site, since there would be more herbivores available to host shift onto the exotics in the more diverse aster patch. Phylogeny was controlled for in this experiment by using invasive and non-invasive species from the same genus, therefore eliminating family related plant differences as confounding factors. That the non-invasive *Centaurea* species were more readily attacked, and hence potentially more preferred, by local herbivores, indicates that they may be less well defended than their highly invasive kin.

The generalist grasshopper, *Melanoplus femurrubrum*, did feed on all knapweeds in a no-choice feeding trial; however there was no pronounced trend between invasive and non-invasive plant material. Two of the highly invasive knapweeds, *C. maculosa* and *C. diffusa*, scored the lowest in terms of herbivory;

however, *C. solstitialis* had the second highest level of damage just under *C. cyanus*. Within the common garden *C. solstitialis* incurred fairly high levels of rosette damage (pers. obs.). However, almost no damage was observed on stem leaves, and initial rosette damage appeared to have little effect on growth, as all *C. solstitialis* plants were flowering at the time of collection. Callaway et al. (2006) found that mechanical defoliation (clipping) of *C. solstitialis* also had no effect on overall growth, as this species fully compensated for biomass after clipping. It is possible that *C. solstitialis* avoids putting energy into herbivory defense (rosette leaves) until it has bolted to ensure that it still has the potential to flower.

The ability of *Melanoplus femurrubrum* to feed on all knapweeds indicates that *Centaurea* spp., both invasive and non-invasive, have not escaped fully from generalist feeders within their new ranges. However, the lower herbivory on *C. maculosa* and *C. diffusa* may be an indicator of better defended leaves, or the presence of secondary compounds that the grasshoppers are less able to detoxify. In a food preference experiment, Siemann and Rogers (2003) found that a native, generalist grasshopper, *Melanoplus angustipennis*, readily accepted the leaves of introduced Chinese tallow tree (*Sapium sebiferum*) within the laboratory setting; however, feeding damage within the introduced range was observed to be very low indicating a reluctance to use it as a food source in the presence of other vegetation. Had preference tests been carried out in this experiment, a larger effect may have been observed. In feeding trials done within its native range, Frank (2003) found that two generalist slugs selectively preferred *C. cyanus* within a common garden over

other local vegetation. This may be an indication that *C. cyanus* lacks potent herbivore defenses against generalist feeders.

Since all species were fed upon both in the field and in the no-choice generalist feeding trial, it can be assumed that knapweed species as a whole have not escaped all generalist feeders within their introduced ranges. Liu et al. (2006) compared herbivore richness on native, non-invasive exotic, and invasive exotic *Eugenia* spp. growing naturally in Southern Florida. Overall, no difference in herbivore richness was found amongst the groups, however, the introduced *Eugenia* did have lower numbers of oligophagous insects than the native species. Since the authors did not examine the amount of herbivory experienced by each group, the true degree of enemy escape could not be assessed. In the case of this experiment, though herbivores could feed on all species, a preference for non-invasive *Centaurea* was apparent. Though it is possible that oligophagous species were present on all knapweeds within the field, preference for non-invasives may exist if these plants possess less potent chemical defenses.

Buschmann et al. (2005) found that herbivory by the slug *Arion lusitanicus* did not differ between native and introduced populations of four Brassicaceae (though differences between species was present) and there was no preference for either in a choice feeding experiment. Slug herbivory was found to reduce viability of seedlings in all species, to reduce fruit production in *Barbarea vulgaris*, as well as alter time of flowering in all species. The fact that the generalist feeder did not prefer introduced over native populations indicates no major differences in chemistry between the subsets (Buschmann et al., 2005). Since plants in the introduced ranges were

generally larger and produced more (*Barbarea vulgaris*) or equal numbers of fruit indicates that there was not a trade-off of defence for growth. Joshi and Vrieling (2005) found that introduced populations of tansy ragwort (*Senecio jacobaeae*) had higher concentrations of pyrrolizidine alkaloids than native populations. These compounds served as deterrents to generalists, but attractants to specialists, indicating that the introduced populations were protecting themselves from local generalist feeders since they have escaped from their natural specialist enemies.

Cnicin from the leaves of *Centaurea maculosa* has been reported as depressing the microbial activity of beneficial bacteria within the digestive tract of sheep (Olson and Kelsey, 1997). This can affect the ability of other grazers to digest plant material and may affect growth rate, thus cnicin may act as an anti-feedant or deterrent for mammals. High cnicin concentrations within artificial diets have been found to be more toxic to generalist feeders (*Spodoptera littoralis*) but not to specialist feeders (*Agapeta zoegana* and *Pterolonche inspersa*) which also preferred to oviposit on cnicin sprayed plants (Landau et al., 1994).

*Centaurea solstitialis* contains several highly potent neurotoxins including solstitialin A, cynaropicrin, and aspartic and glutamic acids (which cause symptoms in horses comparable to human Parkinson's disease) (Roy et al., 1995). It is possible that the small mammals in the field avoided this plant, despite its large size, in preference to the less apparent non-invasives due to this toxic chemistry. Garlic mustard (*Alliaria petiolata*) has been reported as producing levels of cyanide within its leaves that can potentially be toxic to some mammals, this is an uncommon trait amongst the Brassicaceae as many members produce glucosinolates, whereas

cyanogenic glycosides are less common (Cipollini and Gruner, 2007). Alliarinoside, a novel cyanoallyl glycoside found in garlic mustard may be the source of the cyanide (Cipollini and Gruner, 2007).

Escape from above-ground herbivores is not likely the sole release experienced by invasive *Centaurea* in North America. In a study utilizing both soil (and hence soil biota) from its native (France) and introduced (Montana, U.S) ranges, Callaway et al. (2004a) found that *C. maculosa* growth was lower in native soil than in introduced soil, and that soil sterilization to remove pathogens increased *C. maculosa*'s growth rate in native, but reduced growth rate in introduced soils. The fact that *C. maculosa* performed better in North America soil that was not sterilized indicates that it may benefit from symbiosis with local soil flora such as mycorrhizal fungi (Klironomos, 2002; Callaway et al., 2004a).

Herbivores play a pivotal role in the control of plant populations within natural habitats. Herbivory can lower the overall competitive advantage of a plant by depleting tissue and thus reducing the amount of energy available for growth. In this study, invasive knapweeds were less fed upon than non-invasive knapweeds within a common garden and by a generalist feeder. Though the invasive species did not completely escape from herbivory, the preference of herbivores for feeding on the non-invasive knapweeds does indicate that the aggressive invaders may possess chemical defenses which lower the quality of their leaf material for the herbivores.

## CONCLUSIONS

Many hypotheses have been proposed to explain the invasive success of exotic plants in their introduced ranges. Two of the most currently researched are the novel weapons (allelopathy) and enemy release (herbivory) hypotheses. Both of these were examined in this study by comparing introduced invasive and non-invasive members of the genus *Centaurea*.

The release of allelopathic compounds had been proposed as one of the characteristics common amongst the invasive *Centaurea*. However, in this study, no difference in allelopathic potential was observed between the highly invasive and non-invasive exotic knapweeds when their soil leachates were applied to two indicator plant species. The comparison of non-carbon and carbon-treated leachates for each species indicated that no allelochemicals were present since no difference in indicator species growth was observed. These results indicate that allelopathy may not be the driving force in knapweed invasion, as it appears not to differ between the invasives and non-invasives. Though allelopathy has been previously reported in all three of the invasive *Centaurea* studied, it is likely that these findings may be isolated, local incidents influenced by both abiotic and biotic factors.

All *Centaurea* experienced herbivory by a generalist grasshopper, *Melanoplus femurrubrum*, and by local herbivore fauna in a common garden; however, a preference for the non-invasive *Centaurea*, especially within the garden, was observed. This indicates that invasive knapweeds experience an escape from herbivory that is greater than that experienced by the non-invasives. Grasshoppers fed more on non-invasive knapweeds in a no-choice feeding trial, indicating that the

invasive knapweeds may be less palatable. Similarly, the three invasive knapweeds all had significantly lower levels of leaf herbivory than non-invasives in the field. When given a choice, local fauna appeared to selectively feed on the non-invasive knapweeds. The enemy release hypothesis states that some plants become invasive in their introduced ranges because they have left their native herbivore faunas behind. This appears to be one of the factors promoting the invasion of *C. diffusa*, *C. maculosa*, and *C. solstitialis* in North America. It is possible that these species contain feeding deterrents or defensive chemistry which is unique in their introduced habitats, and to which local herbivores have yet to adapt.

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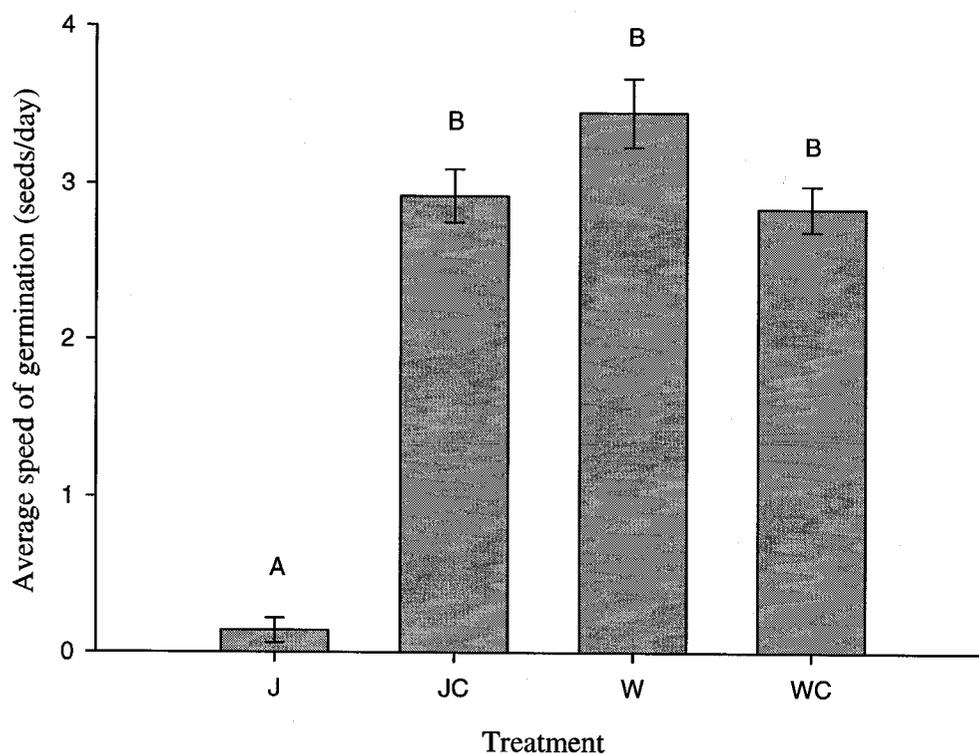


Figure 1: Speed of germination (mean  $\pm$  SE) of radish (*Raphanus sativus*) seeds exposed to 2 mM juglone (J), carbon-treated 2 mM juglone (JC), distilled water (W), or carbon-treated distilled water (WC) over seven days. Treatment was found to be a significant predictor of germination (ANOVA:  $F_{3,39} = 84.7$ ,  $p < 0.0001$ ). Treatments with the same letters have statistically indistinguishable means (Tukey's HSD post hoc comparison).

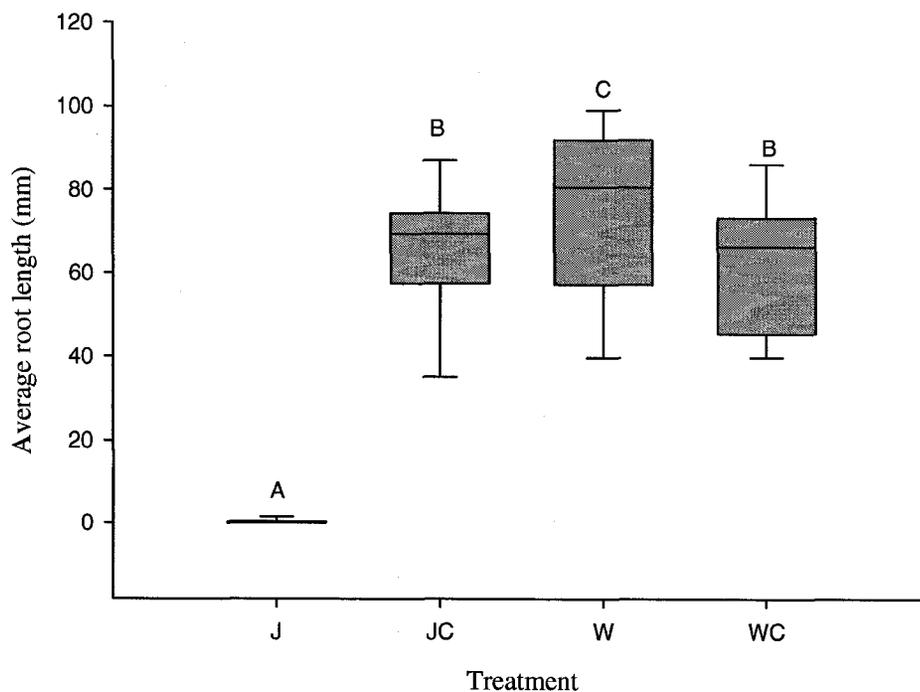


Figure 2: Root length of radish (*Raphanus sativus*) seedlings exposed to 2 mM juglone (J), carbon-treated 2 mM juglone (JC), distilled water (W), or carbon-treated distilled water (WC) after seven days. Treatment was found to be a significant predictor of root growth (Kruskal-Wallis rank sums:  $\chi^2 = 23.9$ ,  $df = 3$ ,  $p < 0.0001$ ). Treatments with the same letters have statistically indistinguishable medians according to Dunn's post-hoc tests (1964). Horizontal lines in box-plots represent median values; 25<sup>th</sup> and 75<sup>th</sup> percentiles are represented by the box; 10<sup>th</sup> and 90<sup>th</sup> percentiles are represented by vertical lines.

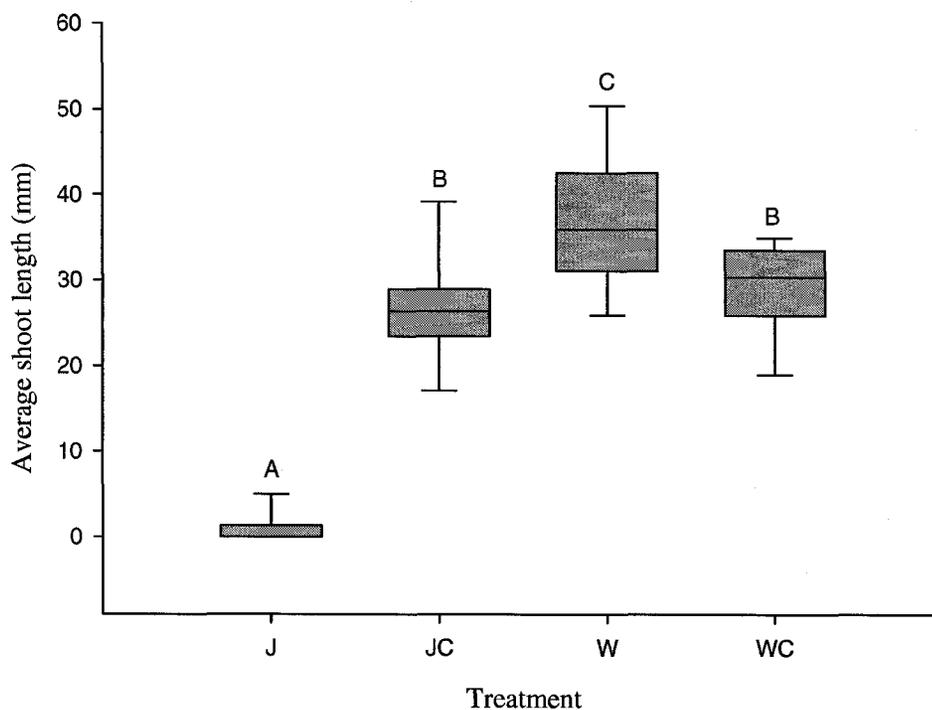


Figure 3: Shoot length of radish (*Raphanus sativus*) seedlings exposed to 2 mM juglone (J), carbon-treated 2 mM juglone (JC), distilled water (W), or carbon-treated distilled water (WC) after seven days. Treatment was found to be a significant predictor of shoot growth (Kruskal-Wallis rank sums:  $\chi^2 = 28.1$ ,  $df = 3$ ,  $p < 0.0001$ ). Treatments with the same letters have statistically indistinguishable medians according to Dunn's post-hoc tests (1964). Horizontal lines in box-plots represent median values; 25<sup>th</sup> and 75<sup>th</sup> percentiles are represented by the box; 10<sup>th</sup> and 90<sup>th</sup> percentiles are represented by vertical lines.

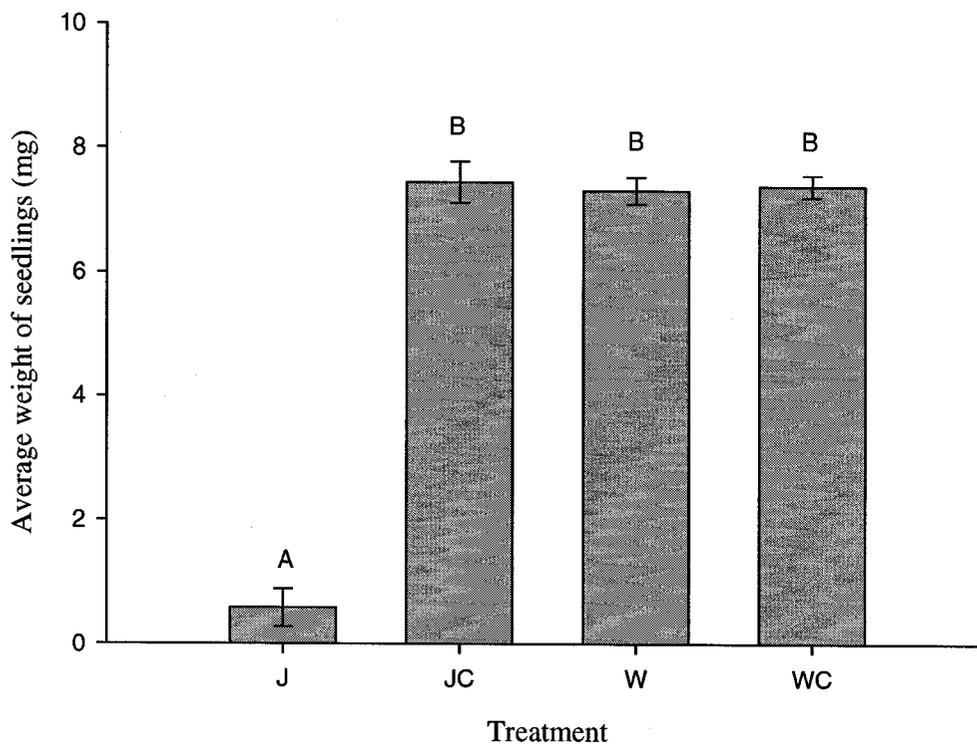


Figure 4: Weight (mean  $\pm$  SE) of radish (*Raphanus sativus*) seedlings exposed to 2 mM juglone (J), carbon-treated 2 mM juglone (JC), distilled water (W), or carbon-treated distilled water (WC) after seven days. Treatment was found to be a significant predictor of radish weight (ANOVA:  $F_{3,39} = 162.7$ ,  $p < 0.0001$ ). Treatments with the same letters have statistically indistinguishable means (Tukey's HSD post hoc comparison).

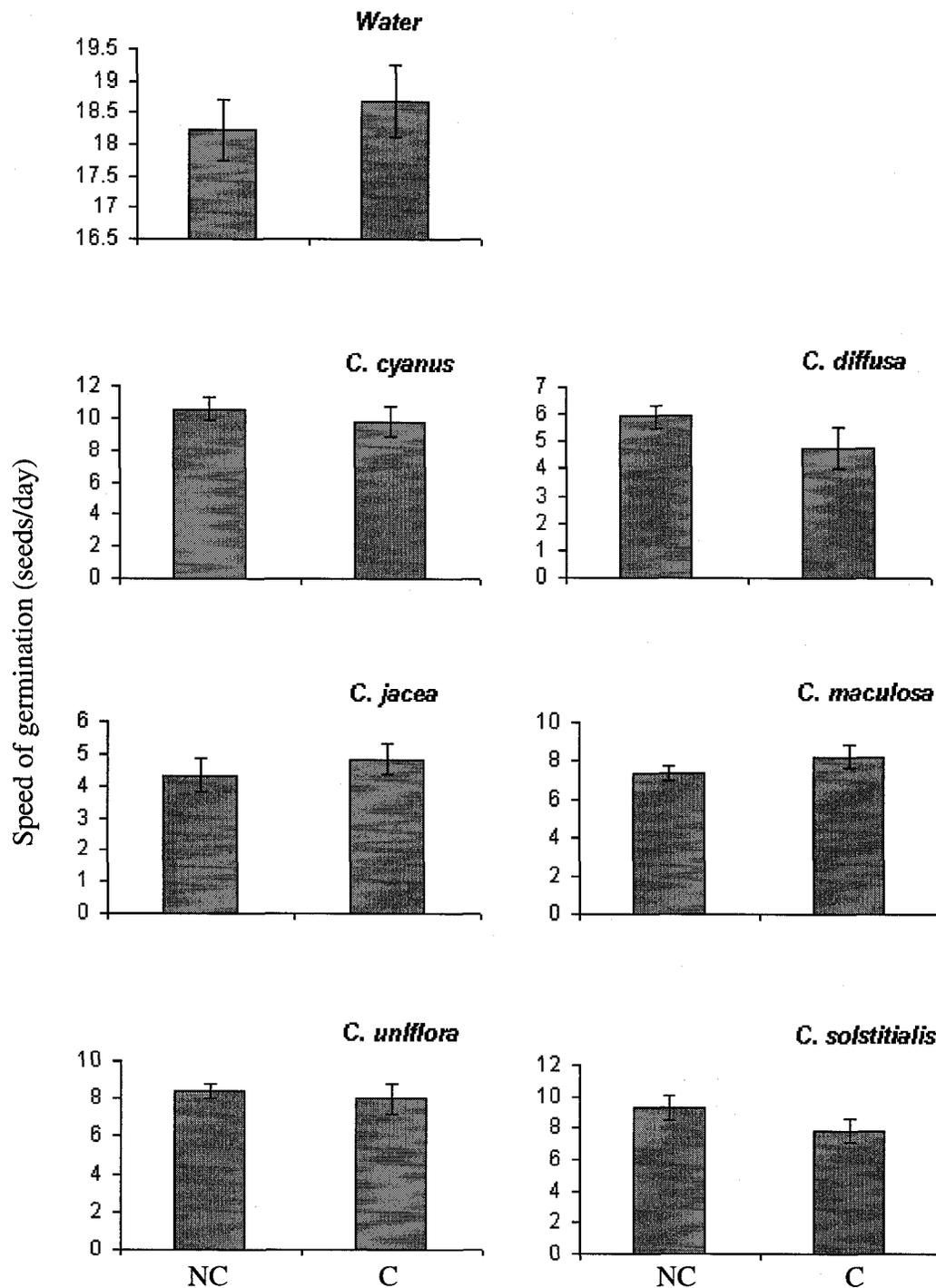


Figure 5: Speed of germination (mean  $\pm$  SE) of lettuce (*Lactuca sativa*) seedlings over 10 days exposed to leachate collected from the soils of three non-invasive (*C. cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds as well as a water control. T-tests found no differences between any groups (Table 1). Note that scales are not equivalent. NC = untreated leachate; C = carbon-treated leachate.

Table 1: Statistical comparisons of non-carbon and carbon-treated leachates collected from the soils of three non-invasive (*Centaurea cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds on the speed of germination of lettuce (*Lactuca sativa*) seeds. All comparisons are t-tests.

<b>Comparison (non-carbon vs. carbon)</b>	<b>Df</b>	<b>t-value</b>	<b>p</b>
Water (control)	8	-0.626	0.607
<i>C. cyanus</i>	8	0.639	0.540
<i>C. jacea</i>	8	-0.724	0.490
<i>C. uniflora</i>	8	0.406	0.695
<i>C. diffusa</i>	8	1.297	0.231
<i>C. maculosa</i>	8	-1.270	0.239
<i>C. solstitialis</i>	8	1.377	0.206

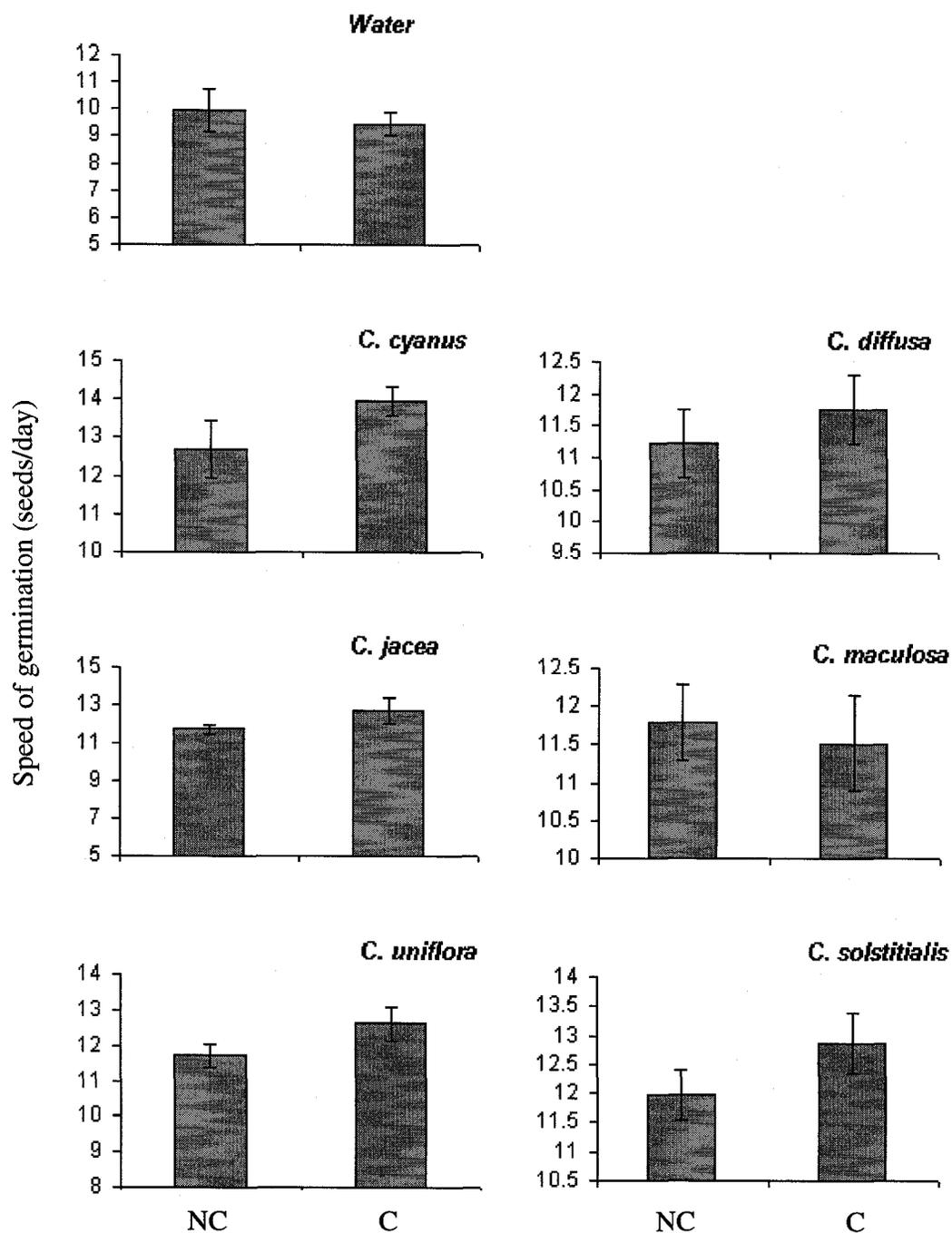


Figure 6: Speed of germination (mean  $\pm$  SE) of radish (*Raphanus sativus*) seedlings over 10 days exposed to leachate collected from the soils of three non-invasive (*C. cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds as well as a water control. T-tests found no differences between any groups (Table 2). Note that scales are not equivalent. NC = untreated leachate; C = carbon-treated leachate.

Table 2: Statistical comparisons of non-carbon and carbon-treated leachates collected from the soils of three non-invasive (*Centaurea cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds on the speed of germination of radish (*Raphanus sativus*) seeds. All comparisons are t-tests.

Comparison (non-carbon vs. carbon)	Df	t-value	p
Water	8	0.535	0.607
<i>C. cyanus</i>	8	-1.502	0.171
<i>C. jacea</i>	8	-1.392	0.201
<i>C. uniflora</i>	8	-1.553	0.159
<i>C. diffusa</i>	8	-0.712	0.497
<i>C. maculosa</i>	8	0.341	0.742
<i>C. solstitialis</i>	8	-1.352	0.213

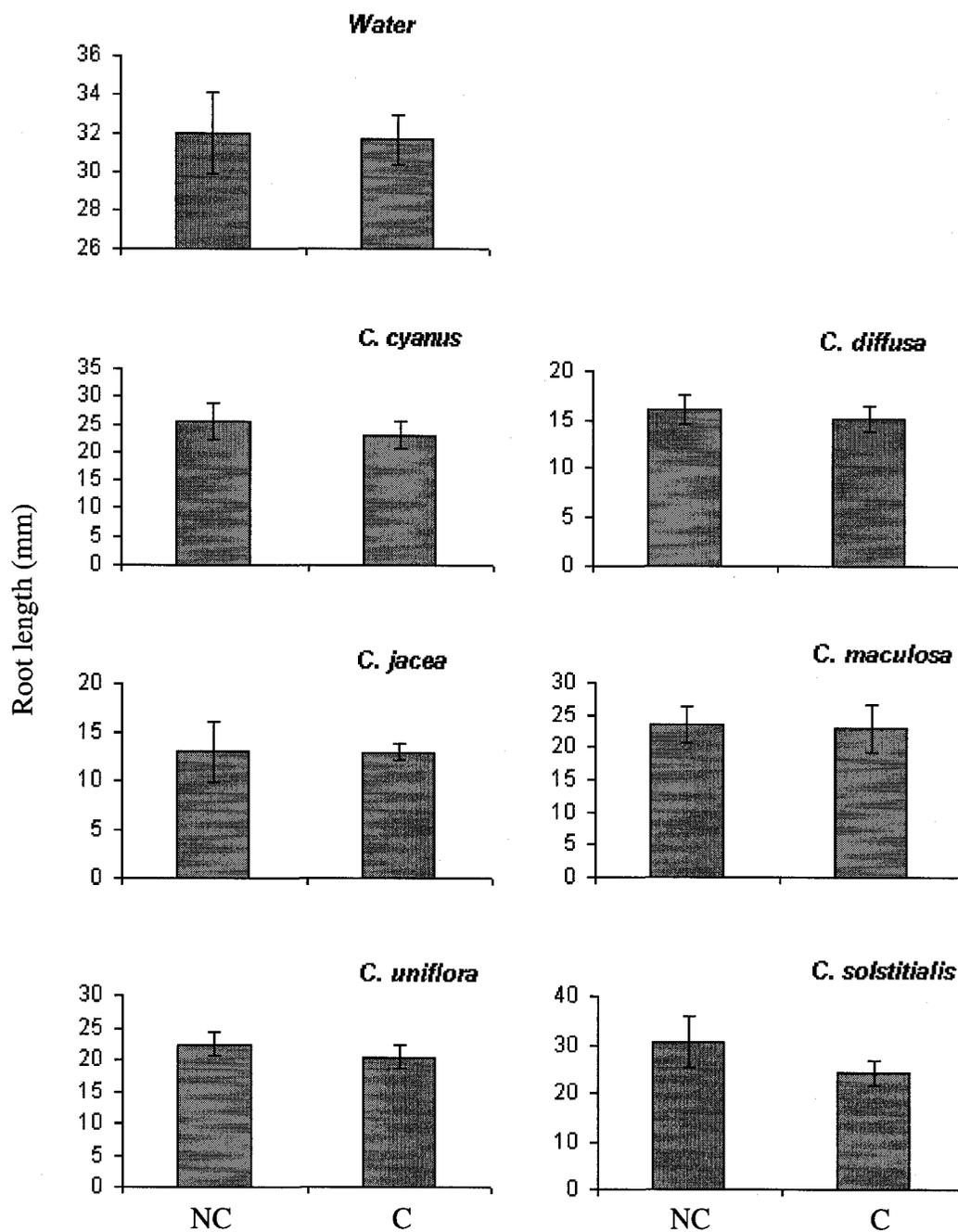


Figure 7: Root length (mean  $\pm$  SE) of lettuce (*Lactuca sativa*) seedlings 10 days after exposure to leachate collected from the soils of three non-invasive (*C. cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds as well as a water control. T-tests found no differences between any groups (Table 3). Note that scales are not equivalent. NC = untreated leachate; C = carbon-treated leachate.

Table 3: Statistical comparisons of non-carbon and carbon-treated leachates collected from the soils of three non-invasive (*Centaurea cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds on root growth of lettuce (*Lactuca sativa*) seedlings. All comparisons are t-tests.

Comparison (non-carbon vs. carbon)	Df	t-value	p
Water (control)	8	0.116	0.910
<i>C. cyanus</i>	8	0.615	0.556
<i>C. jacea</i>	8	0.023	0.982
<i>C. uniflora</i>	8	0.800	0.448
<i>C. diffusa</i>	8	0.474	0.648
<i>C. maculosa</i>	8	0.133	0.898
<i>C. solstitialis</i>	8	1.106	0.301

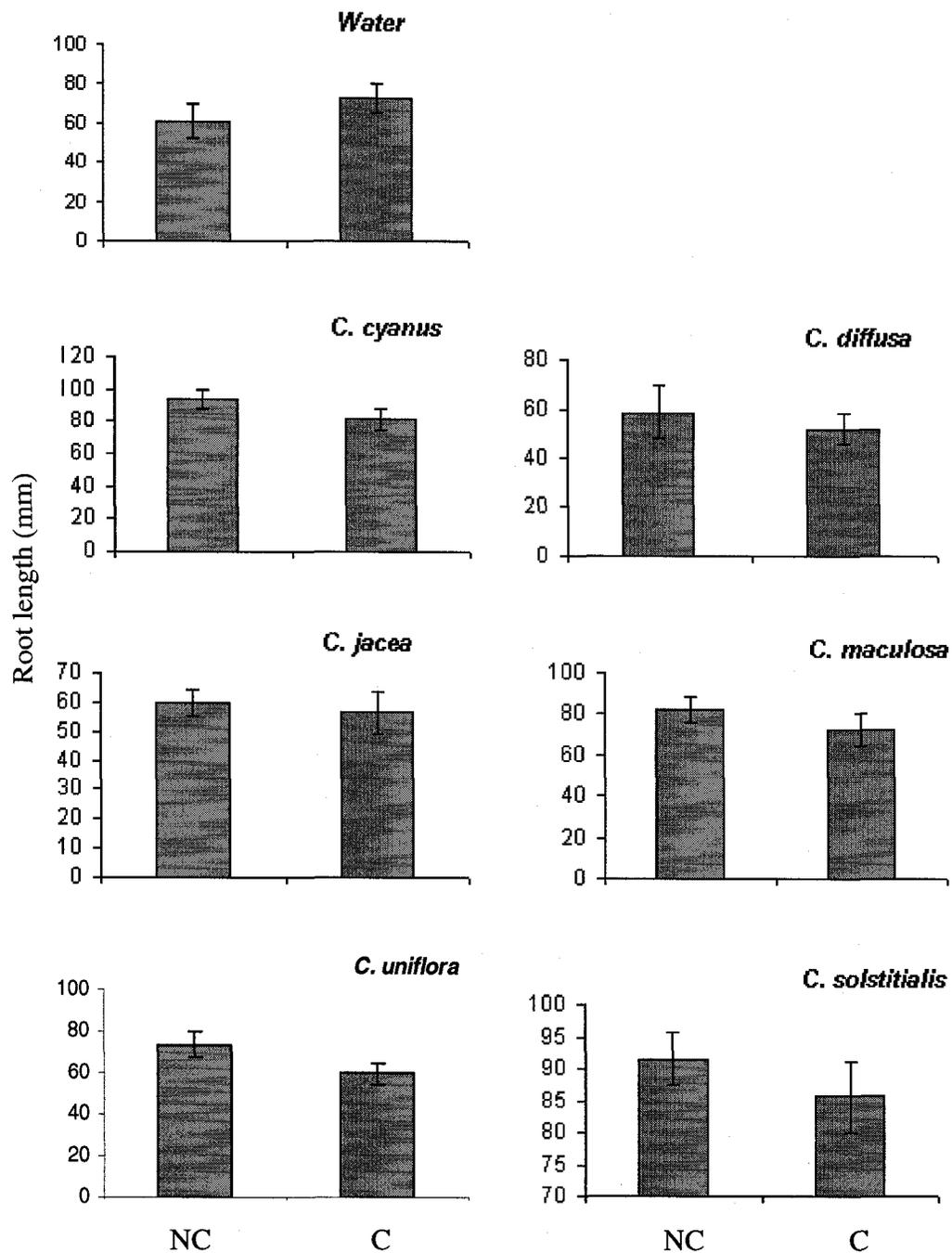


Figure 8: Root length (mean  $\pm$  SE) of radish (*Raphanus sativus*) seedlings 10 days after exposure to leachate collected from the soils of three non-invasive (*C. cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds as well as a water control. T-tests found no differences between any groups (Table 4). Note that scales are not equivalent. NC = untreated leachate; C = carbon-treated leachate.

Table 4: Statistical comparisons of non-carbon and carbon-treated leachates collected from the soils of three non-invasive (*Centaurea cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds on root growth of radish (*Raphanus sativus*) seedlings. All comparisons are t-tests.

Comparison (non-carbon vs. carbon)	Df	t-value	p
Water	8	-1.005	0.344
<i>C. cyanus</i>	8	1.469	0.180
<i>C. jacea</i>	8	0.414	0.690
<i>C. uniflora</i>	8	1.727	0.122
<i>C. diffusa</i>	8	0.509	0.625
<i>C. maculosa</i>	8	0.915	0.387
<i>C. solstitialis</i>	8	0.842	0.424

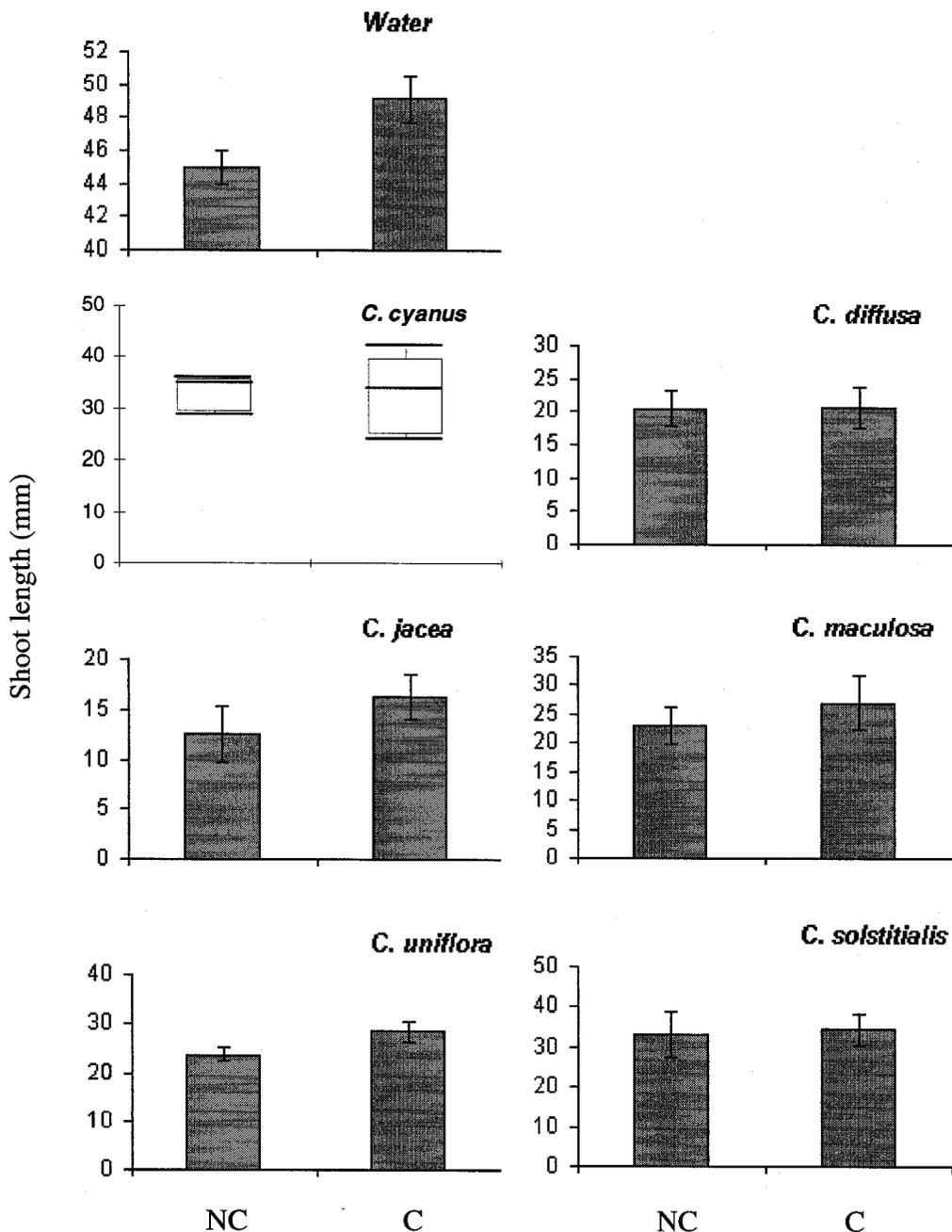


Figure 9: Shoot length (Bars: mean  $\pm$  SE; Boxplots: median and quantiles) of lettuce (*Lactuca sativa*) seedlings 10 days after exposure to leachate collected from the soils of three non-invasive (*C. cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds as well as a water control. *C. cyanus* treatments were compared using Wilcoxon rank sums; all others compared using t-tests. A significant difference was only found for the control water treatment, all other treatments were not statistically different (Table 5). Note that scales are not equivalent. NC = untreated leachate; C = carbon-treated leachate.

Table 5: Statistical comparisons of non-carbon and carbon-treated leachates collected from the soils of three non-invasive (*Centaurea cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds on shoot growth of lettuce (*Lactuca sativa*) seedlings. T-values are reported for parametric data;  $\chi^2$  values are reported for non-parametric comparisons using Wilcoxon rank sums.

Comparison (non-carbon vs. carbon)	Df	t-value or $\chi^2$ *	p
Water (control)	8	-2.359	0.046
<i>C. cyanus</i>	1	0.011 *	0.917
<i>C. jacea</i>	8	-1.044	0.327
<i>C. uniflora</i>	8	-1.858	0.100
<i>C. diffusa</i>	8	-0.049	0.962
<i>C. maculosa</i>	8	-0.708	0.499
<i>C. solstitialis</i>	8	-0.163	0.875

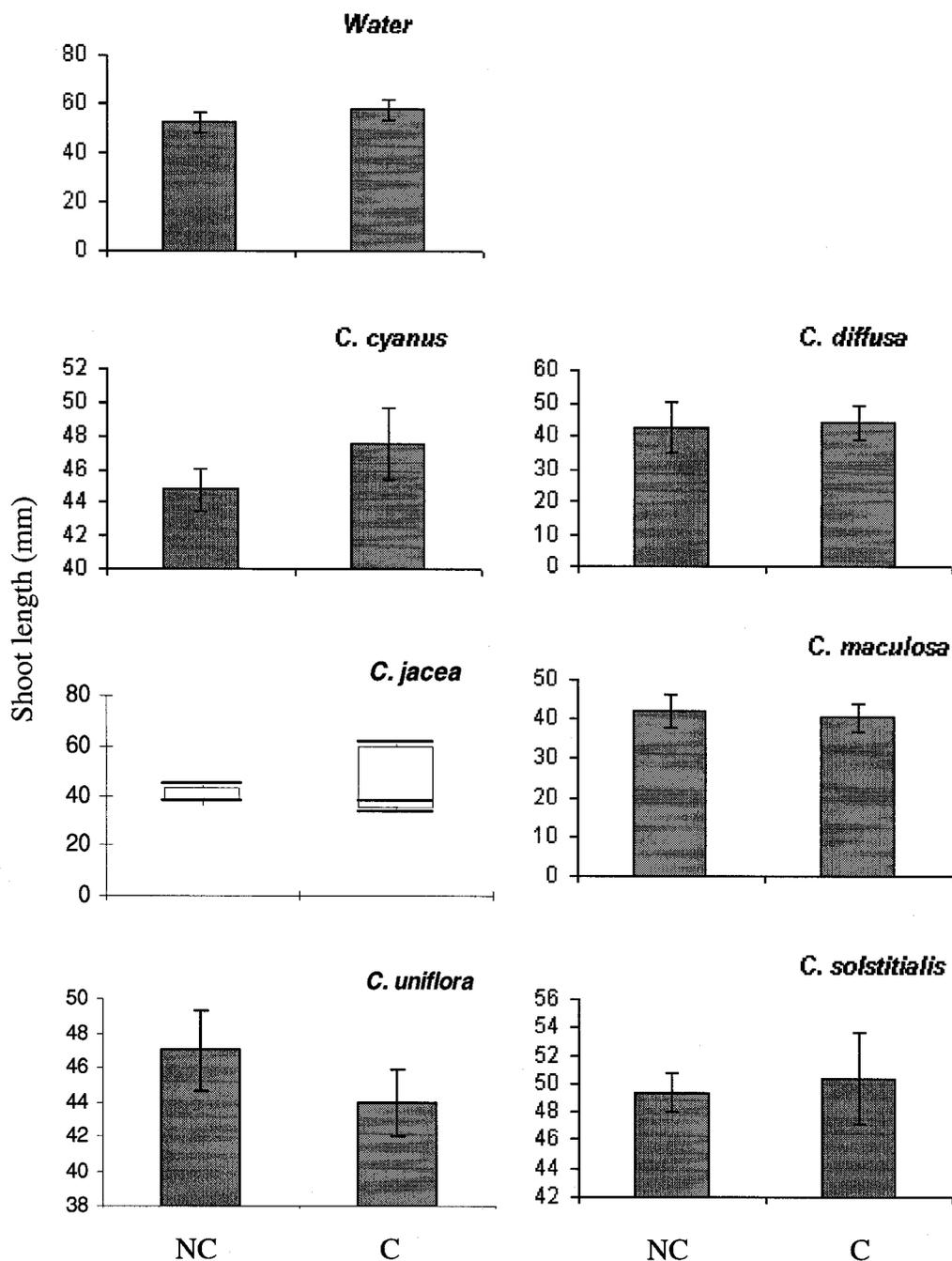


Figure 10: Shoot length (Bars: mean  $\pm$  SE; Boxplots: median and quantiles) of radish (*Raphanus sativum*) seedlings 10 days after exposure to leachate collected from the soils of three non-invasive (*C. cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds as well as a water control. *C. jacea* treatments were compared using Wilcoxon rank sums; all others compared using t-tests. All treatments were not statistically different (Table 6). Note that scales are not equivalent. NC = untreated leachate; C = carbon-treated leachate.

Table 6: Statistical comparisons of non-carbon and carbon-treated leachates collected from the soils of three non-invasive (*Centaurea cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds on shoot growth of radish (*Raphanus sativus*) seedlings. T-values are reported for parametric data;  $\chi^2$  values are reported for non-parametric comparisons using Wilcoxon rank sums.

Comparison (non-carbon vs. carbon)	Df	t-value or $\chi^2$ *	p
Water	8	-0.897	0.396
<i>C. cyanus</i>	8	-1.082	0.311
<i>C. jacea</i>	1	0.098 *	0.754
<i>C. uniflora</i>	8	0.985	0.353
<i>C. diffusa</i>	8	-0.157	0.879
<i>C. maculosa</i>	8	0.279	0.787
<i>C. solstitialis</i>	8	-0.285	0.783

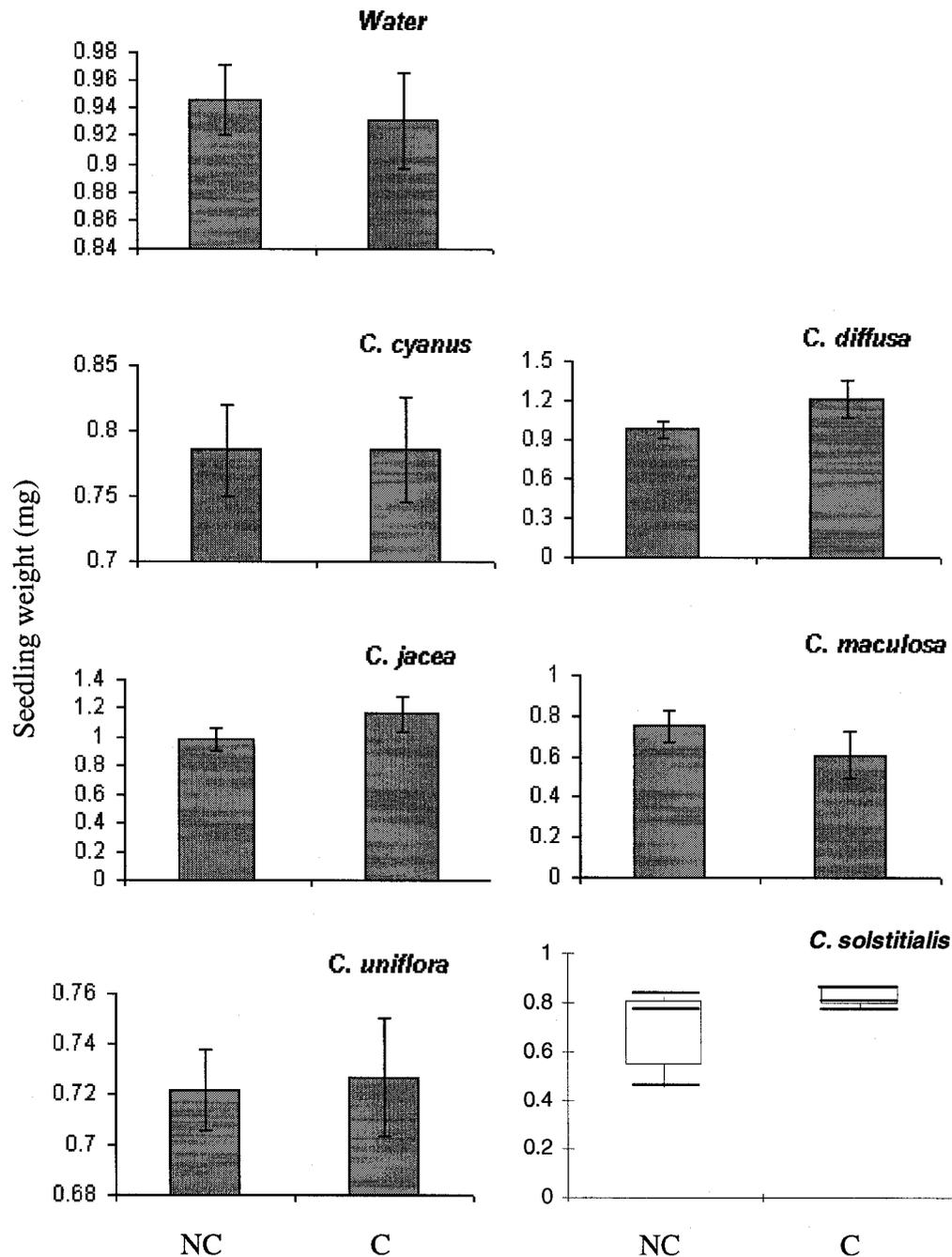


Figure 11: Weight (Bars: mean  $\pm$  SE; Boxplots: median and quantiles) of lettuce (*Lactuca sativa*) seedlings 10 days after exposure to leachate collected from the soils of three non-invasive (*C. cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds as well as a water control. *C. solstitialis* treatments were compared using Wilcoxon rank sums; all others compared using t-tests. All treatments were not statistically different (Table 7). Note that scales are not equivalent. NC = untreated leachate; C = carbon-treated leachate.

Table 7: Statistical comparisons of non-carbon and carbon-treated leachates collected from the soils of three non-invasive (*Centaurea cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds on lettuce (*Lactuca sativa*) seedling weight. T-values are reported for parametric data;  $\chi^2$  values are reported for non-parametric comparisons using Wilcoxon rank sums.

Comparison (non-carbon vs. carbon)	Df	t-value or $\chi^2$ *	p
Water (control)	8	0.343	0.740
<i>C. cyanus</i>	8	-0.0034	0.997
<i>C. jacea</i>	8	-1.211	0.260
<i>C. uniflora</i>	8	-0.178	0.863
<i>C. diffusa</i>	8	-1.485	0.176
<i>C. maculosa</i>	8	1.013	0.341
<i>C. solstitialis</i>	1	2.455 *	0.117

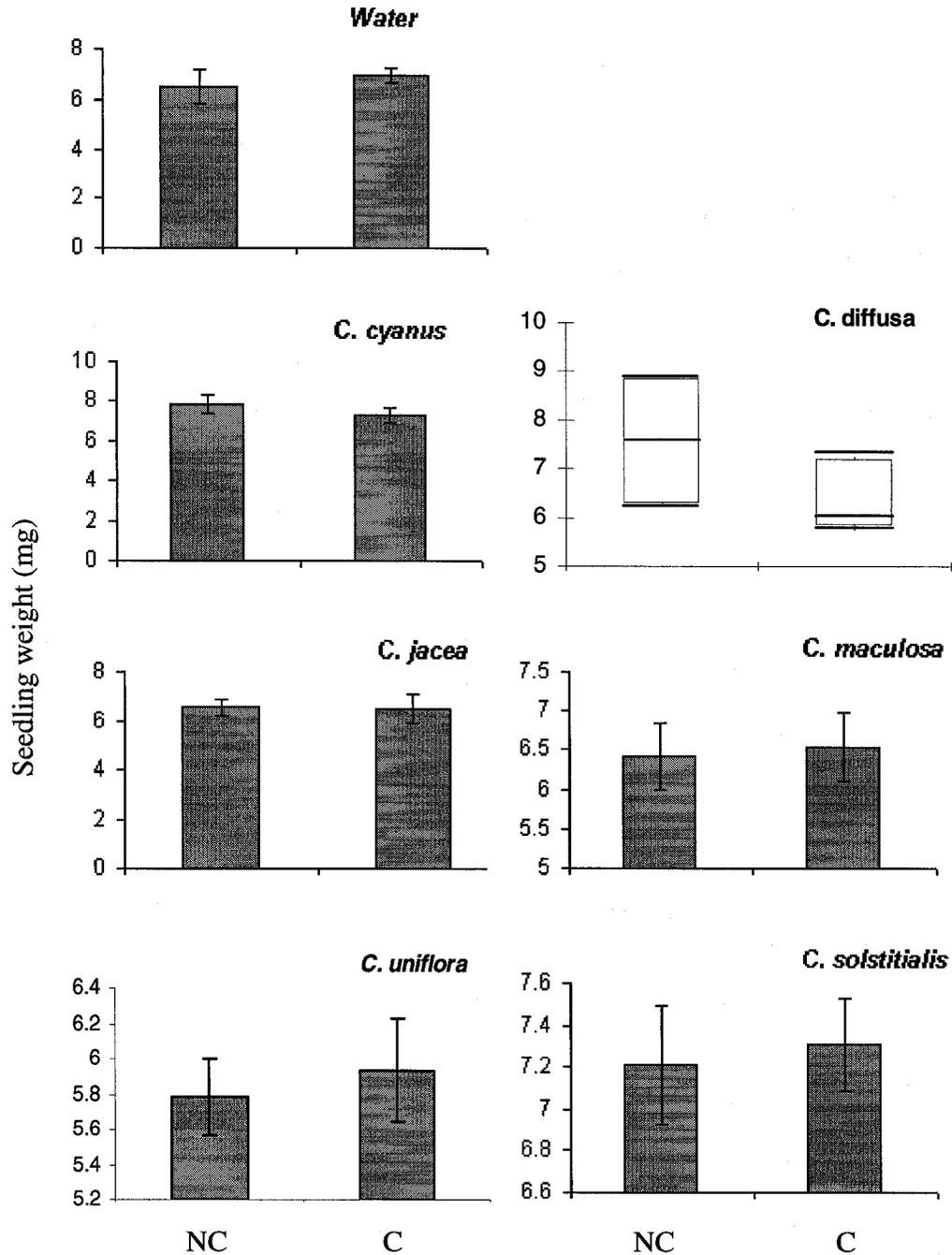


Figure 12: Weight (Bars: mean  $\pm$  SE; Boxplots: median and quantiles) of radish (*Raphanus sativum*) seedlings 10 days after exposure to leachate collected from the soils of three non-invasive (*C. cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds as well as a water control. *C. diffusa* treatments were compared using Wilcoxon rank sums; all others compared using t-tests. All treatments were not statistically different (Table 8). Note that scales are not equivalent. NC = untreated leachate; C = carbon-treated leachate.

Table 8: Statistical comparisons of non-carbon and carbon-treated leachates collected from the soils of three non-invasive (*Centaurea cyanus*, *C. jacea*, and *C. uniflora*) and three invasive (*C. diffusa*, *C. maculosa*, and *C. solstitialis*) knapweeds on radish (*Raphanus sativus*) seedling weight. T-values are reported for parametric data;  $\chi^2$  values are reported for non-parametric comparisons using Wilcoxon rank sums.

Comparison (non-carbon vs. carbon)	Df	t-value or $\chi^2$ *	p
Water	8	-0.630	0.546
<i>C. cyanus</i>	8	0.964	0.363
<i>C. jacea</i>	8	0.068	0.948
<i>C. uniflora</i>	8	-0.432	0.677
<i>C. diffusa</i>	1	2.160*	0.142
<i>C. maculosa</i>	8	-0.215	0.835
<i>C. solstitialis</i>	8	-0.252	0.807

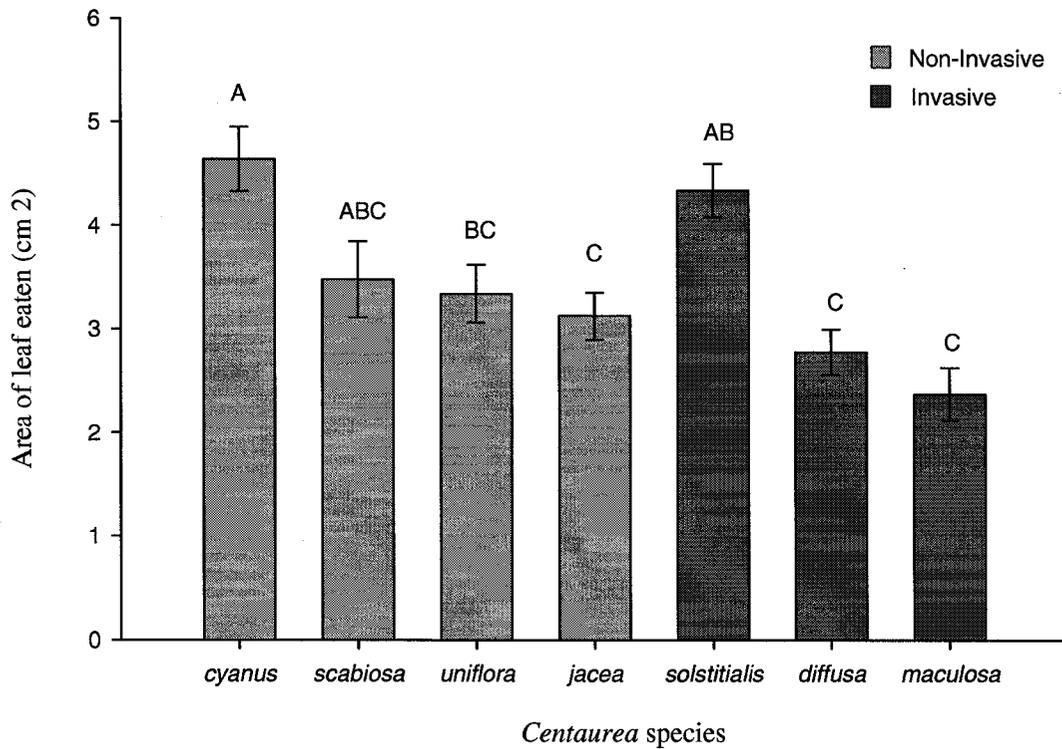


Figure 13: *Melanoplus femurrubrum* leaf feeding damage (mean  $\pm$  SE) on four non-invasive and three invasive knapweeds (*Centaurea* spp.) after two hours in a no-choice feeding experiment. Species was found to be a significant predictor of damage (ANOVA:  $F_{6,174} = 8.47$ ,  $p < 0.0001$ ). Treatments with different letters represent significant differences between species (Tukey HSD post hoc comparisons).

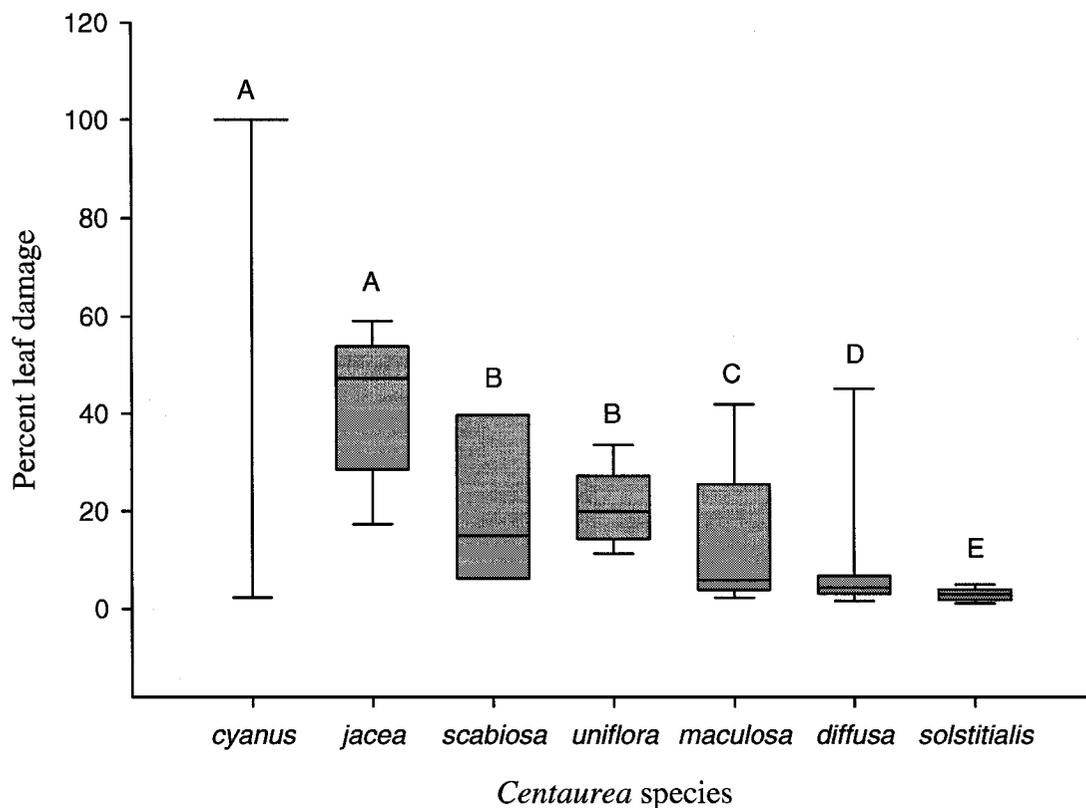


Figure 14: Percent leaf herbivory observed on four non-invasive and three invasive knapweeds (*Centaurea* spp.) after three months grown in a common garden in Ottawa, Ontario during the summer of 2006. Species was found to be a significant predictor of leaf herbivory ( $\chi^2 = 66.1796$ ,  $df = 6$ ,  $p < 0.0001$ ). Treatments with different letters have statistically different medians according to Dunn's post-hoc tests (1964). Horizontal lines in box-plots represent median values; 25<sup>th</sup> and 75<sup>th</sup> percentiles are represented by the box; 10<sup>th</sup> and 90<sup>th</sup> percentiles are represented by vertical lines.